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Bactericidal properties of Tego 103 S and Tego 103 G

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SUMMARY

The bactericidal activity of Tego 103S was compared with that of chlorhexidine in ethanol, and Tego 103G with Halamid. The activity was determined on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Achromobacter anitratus* by various methods. Tego 103S in 1% solution was less active than chlorhexidine 0·5% in 70% ethanol, and Tego 103G in 1% solution was less active than Halamid 0·3%. The presence of serum did not noticeably influence the activity of the Tego preparations.

INTRODUCTION

Tego preparations (Th. Goldschmidt Ltd., Chemical Industries, Essen, Germany) are ampholytic surface-active compounds. Tego 103S and Tego 51 contain dodecyl-di (aminoethyl)-glycine. Tego 103G and Tego 51B are mixtures of compounds with alkyl radicals of different chain lengths. Tegolan is an emulsion of the monoaminopropyl-amino butyric acid derivate. They are variously recommended for disinfecting the skin (Tego 103S and Tegolan) and surfaces particularly in hospitals (Tego 103G) and in the meat and dairying industries (Tego 51 and Tego 51B). Papers concerning bactericidal properties of Tego 103S and Tego 103G are not frequent and the findings about these properties of the preparations are not uniform. Brausz (1953) and Perkins, Darlow & Short (1967) found Tego 103S and Tego 103G effective. However, Naumann (1952) found bactericidal activity more apparent than real. After Dold & Gust (1957) isolated living *Pseudomonas fluorescens* from solutions of Tego, changes have been made in the composition of Tego compounds (Goldschmidt A. G., 1967). Tego 103G, indeed, causes morphological changes in *Ps. aeruginosa* (Lickfeld, 1965), but data about the bactericidal properties are lacking.

In this paper the activities of Tego 103S in 1% solution and of Tego 103G in 1% solution are compared with chlorhexidine 0·5% in 70% ethanol and Halamid (paratoluol-sulphon-chloramid-Na) 0·3%, respectively.

MATERIALS AND METHODS

Organisms and method of culture

The test organisms used were freshly isolated strains of *Ps. aeruginosa* (our phage and pyocine type 12), *Staphylococcus aureus* (phage type 29/52/80) and

Achromobacter anitratus (laboratory no. 1493). Each culture was grown in 10 ml. of nutrient broth No. 2 (Oxoid Ltd, London), enriched with 0·5 % glucose, for 18 hr. at 37° C. The final viable counts for the three organisms were 2·8, 3 and 31 million/ml. respectively, as determined by the pour-plate method. Each culture was then centrifuged and the pellet washed twice with sterile water and finally resuspended in sterile water to the same opacity as before.

The solid media used were glycerol agar (glycerol, 1 %, w/v; Difco proteose-peptone, 0·5 %; K_2HPO_4, 0·04 %; $MgSO_4.7H_2O$, 2 %; $FeSO_4.7H_2O$, 0·003 %; Bacto-agar, 1 %; pH 7·1–7·2), Blood agar Base (Oxoid) and Endo agar (Oxoid) for *Ps. aeruginosa*, *St. aureus* and *Achr. anitratus*, respectively. For agar cylinders we used a consolidated agar (1·8 %).

For neutralization of the carry-over of Tego 103S and Tego 103G we included 0·5 % (w/v) of lecithin in 3 % (w/v) of Tween 80 in the media (Die Deutsche Gesellschaft für Hygiene und Mikrobiologie, 1958). For chlorhexidine and Halamid we used 1 % (w/v) of Lubrol W and 0·5 % of lecithin (Sykes, 1965), and 0·5 % (w/v) of sodium thiosulphate and 0·5 % of Tween 80 (Kayser & van der Ploeg, 1965), respectively. The neutralizing solution, used in a membrane filter test, was prepared by adding the neutralizers to quarter-strength Ringer's solution.

Germicides

The two Tego preparations were both used at a concentration of 1 % in water, as recommended by the manufacturers. The water used had a total hardness of 13·75. The test solutions had a temperature of 22° C. and pH of 7·4. The influence of organic material was determined by adding 10 % of rabbit serum.

Tests

The activities of the germicides were determined in three ways by a suspension test, a membrane filter test (Hirsch, 1950) and by surface tests. Each test was done five times.

Suspension test

In the suspension test 0·15 ml. of a culture was added to 2·5 ml. of germicide and at intervals 0·25 ml. was subcultured into tubes containing 7 ml. of nutrient broth No. 2 containing 0·2 M sucrose. For testing Tego 103S and chlorhexidine in ethanol these intervals were $\frac{1}{2}$, 1, $2\frac{1}{2}$, 5, $7\frac{1}{2}$ and 10 min.; for Tego 103G and Halamid they were 5, 10, 15, 20, 30, 45 and 60 min. The sucrose broth contained suitable inhibitors (see above). After incubation at 37° C. for 48 hr. the tubes were subcultured on solid media.

Membrane filter test

A Sartorius filter apparatus SM 16114 (Sartorius-Membranfilter GmbH, Göttingen, Germany) with type SM 11009 membranes (Sartorius) was used, except for chlorhexidine in ethanol when SM 11106 membranes (Sartorius) were used.

The filters were sterilized and washed before use (Taylor & Burman, 1964). The test cultures, 0·15 ml., were added to 2·5 ml. of germicide and, after the same intervals as were used in the suspension test, 10 ml. of a neutralizing solution was

added. The mixture was then membrane-filtered. The membrane was washed through with 40 ml. of the neutralizing solution and finally cultured on a solid medium. After 48 hr. at 37° C. the number of colonies was counted by means of a modified Quebec counter.

Surface Tests

Surface tests were done with a swabbing test and an agar cylinder method (Kuipers, 1968). The activity of Tego 103S and of chlorhexidine in ethanol were determined on the skin of the forearm of members of the laboratory staff. A small volume, 0·1 ml., of one of the test cultures was spread evenly on the forearm at six marked spots, each with an area of 10 cm.^{2}. A germicide was spread over the contaminated parts of the skin. Tests were made after intervals of $\frac{1}{2}$, 1, $2\frac{1}{2}$, 5, $7\frac{1}{2}$ and 10 min.

Tego 103G and Halamid were tested in a similar way on a polyvinylchloride (PVC) floor, using culture volumes of 0·3 ml. spread over areas of 64 cm.^{2}. Tests were made after intervals of 5, 10, 15, 20, 30, 45 and 60 min.

Swabbing test. The marked spots on the skin and floor were wiped with sterile cotton-wool swabs moistened with nutrient broth No. 2 (Oxoid). The swabs were inoculated in tubes containing 7 ml. of nutrient broth No. 2 containing 0·2 M sucrose and the appropriate neutralizers. After incubation for 48 hr. at 37° C. the tubes of sucrose broth were subcultured on solid media.

Agar cylinder method. Impressions of the marked spots on the skin and the floor were made with agar cylinders with a surface of 10 and 64 cm.^{2} respectively. The agar contained the appropriate inhibitors for the germicides. After incubation for 48 hr. at 37° C. the number of colonies on the slices were counted with a modified Quebec counter.

RESULTS AND DISCUSSION

Tego 103S did not kill *Ps. aeruginosa, St. aureus* and *Achr. anitratus* in a suspension test within 10 min. (Table 1). In a membrane filter test and an agar cylinder test 99·97% of these organisms were not killed within 10 min. (Table 2 and Table 3).

An antiseptic should have an immediate effect within a short time of contact, as the reference antiseptic, chlorhexidine in ethanol, did. In the membrane filter test, when 10% of serum was added to the test solution of chlorhexidine in ethanol we found longer times to kill 99·97% of test organisms (Table 2). Evidently the neutralization in this testing method is less effective than in the suspension test and in the surface tests (Chiori, Hambleton & Rigby, 1965).

Tego 103G in a suspension test killed *Ps. aeruginosa in* 60 min. or less, but failed to kill *St. aureus* in 60 min. (Table 1). The times required to kill 99·97% of these organisms was also 60 min. or more (Table 2). Tego 103G was less active than Halamid, the reference disinfectant, in the three tests. In a suspension test and a membrane filter test Tego 103G and Halamid showed equal activity against *Achr. anitratus*, but in the *in vivo* test the activity of Halamid on this organism was greater (Table 3).

The influence of 10% of serum on the bactericidal properties of Tego 103S could not be determined in intervals up to 10 min. Therefore we made the same tests under the same conditions with Tego 103 in intervals up to 60 min. There

Table 1. *Action of Tego 103S, chlorhexidine in ethanol, Tego 103G and Halamid on test organisms in the suspension test*

Germicide	Concn of test solution (%)	Time (min). to kill *Ps. aeruginosa*	*St. aureus*	*Achr. anitratus*
Tego 103S	1 (v/v)	> 10	> 10	> 10
Tego 103S + serum	1 (v/v)	> 10	> 10	> 10
Chlorhexidine in ethanol	0·5 (w/v) – 70 (w/v)	0·5	0·5	0·5
Chlorhexidine + serum in ethanol	0·5 (w/v) – 70 (w/v)	1	1	1
Tego 103G	1 (v/w)	45	> 60	15
Tego 103G + serum	1 (v/v)	60	> 60	15
Halamid	0·3 (w/v)	30	5	15
Halamid + serum	0·3 (w/v)	60	10	30

Table 2. *The times required (min.) to kill 99·97% of the test organisms by Tego 103S, chlorhexidine in ethanol, Tego 103G and Halamid in the membrane filter test*

Germicide	Concn of test solution (%)	Time (min). to kill 99·97% of *Ps. aeruginosa*	*St. aureus*	*Achr. anitratus*
Tego 103S	1 (v/v)	> 10	> 10	10
Tego 103S + serum	1 (v/v)	> 10	> 10	> 10
Chlorhexidine in ethanol	0·5 (w/v) – 70 (w/v)	2	0·5	0·5
Chlorhexidine + serum in ethanol	0·5 (w/v) – 70 (w/v)	10	7·5	2·5
Tego 103G	1 (v/v)	> 60	> 60	20
Tego 103G + serum	1 (v/v)	> 60	> 60	20
Halamid	0·3 (w/v)	30	20	20
Halamid + serum	0·3 (w/v)	45	30	30

Table 3. *The times required (min.) to kill 99·97% of the test organisms on skin by Tego 103S and chlorhexidine in ethanol and on* PVC *floor by Tego 103G and Halamid*

Germicide	Concn of test solution (%)	Time (min.) to kill 99·97% of *Ps.* aeruginosa	*St. aureus*	*Achr. anitratus*
Tego 103S	1 (v/v)	> 10	> 10	> 10
Chlorhexidine in ethanol	0·5 (w/v) – 70 (w/v)	1	0·5	0·5
Tego 103G	1 (v/w)	> 60	> 60	> 60
Halamid	0·3 (w/v)	60	10	10

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was no great change in the end-points or in the times required to kill 99·97% of the organisms when 10% of serum was added to the test solution.

Tego 103G with 10% of serum was more effective than Halamid with serum, tested against *Achr. anitratus* (Tables 1, 2). The influence of 10% of serum on the activity of Tego 103G on *Ps. aeruginosa* and *St. aureus* was tested at intervals up to 120 min. and no change was found in the end-points or in the times required to kill 99·97% of the organisms.

The findings of Herrmann & Preusz (1949) that Tego preparations were more effective against Gram-negative organisms than against *St. aureus* were supported by some tests we made with Tego 103S and Tego 103G, each used in a concentration of 2%.

We have not tested the activity of the Tego preparations on *Escherichia coli* or on *Proteus vulgaris*. The trade literature according to the statements of Wallhäuser & Schmidt (1967) gives very confused findings. For example, the bactericidal activity of Tego 103S on these organisms at a concentration of 0·5% appears greater than at 1%.

In spite of changes that have been made in the composition of the Tego compounds, the bactericidal properties of Tego 103S and Tego 103G are slight. Tego 103G has moreover the disadvantage that eczema may appear after skin contact (Sanderink & Singelenberg, 1963).

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