A NEW METHOD FOR MEASURING DIFFUSION OF ANTISEPTICS FROM OINTMENT BASES

By G. H. Clark and G. E. Davies

From the Pharmaceutical Research and Service Laboratory, Imperial Chemical Industries, Ltd., Blackley, Manchester

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Although it is not yet possible to determine by laboratory tests the clinical efficacy of medicated ointments, methods have been suggested for the measurement *in vitro* of certain physical properties which may influence their therapeutic action. For instance, it has frequently been found useful to study the rate of liberation of active agent from base under standard conditions. It is realised that data so obtained cannot be related directly to release of drug *in vivo* but it may, nevertheless, serve as a guide to the suitability of bases for specific purposes. The information may also provide a check on possible incompatibility between drug and excipients. Attention has so far been directed chiefly to the study of the diffusion of water-soluble drugs, usually antiseptics, from various bases and two general methods of measurement have been evolved:—(a) Measurement of zones of inhibition observed on inoculated agar following diffusion from a small cup[1]; (b) Estimation by chemical or other suitable methods, of the amount of drug which diffuses into water through a cellophane or similar membrane[2,3].

Method (a) usually involves a minimum period of 24 hours contact between ointment and agar before any readings are made. It is thus not possible to compare different bases in respect of diffusion-rates over shorter periods of, say, 2 hours. There is also the drawback that it is not always easy to fill the cups completely and uniformly, as a result of which there may not be good agreement between duplicate experiments. In addition, the interpretation of results obtained by method (a) is very difficult, since not all antiseptics penetrate the agar gel in the same way. The antiseptic may—(1) diffuse in a simple manner through the agar gel, in which case the concentration of antiseptic falls steadily as the distance from the cup increases; (2) be weakly adsorbed on the agar, giving rise to a gradual fall in concentration of antiseptic from the cup outwards; the penetration of antiseptic is not, however, so extensive as in the first case; (3) be strongly adsorbed on the agar so that, from the cup outwards, there is a narrow zone of substantially constant antiseptic concentration followed by a rapid decrease to zero. Before conclusions can be drawn from any method involving transfusion into agar the precise nature of the process must be known. It is possible to compare different concentrations of the same antiseptic (e.g. penicillin) once the diffusion mechanism is known; it is very difficult to compare different antiseptics which may have dissimilar diffusion mechanisms.

Method (b) has the advantage of being adaptable to all water-soluble drugs, whether bacteriostatic or not, but requires a rather elaborate technique.

The object of the work described here was to devise a simple technique capable of detecting different rates of diffusion over shorter time intervals. It has resulted in the development of a test in which the active agent of an ointment diffuses through cellophane on to inoculated agar, and the minimum time of contact sufficient to cause inhibition of bacterial growth is recorded.

Attempts were made to devise a method of applying an ointment to inoculated agar in such a manner that it would be removable easily and completely after any desired time-interval. After a number of preliminary tests the procedure described below as Method I was selected as the most satisfactory and was thereafter evaluated by:—(a) Confirmation of reproducibility, (b) Comparison against other methods, (c) Utilisation to distinguish between bases of different type.

Method 1. 10 ml. of molten agar is poured into a petri dish and allowed to set. The plate is inverted in an incubator and dried for 2 hours. On to the layer of agar is poured 5 ml. of molten agar containing 1 ml. of a 1 in 10 broth dilution of a 24-hour broth culture of *Staphylococcus aureus*. Similar results are obtained if, instead of using two layers of agar, the culture is pipetted directly on to the surface of alcohol-dried agar, but it is difficult to ensure an even spread of the culture and it may take several hours for the liquid to become absorbed into the agar. The first method of inoculation was therefore considered preferable since it results in a dry, evenly-contaminated surface. By means of sterile forceps, four 1 inch squares of sterile cellophane are placed on the surface of the agar and incubated for 45 minutes. The incubation causes the cellophane to spread evenly on the agar. To absorb moisture a disc of filter paper is trapped in the lid of the dish. The preparation to be tested is applied to the surface of the cellophane by means of an all glass hypodermic syringe without needle, so as to leave a border about $\frac{1}{8}$" wide on the cellophane. The plate is then returned to the incubator and pieces of cellophane with the adherent preparation under test, removed at suitable time-intervals. After incubation overnight the plates are examined for growth-inhibition which is indicated by a clear area of agar at the site of the cellophane square. The minimum time required to cause inhibition of growth is recorded. This period may vary from a

TABLE I

DIFFUSION OF ANTISEPTIC FROM PHENYLMERCURIC NITRATE JELLY 0·001 PER CENT. AS DETERMINED BY CELLOPHANE METHOD

Date Tested	Period of contact between treated cellophane and agar							
	1 min.		5 mins.		10 mins.		15 mins.	
15.1.48	∓	∓	∓	∓	+	+	+	+
16.1.48	−	−	∓	−	+	+	+	+
20.1.48	∓	−	∓	−	+	+	+	+
23.1.48	−	−	∓	∓	+	+	+	+
27.1.48	−	−	+	+	+	+	+	+

+ =complete clearance ; ∓ =partial clearance ; − =no clearance.

few minutes to several hours and sighting experiments are therefore necessary when a preparation is tested for the first time.

Reproducibility. The test was applied to a range of active agents in a variety of bases (see Appendix) and no difficulty was experienced in obtaining replicate results. This is illustrated by Table I, which records successive tests made on a dilute phenylmercuric nitrate jelly.

Comparison with other methods. Although there may be objections to using variations of the agar cup method for evaluation of the diffusion of an antiseptic from ointment bases, there are no other methods available which are based on a biological technique. Accordingly, for comparison, it was decided to use three variations of the agar diffusion method, as detailed below.

Method 2. A cylindrical hole 15 mm. in diameter is cut in an agar plate inoculated as in Method 1, and a few drops of molten agar added, from a Pasteur pipette, to cover the glass surface so exposed. This hole is then filled with the preparation to be tested. The plate is incubated for 24 hours and the diameter of clearance measured.

Method 3. This method is similar to Method 2, but the preparation is placed in a ditch 10 mm. × 40 mm. instead of in the cylindrical hole.

Method 4. Four sterile glass cylinders of internal diameter 10 mm. and depth 13 mm. are filled with the preparation to be tested and placed on the surface of a poured inoculated agar plate.

The results are recorded in Table II.

TABLE II

ANTISEPTIC ACTIVITY AS TESTED BY FOUR DIFFERENT METHODS

Base	Active agent		Method 1	Method 2	Method 3	Method 4
			Minimum time for inhibition	Average diameter or clearance of 4 tests		
		per cent.	minutes	mm.	mm.	mm.
Jelly	crystal violet	1·0	5	20	20	20
	sodium ethyl mercurithiosalicylate	0·02				
Jelly	sulphanilamide	5·0	5	20	18	10
Jelly	4 : 4′ - diamidinodiphenoxypropane di-(hydroxyethanesulphonate)	0·15	10	4	4	4
Oil-in-water emulsion	potassium hydroxyquinoline sulphate	0·2	15	20	18	10
	chlorocresol	0·2				
Petroleum/lanolin ...	benzoyl peroxide	10·0	60	10	14	4
	chlorohydroxy quinoline ...	0·5				
Simple ointment ...	phenylmercuric nitrate ...	0·001	60	7	6	4
			hours			
Glycerin/wool fat ... White soft paraffin ...	Resorcinol	12·5	2	2	4	1
Oil-in-water cream ...	diamidinodiphenoxypropane dihydrochloride	0·1	4	2	4	0

A number of preparations when tested in the same way gave negative results by all four methods, i.e., no diffusion of active agent could be detected. These included Scarlet Red Ointment B.P.C., Ichthamol Ointment B.P.C., Coal Tar Ointment B.P.C., and Salicylic Acid Ointment B.P.C. It will be noticed that a certain parallel exists between the results obtained by Method 1 and those from the remaining three inasmuch as increased times of contact usually correspond to decreased zones of inhibition, but Method 1 provides a much sharper distinction.

Distinction between different bases: Phenylmercuric nitrate and neutral proflavine sulphate were formulated into a series of five different types of base and at different concentrations. The results of applying Method 1 to these preparations are recorded in Table III.

TABLE III

MINIMUM TIME FOR INHIBITION OF BACTERIAL GROWTH BY VARIOUS OINTMENTS WHEN TESTED BY THE CELLOPHANE METHOD

Active ingredient		Fatty Base	Water-in-oil Base	Oil-in-water Base 1	Oil-in-water Base 2	Jelly
	per cent					
Phenylmercuric nitrate	0·001	60 mins.	10 mins.	15 mins.	15 mins.	2 mins.
,, ,, ...	0·002	30 mins.	10 mins.	4 mins.	4 mins.	3 mins.
Neutral proflavine sulphate	0·05	24 hrs.	24 hrs.	4 hrs.	45 mins.	30 mins.
,, ,, ,, ...	0·1	24 hrs.	24 hrs.	4 hrs.	25 mins.	15 mins.
,, ,, ,, ...	0·2	24 hrs.	24 hrs.	4 hrs.	15 mins.	5 mins.

The results obtained with this test seem to indicate that, other factors being equal, a jelly type of base is superior to oil-in-water emulsions, which in turn are better than water-in-oil emulsions or fatty bases. In fact, for water-soluble antiseptics, it would seem that the oil phase is largely redundant, its purpose being solely to increase the consistency of the preparation so that it will remain at the site of application. This function can be fulfilled equally well with the jelly base which has the added advantage of being cheaper and easier to prepare.

DISCUSSION

There will probably always be a wide gap between the clinical evaluation of an ointment and laboratory tests made *in vitro*. Nevertheless, pharmaceutical technique has advanced beyond the stage when an ointment was selected merely on account of its elegance. The latter practice must have inevitably resulted in the formulating of many ineffective preparations whose faults might have remained undetected for a long time.

In the formulating of antiseptic preparations there is often available a series of ointment bases all equally suitable in stability and appearance. The final choice should therefore take into account the rapidity with which the antiseptic is required to be released. It is suggested that the test described above provides a convenient method of doing this since it can be performed with standard apparatus available in most hospital dispensaries and manufacturing laboratories. The test can also be applied as a routine measure to control manufacture.

SUMMARY

1. A test is described for measuring the release of antiseptics from ointments.

2. The significance of the test is discussed and some applications suggested.

REFERENCES

1. Ruehle and Brewer, *U.S. Dept. Agric. Circ.*, No. 198, 1931.
2. Howard, *New Engl. J. Med.*, 1945, 232, 698.
3. Fuller, Hawking and Partridge, *Quart. J. Pharm. Pharmacol.*, 1942, **15**, 127.

APPENDIX

MATERIALS

Ointment Bases:		
Fatty Base:	Simple Ointment B.P.	
Water-in-oil Base:	Hydrous Ointment B.P.	
Oil-in-water Base 1:	Active Agent	a sufficient quantity
	Emulsifying Wax B.P.	7 g.
	Hard Paraffin B.P.	5 g.
	Liquid Paraffin B.P.	40 g.
	Distilled Water, sufficient to produce	100 g.
Oil-in-water Base 2:	Active Agent	a sufficient quantity
	Castor Oil B.P.	20 g.
	Cetyl Alcohol	6 g.
	Diethyleneglycol distearate	9 g.
	Polyglyceryl ricinoleate	5 g.
	*Emulsifying Agent (non-ionic) ...	3 g.
	Distilled Water, sufficient to produce	100 g.

* A condensation product of cetyl alcohol with ethylene oxide.

Jelly:	Active Agent	a sufficient quantity
	Cellofas W.F.Z.	6 g.
	Distilled Water, sufficient to produce	100 g.
Cellophane:	Described as non-waterproof of 0·0009 inch thickness	
Staphylococcus aureus:	N.C.T.C. 4163	per cent.
Culture Medium:	Sodium Chloride	0·125
	Peptone	1·5
	Yeastrel	0·5
	Potassium dihydrogen phosphate (anhydrous)	0·1
	Dipotassium hydrogen phosphate (anhydrous)	0·1
	Dextrose	0·5
	Agar	2·0
	Distilled water (*p*H 7·2 to 7·6) to produce	100