J. Soc. Cosmetic Chemists **19** 531-549 (1968)

Synergism *in vitro* of certain antimicrobial agents

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A lecture delivered before the Society on 4th January 1968.

Synopsis—The need for broad-spectrum antimicrobial systems, active against both gram-positive and gram-negative micro-organisms, which function not only as preservative systems but also exert a rapid bactericidal and fungicidal effect is discussed. The desired antiseptic effect can result from the synergism obtained when using different combinations of antimicrobial agents.

The existence of antimicrobial compounds has been recognized for many years and chemically they represent a very heterogenous group of compounds. By definition, an antimicrobial agent is one that interferes with the growth and activity of microbes; it can be microbicidal (any agent that kills microbes) or microbiostatic (a condition in which the growth of the organism is prevented), or both, depending on the contact time. Although an antimicrobial which is microbiostatic is considered sufficient for the preservation of some cosmetics this is a dangerous state of affairs. A change in storage conditions can lead to a change of state where microbial growth becomes possible and therefore it is always best to use a microbicidal.

The specific nature of activity of any antimicrobial agent, i.e. whether it is essentially active against bacteria, moulds or yeasts or whether it has a more broad-spectrum of activity and is active against bacteria, moulds and yeasts has initially to be considered. One has then to distinguish between antimicrobial agents which are essentially active against gram-positive organisms e.g. *Staphylococci, Streptococci* and gram-negative organisms,

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e.g. *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas pyocyanea* (*aeruginosa*). An example of antimicrobial agents essentially active against gram-positive micro-organisms are bis-phenols of the hexachlorophane type. An example of an antimicrobial agent essentially active against gram-negative organisms is a compound of the phenoxetol type. By using two or more antimicrobial agents in combination, it is therefore possible to obtain a system exhibiting antimicrobial activity against a very broad range of micro-organisms. This combination of antimicrobial agents is said to be synergistic only if the spectrum of activity of the combination is greater than when either agent is used alone or to that which could be attributed to an additive effect. The concentration of each antimicrobial agent required is usually lower than when either is used alone. One is able to determine experimentally whether any given combination is synergistic in its antimicrobial effectiveness. Death of the entire population does not occur instantaneously but follows a definite predictable pattern. Irrespective of the initial population, under uniform conditions, the number of organisms will be reduced by the same percentage during each equal period of time. Thus for each unit of time the percentage of the population killed will be constant, e.g. if the initial population was 100,000 and 1,000 after 5 min, then after a further 5 min, 100 will remain; in each case the populations is reduced by 90%. This is known as the logarithmic death rate and is illustrated by *Fig. 1.*

An antimicrobial agent is influenced by the following factors:

1. Concentration of antimicrobial agent. The higher the concentration, the greater the rate of kill.
2. Time. The longer the time contact between the agent and organism the greater the number of organisms destroyed.
3. Temperature. An increase hastens the destruction.
4. Number of organisms. From *Fig. 1* it can be concluded that the larger the population, the longer the time required to kill all the micro-organisms providing all other conditions remain uniform.
5. Kind of organisms. Micro-organisms differ in their susceptibility to chemical and physical agents. Growing vegetative cells are most susceptible whereas spore forms are extremely resistant.
6. Nature of material bearing the organisms. The physical and chemical properties of the medium carrying the organisms has a profound influence on the rate and the efficacy of microbial destruction. The effectiveness of heat is greater in acid than in alkaline material and high con-

centrations of carbohydrates generally increase thermal resistance. The presence of extraneous organic matter can reduce efficacy of an antimicrobial by inactivating it or protecting the micro-organism from it.

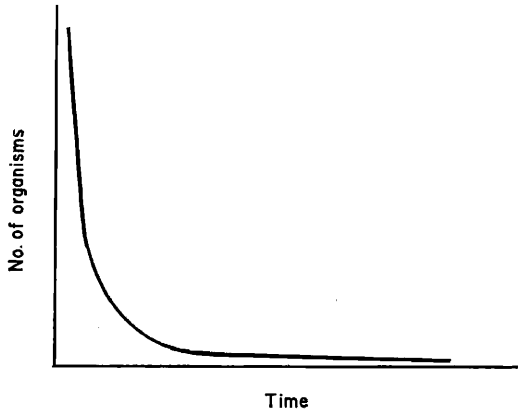

Figure 1

Accordingly, when an antimicrobial is required for a system one has to consider what factors remain constant. In cosmetics, the temperature of the product, the nature of the material-bearing organisms, and very often the kind of organisms present will be relatively constant for a given product. Thus one must select the best antimicrobial which will cope with the population likely to be encountered in the system, in a reasonable time. Sometimes a single compound is effective, but usually it has been found that widespread use of such an agent e.g. hexachlorophane, although initially very effective, brings additional problems. The vacuum left by one species of organisms which the antimicrobial is effective against, is often filled by another organism against which the agent has no effect. This problem is now tackled by synergism of antimicrobials, illustrated by the following:

The widespread use of hexachlorophane as a 3% emulsion in many commercial products as a means of providing an emulsion cream system for use in hospitals as an effective sterilising agent, e.g. as a surgical scrub, has recently been shown by many workers as well as by ourselves to be insufficiently effective for the application intended. Unopened containers have been found to be contaminated by organisms resistant to the antimicrobial used.

Hexachlorophane is essentially active against gram-positive bacteria, e.g. *Staphylococci* and consequently its use alone as a 3% emulsion in creams for the treatment of surgeons' hands in pre- and post-operative situations has its limitations. The question arises as to the effect of these systems containing 3% hexachlorophane against pathogenic gram-negative micro-organisms, such as *Pseudomonas pyocyanea, Escherichia coli* and *Proteus*. A scrub which is not effective against a broad range of organisms is potentially dangerous. During production the scrub can become contaminated with organisms, resistant to the antimicrobial present, which can cause an infection when used. In addition, an initially sterile scrub can pick up resistant strains during use. It is therefore important that the antimicrobials used should have the greatest possible spectrum of activity and thus the *in vivo* as well as the *in vitro* effect of the scrub is important.

It has been observed, for example by Knight *et al* (1), that a container of a 3% hexachlorophane emulsion was contaminated with gram-negative organisms and retained these organisms for several days. The use of such a product therefore in pre-operative "surgical scrubs" as a cleanser for the mucous membranes of infants, children, adults and for the dry wash of new born babies or as a surgical lubricant in gynaecology, should be rejected for hospital use if laboratory tests show the presence of such gram-negative organisms in unopened containers.

Anderson (2) has observed the persistent growth of gram-negative organisms (*Proteus* and *Pseudomonas*) in hexachlorophane soaps. He has emphasised that the suppression of pathogenic *Staphylococci* by several active antimicrobials, such as hexachlorophane, has given rise in many cases, to their replacement by gram-negative organisms. In particular, *Pseudomonas pyocyanea* and *Proteus* cause cross infection and are more likely to appear if the antimicrobial in use has no activity against gram-negative microbes. In considering the increase of gram-negative infection of the urinary tract, workers in Amsterdam (3) were able to relate this increase to the use of chlorhexidine as an antimicrobial agent. Most strains of *Proteus* investigated were insensitive to chlorhexidine at the recommended concentration. It is interesting that these workers (3) found that a combination of kanamycin (or neomycin) with tetracycline was synergistic against these organisms. The action of kanamycin (50μg ml^{-1}) with tetracycline (20 μg ml^{-1}) was bactericidal on all the strains of *Proteus* investigated, although such concentrations alone were ineffective.

Earlier work has shown the synergistic effect of using combinations of penicillin, tyrothricin, sulphonamides, acridines, quaternary compounds

and the phenoxetols. For example, Kaiser (4) has recognised the synergistic activity between 0.1% tyrothricin and 0.5% phenoxetol in the control of bacterial infection in medicinal products. Similar results were apparent when using 2, 7 diamino acridine and quaternary ammonium compounds.

The need therefore to consider an antimicrobial system with activity against both gram-positive and gram-negative organisms is clearly essential. In addition, the time necessary for the complete destruction of these organisms is of utmost importance in dealing with cream products of the above type. It is therefore necessary to consider the potential combinations of antimicrobial agents for this purpose, which based on a knowledge of their inherent activity, should, when combined, provide an antimicrobial system active against both gram-positive and gram-negative organisms. It is not possible however, purely on the basis of theoretical considerations, to say "x" is active against gram-positive organisms and "y" is active against gram-negative organisms, and therefore a combination of "x" and "y" will show activity against both, as well as a synergistic effect. Initially, this has to be determined experimentally by bacteriological methods.

We carried out tests, where known numbers of organisms likely to be encountered during use, were innoculated into given quantities, under sterile conditions. Samples were then removed at known time intervals on which plate counts were carried out using nutrient agar containing 3% *Tween 80* as a quenching agent. From the results obtained each antimicrobial combination was evaluated.

One has, of course, to distinguish between a purely additive and synergistic effect. Synergism is only apparent if, when used in combination to provide a more effective bactericidal or fungicidal effect, a lower concentration of each constituent is necessary than when any of the constituents are used independently.

For example, a 3% hexachlorophane emulsion (o/w type containing alkyl aryl polyether sulphonate, lanolin cholesterols, and petrolatum) when used alone was ineffective in destroying *Pseudomonas pyocyanea* within 30 min. However, when this emulsion was combined with 1% *Phenoxetol**, the *Pseudomonas pyocyanea* was destroyed within 3 min. *Phenoxetol* when used alone at 1% required 30 min to destroy the same organism. (*Table I*).

Tribromosalicylanilide, another antimicrobial agent, when used as a 1% emulsion was only effective in destroying *Pseudomonas pyocyanea* in 30 min, whereas a combination of this product together with 1% *Phenoxetol*

**Phenoxetol:* β-phenoxyethanol

destroyed the same organism within 5 min. When using 0.3% *p*-chlorophenoxetol together with 1% tribromosalicylanilide this organism was destroyed within 2 min (*Table II*).

Table I

Pseudomonas pyocyanea

	Time in minutes						
	1	2	3	5	10	15	30
3% Hexachlorophane emulsion alone	+	+	+	+	+	+	+
3% Hexachlorophane emulsion plus 1% phenoxetol	+	(+)	—	—	—	—	—
2% Phenoxetol alone	(+)	—	—	—	—	—	—
1% Phenoxetol alone	+	+	+	+	+	+	+
Control	+	+	+	+	+	+	+

\+ strong growth
(+) slight growth
— no growth

Table II

Pseudomonas pyocyanea

	Time in minutes						
	1	2	3	5	10	15	30
1% emulsion of tribromosalicylanilide	+	+	+	+	+	+	—
1% emulsion of tribromosalicylanilide +1% *Phenoxetol*	+	+	(+)	—	—	—	—
1% emulsion of tribromo salicylanilide +0.3% *p*-chloro-phenoxetol	+	—	—	—	—	—	—
1% *Phenoxetol*	+	+	+	+	+	+	—
0.3% *p*-chloro-phenoxetol	+	+	+	—	—	—	—
Control	+	+	+	+	+	+	+

\+ strong growth
(+) slight growth
— no growth

It should be noted that both the 3% hexachlorophane and 1% tribromosalicylanilide emulsions, when used alone, were very effective in destroying *Staphylococcus*, kills being obtained within 60 s. In comparison 2% *Phenoxetol* does not destroy these organisms in 60 min. Against *Escherichia coli* a combination of tribromosalicylanilide and *Phenoxetol* or *p*-chlorophenoxetol exhibited similar results (*Table III*).

Table III

Escherichia coli

	Time in minutes						
	1	2	3	5	10	15	30
1% tribromosalicylanilide emulsion	+	+	+	+	+	+	+
1% tribromosalicylanilide emulsion +1% *Phenoxetol*	+	+	(+)	—	—	—	—
1% tribromosalicylanilide emulsion +0.3% *p*-chlorophenoxetol	+	+	(+)	—	—	—	—
1% *Phenoxetol*	+	+	+	+	+	+	+
2% *Phenoxetol*	+	—	—	—	—	—	—
0.3% *p*-chlorophenoxetol	+	+	+	+	(+)	—	—
Control	+	+	+	+	+	+	+

\+ strong growth
(+) slight growth
— no growth

These bacteriological results give a clear indication of the value to be attached to combinations of this type for the purpose of combating infection by both gram-positive and gram-negative organisms. Repeated evaluations confirmed the results detailed in *Tables IV-VII*.

Tables IV-VII show the bactericidal properties of

(a) a commercially available 3% hexachlorophane emulsion,
(b) a commercially available 1% tribromosalicylanilide emulsion,
(c) aqueous solutions of various concentrations of *Phenoxetol* and *p*-chlorophenoxetol,
(d) hexachlorophane emulsions into which *Phenoxetol* has been incorporated, and
(e) tribromosalicylanilide emulsions into which *Phenoxetol* or *p*-chlorophenoxetol has been incorporated.

Table IV

Test organism—*Staphylococcus aureus* (a gram-positive organism)

Material	Time in minutes						
	1	2	3	5	10	15	30
A	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—
(2% *Phenoxetol*)	+	+	+	+	+	+	+
D+1% *Phenoxetol*	—	—	—	—	—	—	—

Table V

Test organism—*Pseudomonas pyocyanea* (a gram negative organism)

	Time in minutes						
	1	2	3	5	10	15	30
A	+	+	+	+	+	+	+
B	+	+	+	+	+	+	—
C (2% *Phenoxetol*)	—	—	—	—	—	—	—
C (1.5% *Phenoxetol*)	+	+	+	+	(+)	—	—
C (1.0% *Phenoxetol*)	+	+	+	+	+	+	—
C (0.5% *p*-chlorophenoxetol)	—	—	—	—	—	—	—
C (0.3% *p*-chlorophenoxetol)	+	+	+	+	(+)	—	—
D (+ 1% *Phenoxetol*)	+	+	(+)	—	—	—	—
E (+ 1% *Phenoxetol*)	+	+	—	—	—	—	—
E (+ 0.3% *p*-chlorophenoxetol)	+	+	—	—	—	—	—

Table VI

Test organism—*Escherichia coli* (a gram-negative organism)

	Time in minutes						
	1	2	3	5	10	15	30
A	+	+	+	+	+	+	+
B	+	+	+	+	+	+	+
C (2% *Phenoxetol*)	+	—	—	—	—	—	—
C (1.5% *Phenoxetol*)	+	+	+	+	+	—	—
C (0.5% *p*-chlorophenoxetol)	+	—	—	—	—	—	—
C (0.3% *p*-chlorophenoxetol)	+	+	+	+	(+)	—	—
D (+ 1% *Phenoxetol*)	+	+	(+)	—	—	—	—
E (+ 1% *Phenoxetol*)	+	+	(+)	—	—	—	—
E (+ 0.3% *p*-chlorophenoxetol)	+	+	(+)	—	—	—	—

Table VII

Test organism—*Proteus vulgaris* (a gram-negative organism)

	Time in minutes						
	1	2	3	5	10	15	30
A	+	+	+	+	+	+	+
B	+	+	+	+	+	+	+
C (2% *Phenoxetol*)	+	—	—	—	—	—	—
C (1.5% *Phenoxetol*)	+	+	+	+	+	+	—
C (0.5% *p*-chlorophenoxetol)	+	—	—	—	—	—	—
C (0.3% *p*-chlorophenoxetol)	+	+	+	+	+	(+)	—
D (+ 1% *Phenoxetol*)	+	+	—	—	—	—	—
E (+ 1% *Phenoxetol*)	+	+	—	—	—	—	—
E (+ 0.3% *p*-chlorophenoxetol)	+	+	—	—	—	—	—

\+ strong growth
(+) weak growth
— no growth

The results of the tables can be summarised as follows:

(1) Against gram-positive organisms, A and B are very effective, whilst C is not.

(2) Against gram-negative organisms, A and B are ineffective, whilst the higher concentrations of C are very effective.

(3) Combinations of A or B with C showed synergistic "broad spectrum" bactericidal properties, even when using lower concentrations of C, and in fact the results were virtually identical when the combinations were made up with 2% and 1% *Phenoxetol* or 0.5% and 0.3% *p*-chlorophenoxetol.

From the results of this work, and other published data, it is clear that germicidal skin cleansers and surgical scrubs based on hexachlorophane or tribromosalicylanilide are not effective against gram-negative bacteria whilst combinations of the above with the *Phenoxetols* are broad spectrum bactericides. From the above discussion and the results shown, the reason for finding a synergistic system, and its possible application are evident.

The problems and results described above are significant in the pharmaceutical industry, and further reference to applications in this field will be made below.

Application to cosmetic products

In the preservation of cosmetic products, the reason for the ever increasing range of antimicrobial agents available commercially and for which suggested applications are stated, is the constant search to find something to cover all eventualities. It should be emphasised that even if one day someone finds the ideal, this will never replace the absolute necessity for sterile and hygienic working conditions throughout the manufacture of a cosmetic product. Mrs. Wedderburn (5) has reviewed this topic. An antimicrobial agent should be an extra insurance in preventing contamination in a finished product and not a means of replacing sterile and hygienic manufacturing methods.

The esters of *p*-hydroxybenzoic acid are perhaps the most well-known antimicrobial agents used in the cosmetic industry. Several authors (6-12) have referred to the enhanced effectiveness of using a combination of esters in providing an antimicrobial agent with a broad spectrum of activity effective against yeasts, mould and bacteria. Furthermore, these ester combinations maintain their effectiveness in weakly acid, neutral and alkaline media, i.e. in a pH range of 6-9, in contrast to benzoic and sorbic acids which are ineffective in this pH range.

Further interest in this field is the synergism which exists not only between these esters themselves, but also the synergism apparent when using them with other antimicrobial agents such as dibromo-propamidine isethionate, merthiolate, phenoxetol, and its derivatives.

The bacteriological results obtained are shown in *Tables VIII* and *IX*.

The synergistic activity between Nipastat and Phenoxetol*

Table VIII
Bactericidal effect at 22°C, pH 6.9
B.6 *Pseudomonas pyocyanea*

	minutes				hours			days				
Material	3	5	10	30	1	3	5	1	5	7	21	28
0.25% *Phenoxetol*	+	+	+	+	+	+	+	+	+	+	+	—
2.0% *Phenoxetol*	—	—	—	—	—	—	—	—	—	—	—	—
0.09% *Nipastat*	+	+	+	+	+	+	—	—	—	—	—	—
0.2% methyl *p*-hydroxybenzoate	+	+	+	+	+	+	+	+	+	+	+	+
0.25% *Phenoxetol* +0.09% *Nipastat*	—	—	—	—	—	—	—	—	—	—	—	—
0.25% *Phenoxetol* +0.15% methyl *p*-hydroxybenzoate	+	+	+	+	+	+	+	—	—	—	—	—
Control	+	+	+	+	+	+	+	+	+	+	+	+

\+ strong growth
(+) slight growth
— no growth

Table IX
The synergistic activity between dibromopropamidine isethionate and *Nipastat*
Bactericidal effect at 22°C-pH 6.9

	Micrococcus Epidermidis					*Pseudomonas*				
	hours			days		hours			days	
Material	1	3	24	2	5	1	3	24	2	5
0.08% *Nipastat*	+	+	—	—	—	+	+	(+)	—	—
dibromopropamidine isethionate	+	+	+	+	+	+	+	+	+	+
0.05% *Nipastat* +0.01% dibromopropamidine isethionate	—	—	—	—	—	—	—	—	—	—
Control	+	+	+	+	+	+	+	+	+	+

**Nipastat:* a combination of esters of *p*-hydroxybenzoic acid

It will be clear from *Tables VIII* and *IX* that one is able to go some way in approaching an all-purpose preservative by using combinations of materials.

We have now obtained a product with a considerably enhanced antimicrobial system and in considering further possibilities of synergism this combination rather than a single ester should be used. In our work, therefore, in considering possible bacteriological experiments, an ester combination has been used as the one component of further mixtures, as already indicated in *Tables VIII* and *IX*.

I wish to emphasise that in our bacteriological tests we have laid considerable stress on determining the effectiveness of combinations on the organism *Pseudomonas pyocyanea* and other gram-negative organisms. The reasons for this have been our observations of the notable increase of this organism in cosmetic preparations. One suggestion put forward for the ever increasing incidence of *Pseudomonas pyocyanea* is the widespread use of hexachlorophane. Continued use of this material considerably reduces the degree of gram-positive skin bacteria, but clears the way for infection by gram-negative organisms such as *Pseudomonas pyocyanea, Proteus, Coli,* etc., against which this product is ineffective. This finding, amongst others, has led to the present bacteriological examination of combinations of the above type. A report (13) refers to a possible connection between the increase in pyocyaneus infections and the widespread use of disinfectants containing hexachlorophane which are ineffective against gram-negative organisms.

Recently, Savin (14) in an investigation of *Pseudomonas pyocyanea* infections in a hospital skin ward, found a diluted steroid cream to contain large numbers of these organisms. It is resistant to most antibiotics and able to fill the biological vacuum left when these are used. Since the introduction of antibiotics, *Pseudomonas pyocyanea* has played an increasing role in human infection.

Reference (15) was made early last year to several instances of infection by *Pseudomonas* which developed despite the fact that treatment had been carried out with creams containing antibiotics, such as hydrocortisone, amphomycin and neomycin to which this organism is usually sensitive. After these cases had been investigated further, it was found that opened tubes of the used ointment were themselves infected with *Pseudomonas pyocyanea* and that several other gram-negative bacteria are able to survive in what might be regarded as an unfavourable environment, providing a trace of moisture is present. Last year Burdon and Whitby (16)

reported the contamination by *Pseudomonas* species of two commercially available preparations, one containing chlorhexidine and the other a combination of chlorhexidine and cetrimide, these preparations being recommended as the disinfectants of choice against *Pseudomonas*.

In all these investigations, *Pseudomonas* is not the only offending micro-organism. Others which have been found include *Staphylococcus pyogenes*, *Escherichia coli* and different *Salmonella* strains.

In further consideration of all these facts, it is necessary not only to determine the actual sterility of a final preparation, but also to determine the resistance of a preparation when inoculated with high concentrations of micro-organisms of the above type. We have carried out much work on the determination of observing the percentage reduction of a given inoculant concentration over short intervals.

This work has involved emulsions containing various concentrations of hexachlorophane, tribromosalicylanilides, alone and in combination with *Phenonip**, a liquid broad spectrum antimicrobial system. Our investigations have been concerned with short time kills, i.e. less than 10 min and the results are shown in *Fig. 2*.

A combination of a 3% hexachlorophane emulsion with 1.0% *Phenonip* thus provides the desired kill rate more closely than 3% hexachlorophane emulsion by itself, against both gram-positive and gram-negative bacteria.

Phenonip itself is a synergistic combination of certain antimicrobial agents. It inhibits the growth of spoilage organisms at concentrations well within its maximal water miscibility of 0.5%. Lethal concentrations are higher but killing times are impressive at maximum solubility. Nonionic surfactants can be used to solubilise *Phenonip* providing sufficient concentrations which are highly effective. Tests with anionic surfactants had a negligible interference effect on the product, in contrast to quaternaries and compounds such as chlorohexidene, which are incompatible with anionics. Generally speaking the product shows inherent efficiency against a broad-spectrum of spoilage micro-organisms. The lethal effects are impressive and inhibiting activity is maintained at low concentrations.

Recent work by Tracy, Glass, Nicholson and Pivnick (17) on preservatives for poliomyelitis vaccine, has demonstrated the effectiveness of using combinations of antibiotics with formaldehyde, and the esters of *p*-hydroxybenzoic acid and the phenoxetols.

These workers found, for example, that the failure of low concentrations

*_Phenonip:_ based on *p*-hydroxybenzoates

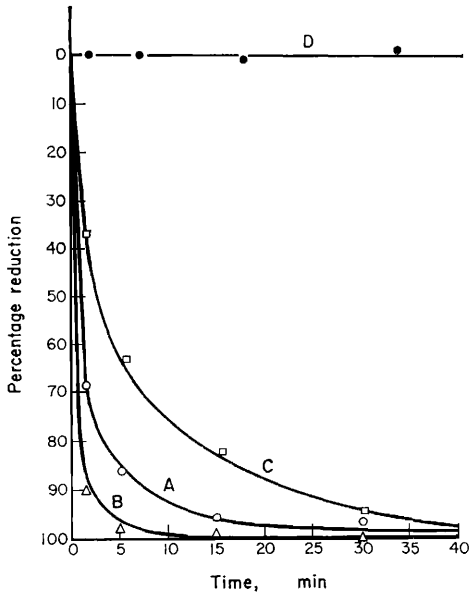

Figure 2

A—Emulsion base +3% hexachlorophane
B—Emulsion base +3% hexachlorophane + 1% *Phenonip*
C—Emulsion base +1% *Phenonip*
D—Emulsion base

of formaldehyde to inhibit yeasts and moulds necessitated the addition of an antimycotic compound. They showed that a mixture of esters of *p*-hydroxybenzoic acid with formaldehyde inhibited the growth of all bacteria, and six out of seven yeasts and moulds. When used alone, however, the esters of *p*-hydroxybenzoic acid exhibited only a mild antibacterial effect at the same concentrations. Their results are shown in *Table X*, which demonstrates the value of combinations and the synergism which is obtained.

A further paper by Pivnick, Tracy, Tosoni and Glass (18) demonstrates, for example, that vaccines containing antibiotics were inadequate for preventing the growth of heavy contamination with bacteria or light contamination with fungi. They showed that the addition of 0.375% *Phenoxetol* to poliomyelitis vaccines presented a stable mixture of preservatives (streptomycin, neomycin and *Phenoxetol*) which was inhibitory

Table X
Antimicrobial effect of *p*-hydroxybenzoates mixed with formaldehyde (18)

% *p*-hydroxy-benzoates	Formaldehyde ppm	Dilution of challenge culture	No. of tubes with growth / No. of tubes challenged	
			Bacteria	Yeasts and moulds
0	8	None	13/13	7/7
		10-3	13/13	5/7
		10-6	13/13	5/7
0	53	None	10/13	7/7
		10-3	4/13	4/7
		10-6	0/13	3/7
0.165	8	None	7/13	3/7
		10-3	5/13a	0/7
		10-6	3/13b	0/7
0.165	54	None	0/13	1/7
		10-3	0/13	0/7
		10-6	0/13	0/7

to both bacteria and fungi. They also showed that *p*-chlorophenoxetol at 0.2% provided very favourable results. The authors chose *Phenoxetol* for their investigations because of its known activity against *Pseudomonas pyocyanea*— a serious potential pathogen which may grow in vaccines and other medicinals. In their conclusions, these authors stated that *Phenoxetol* supplements the antibacterial activity of antibiotics, (a point already referred to above in considering penicillin-*Phenoxetol* systems) provides adequate antifungal activity and has excellent stability. Furthermore, this product is not inactivated by materials rich in protein, in contrast to bithionol and quaternary antibacterial agents, e.g. cetyl pyridinium chloride, which are inactivated by fatty acids present in protein (19). No benefit is obtained when using a combination of a material such as benzalkonium chloride with hexachlorophane, where a loss of antibacterial activity is observed (20). When considering a particular component for a potentially synergistic combination for use in preservation, the components must be compatible, firstly with each other and secondly, with the material into which they are incorporated and which they are intended to preserve.

Another recent publication describing the synergism of combinations of antibiotics and other well-known antimicrobial agents (21) describes the combined use of an antibiotic, e.g. neomycin and a product of the *Phenoxetol* type, in providing an effective system for the treatment of ear

infections. This preparation was both bactericidal and bacteriostatic against gram-negative and gram-positive organisms.

In the cosmetic and semi-cosmetic/pharmaceutical fields much has to be recommended on the basis of the bacteriological results given above for using such effective systems in products such as body deodorants, anti-dandruff preparations, shampoos, antimycotic foot preparations, after-shave preparations and cosmetic-serum products. Janistyn (22), for example, has especially recommended the use of a combination of *Phenoxetol* with other antiseptic agents in shaving lotions and other preparations required for use as effective antiseptic agents in the treatment of infectious skin diseases, e.g. dermatitis.

Considerable work by Clausen and Raugstad (23) has included combinations of many different antimicrobial agents including benzalkonium chloride, aminacrine hydrochloride, esters of *p*-hydroxybenzoic acid, benzyl alcohol, *Phenoxetol* and its derivatives, dibromo-propamidine isethionate, etc.

Some of the results obtained by Clausen *et al* (23) are shown in *Tables XI-XIII*.

The synergistic combination of propylene *Phenoxetol* at 1.0% and aminacrine hydrochloride at 0.05% in aqueous solution in the absence, or presence, of normal serum has an excellent bactericidal effect. The compatibility of this system with serum (i.e. a protein rich medium) is another feature worth mentioning, as this is in contrast to products such as quaternary antimicrobial agents which are inactivated by such media. This combination has an excellent effect on *Pseudomonas pyocyanea* which, as we have seen, appears to be highly resistant especially in the presence of organic matter. This synergistic combination was also effective against *E. coli*, although a weaker effect was displayed against gram-positive micro-organisms.

The results in *Tables XI-XIII* were obtained from tests conducted in 20% horse serum. Since the antimicrobial effect of many substances is considerably reduced by blood, the results obtained give an idea of the antiseptic effect of the products involved, i.e. a determination of their usefulness in preventing infection in topical therapy. We have already seen that the glycol ethers, e.g. *Phenoxetol* and its derivatives, are readily compatible with organic material of this type and combined with their non-irritant effect on the skin they may be considered well suited to external therapy. Quaternary compounds on the other hand, e.g. benzalkonium chloride or cetyl pyridinium chloride, as well as organic mercury com-

Table XI

Test bacteria	Time of action	Propylene *Phenoxetol* 10^{-2} dissolved in water pH 4.7	Aminacrine hydrochloride $1/2 \cdot 10^{-3}$ dissolved in water pH 6.0	Propylene phenoxetol 10^{-2} + aminacrine hydrochloride $1/2 \cdot 10^{-3}$ dissolved in		
				water pH 5.9	serum 20% pH 7.6	serum 50% pH 7.9
Ps. aerug	1 min	+	+	—	+	+
	5 min	+	+	—	—	—
	10 min	—	+	—	—	—
	15 min	—	+	—	—	—
	30 min	—	+	—	—	—
	1 hr	—	—	—	—	—
	2 hr	—	—	—	—	—
E. coli	1 min	+	+	+	+	+
	5 min	+	+	—	+	+
	10 min	+	+	—	—	+
	15 min	—	+	—	—	—
	30 min	—	+	—	—	—
	1 hr	—	+	—	—	—
	2 hr	—	+	—	—	—
Staph. aureus	1 min	+	+	+	+	+
	5 min	+	+	+	+	+
	10 min	+	+	+	+	+
	15 min	+	+	+	+	+
	30 min	+	+	—	+	+
	1 hr	+	+	—	—	+
	2 hr	+	+	—	—	—
Strept. faecalis	1 min	+	+	+		
	5 min	+	+	—	+	+
	10 min	+	+	—	+	+
	15 min	+	+	—	+	+
	30 min	+	+	—	+	+
	1 hr	+	+	—	—	+
	2 hr	+	+	—	—	—

Table XII

Bactericidal effect of a combination of benzalkonium chloride and benzyl alcohol against *Escherichia coli*, at 22°C (pH 4.7)

	hours		
	1	3	24
Benzalkonium chloride at 0.01%	+	+	—
Benzyl alcohol at 0.5%	+	+	+
Benzyl alcohol at 0.5% + benzalkonium chloride at 0.01%	—	—	—
Control	+	+	+

+ growth
— no growth

Table XIII

Bactericidal effect of a combination of benzalkonium chloride plus propylene *Phenoxetol* in 20% serum against *Pseudomonas pyocyanea* at 22°C (pH 6.5)

	min	hours				days
	20	1	3	24	48	3
Benzalkonium chloride at 0.1%	+	+	+	+	+	+
Benzalkonium chloride at 0.2%	+	+	—	—	—	—
Propylene *Phenoxetol* at 1.0%	+	—	—	—	—	—
Benzalkonium chloride at 0.1% + propylene *Phenoxetol* at 1.0%	—	—	—	—	—	—
Benzalkonium chloride at 0.1%+ propylene *Phenoxetol* at 0.5%	+	—	—	—	—	—
Control	+	+	+	+	+	+

pounds exhibit considerably reduced antimicrobial activity in the presence of organic matter and by using these products in combination with products not inactivated in this way, a system is obtained that is not only synergistic but also more effective in the presence of organic material. Clearly, however, the compatibility of each component of these synergistic systems and also their final intended application must be ascertained before such systems are used in any given preparations.

It will therefore be evident that several possibilities exist. Apart from the foregoing results some other references may be summarised as follows:

Osman and Mariah (24) studied the effect of combining salicylic acid with other preservatives, and they were able to conclude from their work "that combinations of salicylic acid and any of the other preservatives investigated proved to be more effective than any of these substances used singly".

Myddleton (25) stated that "mixtures of different antiseptics sometimes reinforce each other and notably the esters of *p*-hydroxybenzoic acid have been found to exhibit higher preservative action than the sum of the activities of the components of the mixture (this therefore is a synergistic effect rather than a purely additive effect) both in the absence and presence of nonionic surface-active agents".

Gershenfeld (26) has pointed out the advantages of using combinations.

Maccacaro (27) has written a review which defines and discusses synergism, and gives a good idea into the effects of the interaction between antimicrobial agents.

Other references to the use of combinations have also been made (6, 28-30).

Reference has been made above to the use of antibiotics together with esters of *p*-hydroxybenzoic acid in connection with the stabilisation of vaccines. Another interesting application is the use of these esters together with an antibiotic in the course of antibiotic therapy.

Moniliasis, an infection by *Candida albicans*, which arises after continued antibiotic therapy can be successfully treated when using esters of *p*-hydroxybenzoic acid, referred to in a patent (31). The specification states that with the combined addition of esters of *p*-hydroxybenzoic acid to preparations containing tetracyclin, undesirable side effects of antibiotic therapy are inhibited. Wegmann (32) has recently confirmed the effectiveness of applying a combined treatment of antibiotics and esters of *p*-hydroxybenzoic acid in preventing the disturbing side effects resulting from continued antibiotic therapy.

(Received: 24th January 1968)

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