ANTAGONISM BETWEEN NON-IONIC DETERGENTS AND ANTISEPTICS

By Mlle. A. Bolle and A. Mirimanoff

From the Laboratory of Galenical Pharmacy the University of Geneva

Received May 26, 1950

The question of detergents has been the subject of numerous papers in scientific literature, and their main properties have recently been summarised for the pharmacist by N. J. Harper[1]. From the point of view of their germicidal properties, cationic detergents are often powerful antiseptics; whereas non-ionic detergents have no such activity owing to the fact, noted by Glassman[2], that they do not denature proteins. As to the anionic derivatives, they have in an acid medium a certain bactericidal action against Gram-positive micro-organisms. We have shown in an earlier paper[3] that the toxicity of these substances did not depend on their surface-active or wetting properties, and that this toxicity varies considerably with the type of plant cell to which it is applied; certain fungi, for example, being more sensitive to the action of anionic wetting agents than the higher forms of plant cells. Finally, when an anionic wetting agent of the type of sodium laurylsulphate is added to an antiseptic—the composition of the latter can vary considerably—a synergistic action is frequently obtained, since the wetting agent increases the fungistatic action of the antiseptic. This does not occur with the higher plant cells and rarely with bacteria. Engler[4] observed that non-ionic detergents not only possess no proper fungistatic action in themselves, but they stimulate the growth of several kinds of moulds. Moreover, in the presence of antiseptics, the fungistatic action is clearly inhibited.

These incomplete observations have led us to take up this latter problem on a more general basis, in relation to the following two points: (a) non-ionic detergents are increasingly used in pharmacy as creams or ointments, (b) if added to an antiseptic preparation, what will be the real value of the latter?

Experimental

In such investigations there are three main variables: (a) the micro-organism; (b) the detergent; (c) the antiseptic.

To limit the number of experiments we restricted the first variable to 2 strains of fungi, the second to 7 detergents selected from amongst those most commonly employed, and the third to 3 antiseptics of very different chemical composition. In certain instances the tests were carried out for definite control purposes or to check either a working hypothesis or an interpretation of results. For most of our tests we worked with a liquid medium, using the Jaag medium, which is favourable to the development of mycelium and has the following composition:—sodium nitrate 3 g., potassium dihydrogen phosphate 1 g.,

potassium chloride 0·5 g., magnesium sulphate 0·5 g., ferrous sulphate 0·01 g., sucrose 50 g., water to 1,000 g.

A suspension of spores (*Penicillium* spp. *Aspergillus niger*) of constant age (10 days) and constant average density is added to 50 ml. flasks containing 10 ml. of Jaag medium. This is the control series. Into similar flasks either the selected antiseptic in progressive dilution or the wetting agent is poured, making a separate series for each. A last series of flasks contains the spores, the antiseptic and the wetting agent all together. The flasks are put in an incubator at 28°C. and observations made at the end of 10 days. It is evident that if the addition of a wetting agent or an antiseptic or a mixture of the two can change the Jaag medium, by diluting it, the control series must undergo a similar modification. Each series of tests is carried out with at least 2 flasks for each variable, and, in the series requiring the weighing of mycelium, 3 flasks are used.

Results

Our first experiments having shown qualitatively that the presence of certain non-ionic detergents favoured the development of mycelium, we have attempted to bring out this point by tests of a quantitative nature. For this purpose we compared the weights of the clumps of mycelium obtained from the control series with those of mycelium from the media with added detergent, at a 2 per cent. concentration. Without going into the details of these tests, of which only the comparative results are of any interest, we shall simply indicate the weight of the mycelium cultivated in the presence of a detergent as compared with that of the control mycelium, the weight of the latter being arbitrarily represented as unity. In classifying, in the order of their growing action, the detergents which favour the development of mycelium we obtain the following series, in which the figures represent the average of 3 weighings.

	Weights
Control	1
+ Carbowax 1500	1·5
+ Tween 80	1·5
+ Span 20	2
+ Crillex 6	2
+ Crillex 11	2
+ Crillex 16	2
+ Oleic acid	2
+ Tween 40	3

Interpretation of results is not quite so simple as might at first appear. Molho and Lacroix[5], in a paper which we received during our experiments, rightly remark that the methods for testing fungistatics are particularly delicate when they deal with the estimation of the weight of the mycelial growths.

These authors have, therefore, made use of a non-ionic wetting agent,

Tween 60, which is a sorbitol monostearate condensed with a long chain of ethylene polyoxide, to render homogeneous the suspension of spores in a liquid medium. The addition of 0·02 per cent. of this product gives good results but upsets the metabolism of the fungus. Observations must therefore be limited to a very definite period of time as this concentration of Tween retards the fungus up to the sixth day. After this period an acceleration of development is to be observed, which depends both on the form of the containers and on the aeration surface.

In our tests we used non-ionic detergents of 100 times higher concentration (2 per cent.) as our object was not so much to obtain a better homogeneity of suspension as to work with quantities equivalent to those used in pharmaceutical practice. The remarks of the French scientists nevertheless remain true, and it is important to work with well-defined conditions and within convenient time limits (10 days in our experience). Further, although our quantitative results very clearly showed the stimulating role of different non-ionic wetting substances on the growth of the *Aspergillus niger* organism (we also made qualitative tests on *Penicillium* spp. *Botrytis cinerea, Achorion quinckeanum*) the main purpose of the present work was to observe the behaviour of some of these detergents in the presence of antiseptics.

The results thus appear clearly and in a perfectly reproducible manner, for if the detergent inhibits the antiseptic at a given dilution of the latter the development of mycelium will take place and a greater quantity of antiseptic is required to obtain the necessary fungistatic effect. Tables I and II summarise the results of a great number of tests.

The following conclusions may be drawn:—

(1) All non-ionic detergents added by themselves to a culture medium have a stimulating action on the development of mycelium.

(2) The antiseptics used are partly inhibited by the detergents added to the culture medium. It should be noted that if inhibition is relatively slight for nipagin, with the detergents used, it is more pronounced for oxyquinoline sulphate and extremely high for G.4 (dioxydichlorodiphenylmethane). At a concentration of 1 in 10,000 this last product is fungistatic for *Aspergillus niger:* it is not so at 1 in 1,000 in the presence of Crillex, Spans and Tweens.

(3) Carbowax 1500 has no appreciable inhibitory action on the antiseptic. It is a polyethylene glycol polymer.

(4) Mannite, tested because of its chemical relationship with certain non-ionic detergents, produces no effect.

Cystein and gelatin have a strong inhibitory action on oxyquinoline sulphate (no doubt because of their SH function), but do not interfere with G.4.

(5) Oleic acid behaves in the same way as the detergents used. Since these frequently contain a certain quantity of free oleic acid, it would appear that this acid can be considered as the essential cause of this

-Aspergillus niger. THE BEHAVIOUR OF THREE ANTISEPTICS IN THE PRESENCE OF DIFFERENT DETERGENTS AND OF SUBS
INHIBITORY TO ANTISEPTICS

ded Antiseptic		Absence of surface-active agent	Carbowax 1500 2 per cent.	Crillex 6 2 per cent.	Crillex 11 2 per cent.	Crillex 16 2 per cent.	Span 20 2 per cent.	Tween 80 2 per cent.	Tween 60 2 per cent.	Oleic Ac. 2 per cent.	Sodium Oleate 2 per cent.	Mannite 2 per cent.
... ...		+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +
	0·2 per cent.	−			−	+			−			
	0·1 per cent.	−			+ +	+			+ +			
	0·05 per cent.	+			+ +	+ +			+ +			
	0·1 per cent.	+ +			+ +	+ +			+ +			
sulphate	0·1 per cent.	−	−	−	−	−	−	−	−	−	−	−
„	0·05 per cent.	−	−	−	−	−	−	−	−	−	−	−
„	0·02 per cent.	−	−	+ +	+ +	+ +	−	+ +	+	+ +	−	−
„	0·01 per cent.	+	−	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	−
„	0·005 per cent.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
diphenylmethane	0·1 per cent.	−	−		+ +	−	+ +	+ +	+ +	+ +	−	−
„	0·05 per cent.	−	−		+ +	+ +	+ +	+ +	+ +	+ +	+	−
„	0·02 per cent.	−	−		+ +	+ +	+ +	+ +	+ +	+ +	+	−
„	0·01 per cent.	−	+		+ +	+ +	+ +	+ +	+ +	+ +	+	−
„	0·005 per cent.	+	+		+ +	+ +	+ +	+ +	+ +	+ +	+	+

Dotted line shows zone of action of the antagonist. Broken line (L.A.) shows limit of action of the antiseptic alone.

+ + normal development, similar to that of the control.
+ development below that of the control.
+ + + development above that of the con
− no development (fungistatic action).

TABLE II

COMPARISON BETWEEN *Aspergillus niger* AND *Penicillium* SPP.

Antiseptic Added	*Aspergillus niger*						*Penicillium* spp.			
	Absence of surface active agent	Crillex 16 2 per cent	Span 20 2 per cent.	Tween 80 2 per cent.	Sodium Oleate 2 per cent.	Cystein 2 per cent.	Absence of surface active agent	Crillex 16 2 per cent.	Span 20 2 per cent.	Tween 80 2 per cent.
... ...	+ +	+ + +	+ + +	+ + +	+ +	+	+ +	+ +	+ +	+ +
sulphate :—										
0·1 per cent.	−	−	−	−	−	+ +	−	−	−	−
0·05 per cent.	−	−	−	−	−	+ +	−	−	−	−
0·02 per cent.	−	+ +	−	+ +	−	+ +	−	−	−	+ +
0·01 per cent.	+	+ +	+ +	+ +	+	+ +	−	+	+	+ +
0·005 per cent.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
diphenylmethane										
0·1 per cent.	−	−	+ +	+ +	+ +	−	−	+ +	+ +	+ +
0·05 per cent.	−	+ +	+ +	+ +	+ +	−	−	+ +	+ +	+ +
0·02 per cent.	−	+ +	+ +	+ +	+ +	−	−	+ +	+ +	+ +
0·01 per cent.	−	+ +	+ +	+ +	+ +	−	−	+ +	+ +	+ +
0·005 per cent.	+	+ +	+ +	+ +	+ +	−	−	+ +	+ +	+ +

Dotted line indicates the zone of action of the antagonist and the broken line (L.A.) shows the limit of activity of the antiseptic alone

phenomenon. Complementary tests, not shown in the Table, with a concentration of 2 in 10,000, have confirmed these results.

The results in Table II are of the same order, only the "growth" factor of the detergents by themselves being less marked. It is of interest to note that the inhibitory action of the antiseptic, weaker with oxyquinoline sulphate, is extremely energetic with G.4. This fact indicates that a detergent need not have stimulating properties to inhibit an antiseptic. It seems as if the detergent reacted chemically with the antiseptic to destroy or diminish the toxicity of the latter. One can correlate this fact to the "neutralisation" of oxyquinoline sulphate by cystein, a substance which is equally antagonistic to penicillin and to mercurial bactericides.

Discussion

Dubos and Davis[6], who seem to have been the first authors to use a non-ionic detergent (Tween 80) in a culture medium with a concentration of 0·1 per cent., had observed the favourable action of this product on the growth "in vitro" of a strain of tubercular bacilli. They attribute this diffuse growth in a culture medium to the surface-action properties of Tween 80 and observe that the non-surface-active esters of oleic acid are indifferent. Whitehill, Oleson and Subbarow[7] observe that free oleic acid can replace biotin as a growth-promoting factor for lactobacillus. Williams, Broquist and Snell[8] remark that oleic acid, although indispensable to the growth of certain micro-organisms, shows a definite toxicity according to the concentration employed and the *p*H to which it is used. On the other hand, the addition of Tween 40 eliminates the toxicity of oleic acid and gives rise to its growth-promoting action. These observations indicate that it is difficult to be precise about the mechanism of interference of these detergents, and that it varies, as one might expect, according to the nature of the metabolites and the culture medium.

Kodicek[9] has demonstrated that the more complete influence of fatty acids on the Gram-positive bacteria depends, amongst other things, on the presence of surface-active substances with which the acids can form complexes. These may be in competition with the substances of the cortex of the bacteria for inhibiting substances. To the best of our belief, facts of this nature concerning fungi have not been made known, and moreover one finds few observations on the subject of the interference produced by the wetting substance on an antiseptic. Mme. Chaix and L. Lacroix[10] have, however, pointed out that Tween 20 and 80 completely protect the cells of *Glaucoma pyriformis* (monocellular ciliated organism) against the action of gammexane. These authors attribute this phenomenon to physical protection.

In the case of our moulds we find it difficult to interpret the phenomena as the above-quoted bacteriologists have attempted to do, for it must not be forgotten that detergents are technical products whose chemical composition—and impurities—are not always precisely known. It seems,

as we have already pointed out, that the antagonistic action of detergents towards antiseptics is partly due to the presence of free, non-saturated, fatty acids side by side with an unesterfied fraction. This can be realised by comparing the action of pure oleic acid with that of sodium oleate. However, this does not explain the behaviour of Crillex 6, which is an ester of lauric acid (saturated), nor of Span 20 (sorbitol monolaurate). It would be useful to increase the number of tests by using chemically pure substances so as to give a more accurate interpretation of these phenomena. But this would be outside the scope of the present work which is devoted to pharmaceutical realities.

SUMMARY

(1) The non-ionic detergents which have been examined in this paper favour the development of moulds in a liquid medium.

(2) With the exception of Carbowax 1500, these detergents decrease the fungistatic action of the antiseptic added to the culture medium. The degree of inhibition varies with the nature of the antiseptic.

(3) It seems that the fatty nature of these detergents is responsible for this phenomenon. Oleic acid by itself, even at a concentration of 2 per 1000, favours the development of mycelium and has an inhibitory effect on the antiseptic action. This may explain the inactivity of Carbowax 1500.

(4) These facts must be considered in the utilisation of non-ionic detergents in pharmacy and cosmetics. These preparations will tend to become mouldy rather quickly. In emulsions and antiseptic ointments with a non-ionic detergent base, control of fungistatic or bacteriostatic action seems indispensable.

REFERENCES

1. Harper, *Pharm. J.*, 1950, **164,** 265.
2. Glassman, *Bact. Reviews,* 1948, **12,** 121.
3. Mirimanoff and Bolle, *Festschrift Casparis,* 1949, 149.
4. Engler, Thèse de Doctorat, No. 1149, Geneva, 1950, 30.
5. Molho and Lacroix, *Bull. Soc. Chim. biol.*, 1949, **31,** 1348.
6. Dubos and Davis, *J. exp. Med.*, 1946, **83,** 409.
7. Whitehill, Oleson and Subbarow, *Arch. Biochem.*, 1947, **15,** 31.
8. Williams, Broquist and Snell, *J. biol. Chem.*, 1947, **170,** 619.
9. Kodicek, *Bull. Soc. Chim. biol.*, 1948, **30,** 946.
10. Chaix, Lacroix and Fromageot., *Biochem. Biophys. Acta,* 1948, **2,** 57.

DISCUSSION

An abstract of the paper was read by Professor Mirimanoff.

DR. I. MICHAELS (London) asked whether the authors could give a summary of the technique of weighing the mycelium.

PROFESSOR H. BERRY (London) asked whether they had measured interfacial tension under the conditions of the experiments. This had been shown to be a very important factor by various workers.

DR. F. HARTLEY (London) commented on the use of "tweens" in microbiological assays, and asked whether the authors accepted the explanation that non-ionic detergents exerted not so much a direct stimulating action on the growth of the organism, but made the nutrient materials more readily available. The latter theory would link up with Professor Berry's point about the effect of interfacial tension between the different phases.

PROFESSOR A. MIRIMANOFF replied by first describing the technique of weighing the mycelium. The method was that described by the French authors Molho and Lacroix. With regard to the influence of the interfacial tension, a whole series of wetting agents were studied for their toxicity on micro-organisms and higher vegetative organisms, by plasmolysis and cytoplasmic movement. No relation had been found between interfacial tension and antiseptic action. For example, a non-ionic "tween" could have a much higher interfacial tension than an anionic agent and not possess toxicity, whereas the anionic agent could be toxic.

The stimulating action of the non-ionic detergents must primarily be a nutritive action, due to the presence of free oleic acid, which was difficult to prove in the commercial products. Substances such as carbowax, which do not contain this acid, do not possess this activity. There must be also a physicochemical action which was linked up with the experiments of Dubos in the study of tuberculosis. Kodicek had published some extremely interesting work on the subject, but its interpretation was very difficult.