The Microbiological Availability of Soap Bacteriostats

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Abstract

The subject of substantivity as applied to bacteriostats used in soap is reviewed, and the concept of microbiological availability is advanced to describe the interaction of the inherent effectiveness of a bacteriostat with the quantity deposited on a substrate. A method for studying microbiological availability is presented together with data for four soap bacteriostats.

Introduction

IN EVALUATING THE UTILITY of an antimicrobial agent intended to be used in soap, several properties aside from toxicity must be considered, and of these the most fundamental are the inherent antimicrobial effectiveness of the chemical and its tendency to be deposited onto skin from a solution of soap, that is, its substantivity. Both of these characteristics are predictive of the utility of a bacteriostatic or bactericidal agent, and both are necessary if the agent is to be practically useful.

Little need be said about inherent antimicrobial effectiveness. It is obvious that materials differ from one another in their ability to inhibit the growth of micro-organisms. All of the commonly used soap bacteriostats are superior in this regard (Figure 1, Table I).

Although virtually nothing is known about the mode of action of soap germicides which would enable one to predict the relative effects of materials in inhibiting the growth of bacteria, the measurement of this property, as, for example, the smallest amount of agent required just to prevent growth, the so-called minimum inhibitory concentration, is straightforward, and the values themselves are simple to interpret.

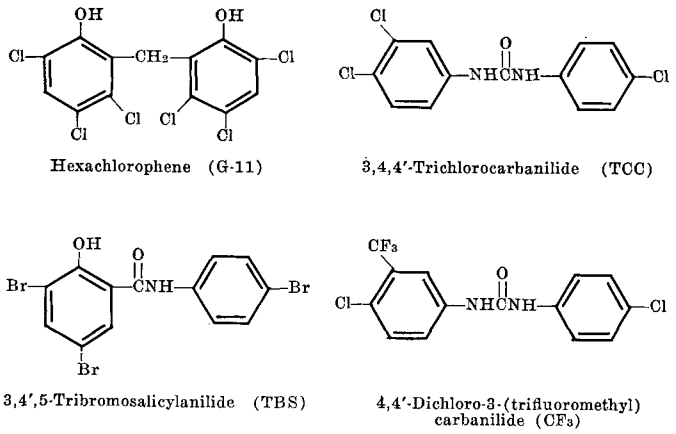
The same cannot be said about substantivity. Although techniques have been published which are said to measure this property of soap germicides, the fact is that what is usually determined is a composite of factors, only one of which is substantivity. Properly speaking, substantivity is concerned with the mechanisms whereby materials are bound to a substrate, the kinetics of the sorption process, and the kinetics and mechanisms of desorption. This kind of information can be obtained by a number of physical methods, and the literature on the general subject is vast, yet nothing seems to have been published in regard to using soap solutions of germicides. Programs currently in progress in this laboratory are designed to fill this gap.

TABLE I

Inherent effectiveness of soap germicides against *Staphylococcus aureus* ATCC 6538

Name	Minimum inhibitory concentration ppm of soap[1]
Hexachlorophene	30
3,4,4'-Trichlorocarbanilide	20
3,4',5-Tribromosalicylanilide	60
4,4'Dichloro-3-(trifluoromethyl) carbanilide	7

[1] Culture transferred three times. A 4-mm loopful of a 24-hour culture then streaked across agar containing the bacteriostat. Soap contained 1% by weight of germicide.



Hexachlorophene (G-11) 3,4,4'-Trichlorocarbanilide (TCC)

3,4',5-Tribromosalicylanilide (TBS) 4,4'-Dichloro-3-(trifluoromethyl) carbanilide (CF_3)

FIG. 1. Soap germicides.

Probably the most widely used methods for evaluating the so-called substantivity of soap bacteriostats are attributable to Vinson and his co-workers (1). In one method, calfskin disks are steeped in a solution of a bacteriostatic soap under standard conditions of time, temperature, and concentration of soap, rinsed thoroughly, then placed for a short time on agar which has been inoculated with bacteria. The agar plate is then incubated overnight. The presence of bacteriostat is indicated by the appearance of a more or less clear zone at the site previously occupied by the disk; this inhibition in the so-called contact zone represents a reduction in growth of micro-organisms and is caused by diffusion of desorbed bacteriostat. The zones are rated according to the sharpness of their outlines; "excellent activity" is characterized by a sharply outlined clear area. In another procedure, a variation of the first, fingers are dipped into a solution of a bacteriostatic soap, rinsed, dried, then pressed briefly on inoculated agar.

Although Vinson found the correlation between these tests and reduction of the bacterial population of the hands, after the use of antibacterial soaps, to be good, it is not always possible to predict which of two products will perform better, based on the zone rating, especially when the ratings are close or identical.

To designate the zone ratings as being indicative of substantivity is misleading, for much more than substantivity is involved. Inherent effectiveness, the ease with which the bacteriostat can be leached from the surface of the skin, and its tendency to diffuse through the agar also must be considered. It is known, for example, that agar interferes with the diffusion of some bacteriostats and inhibits the antimicrobial effects of others (2).

The interaction of these several factors must be complex indeed, and it is realistic, rather than pessimistic, to assume that considerable time will elapse before the precise nature of the interactions will be elucidated. To designate this complexity of interactions the term "microbiological availability" or simply "availability" is proposed to replace the word "substantivity," which has been used heretofore in this connection. It is also proposed that "substan-

tivity" be used in its correct sense of "sorbability." More descriptive of the experimental event, the suggested term also defines a major goal of research in the field of soap germicides. From the use point of view, microbiological availability identifies the one characteristic of a soap germicide which is of supreme importance.

Two methods have been described recently by McNamara and his co-workers (3) as microbiological techniques for evaluating the "substantivity" of germicides on calfskin. In one, the minimum inhibitory concentration of a solution to which disks of calfskin have been exposed, is measured; any change in this value is attributable to the sorption of bacteriostat. This elegant method carries with it the implicit assumption that the relationship between inhibitory activity and concentration of germicide is linear; to the extent that this is not true, comparisons of bacteriostats might be misleading. The same comment can be made for a second procedure in which the rate of elution of germicide from calfskin is determined bacteriologically. "Microbiological availability" would seem to be a more accurate designation for these experimental events than "substantivity."

However intricate the property of microbiological availability may be, it must presumably be a function of a number of variables—time and concentration, for example—if only because substantivity, and therefore adsorption, are involved. Vinson's calfskin method, which, in principle, would be suitable for studying microbiological availability, is too unresponsive to slight changes in experimental conditions to be useful. It seemed however that the measurement of the inhibition of bacterial growth by some counting procedure would be suitable, and a simple, satisfactorily reproducible technique was developed which embodies this principle.

In brief, the surface of calfskin which has been steeped in a solution of a bacteriostatic soap and rinsed is covered with a thin layer of agar containing a known number of bacteria. After incubation the reduction in bacterial population is calculated. Except for the diffusibility of bacteriostat through agar and perhaps (although this is a moot point) the influence of agar on the desorption of the bacteriostat, the procedure is, in effect, a model for skin degerming in vivo. Differences between calfskin, the protein of which is mostly collagen, and human skin, the surface of which is keratin, may or may not be significant in this connection, and this problem is now being studied.

In the early exploration of this approach, which is similar to one described by Quinn (4) for evaluating antibacterial finishes on fabric, it became apparent that certain major mechanical problems had to be solved. Most importantly, it was necessary to prevent the calfskin from swelling or otherwise becoming distorted during the incubation period. It was also necessary to make certain that the skin was flat so that agar could be applied at a uniform thickness and in such a way that all of the agar, which is liquid when added to the skin, is confined to a predetermined area.

Experimental Section

The device designed to accommodate these requirements in shown in Figure 2. Circles ½ in. thick, 2¼ in. OD, and 1¼ in. ID are machined from Type 304 stainless steel (18-8 round) and drilled for four stainless steel Allen socket head machine screws; the holes of one circle of each pair are threaded.

To prepare the substrate, pickled calfskin (W. C. Laurence Leather Company, Peabody, Mass.) is depickled according to the method of Vinson (1) and modified according to Ambye (5) so as to make certain that the internal pH of the skin is between 5.5 and 6.0. This involves soaking the skin in a solution of 5% sodium chloride and 2% sodium acetate, then rinsing several times with distilled water, followed by treatment with 95% ethyl alcohol and acetone. After drying, squares of skin 2½ in. on each edge are measured for thickness (with a Starrett Dial Indicator Pocket Gage, range 0–⅜ in., L. S. Starrett Company, Athol, Mass.); only pieces measuring between 0.03 and 0.06 in. are used. At the outset of every experiment each piece of skin is soaked in 45C water for 10 minutes to make it pliable, then stretched between pairs of the steel circles. With a cork borer, holes are drilled through the skin, using the screw holes in the circles as guides. The skin is secured to the apparatus by connecting the two circles with the screws.

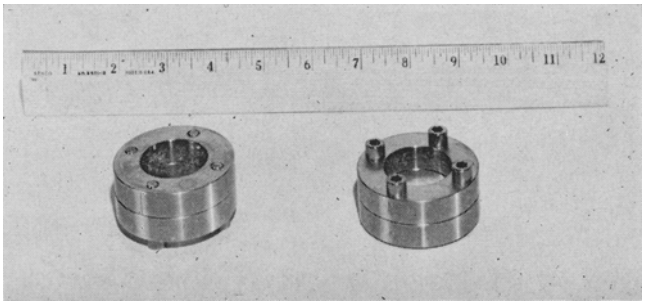


FIG. 2. Apparatus for studying the microbiological availability of bacteriostats on calfskin, assembled.

In order to sterilize the skin and the apparatus, 95% ethyl alcohol is added to the well, screw heads down, and the device is let stand for 30 minutes. After decanting, the well is rinsed for 15 minutes in a constant stream of 45C water. The unit is shaken vigorously so as to remove excess water, and the soap solution to be examined is added until flush with the top of the well (about 10 ml). The solution is added at 45C, and the temperature is allowed to fall to ambient. After remaining in contact with the skin for a predetermined time, the soap solution is emptied from the container and the well is again rinsed for 15 minutes in a stream of water maintained at 45C. Water is shaken out thoroughly and the unit, screw heads down, is placed in a 250-ml beaker containing one ml of water. The function of the water is to provide sufficient humidity so as to minimize the drying of the skin. The beaker is covered with a sterile Petri dish cover.

Suitable dilutions of a 24-hour culture of *Staphylococcus aureus* ATCC 6538, which has been transferred on three consecutive days, are made in broth with the aid of turbidimetric measurements. The adjusted broth culture is added to phenol red dextrose agar so that the latter contains $2–4 \times 10^3$ cells/ml. One ml of inoculated agar, held at 45C, is added to the well while the unit is tilted carefully so as to spread the liquid evenly on the skin surface. The hardened plates are incubated for 48 hours at 37C. For control, skins washed with Ivory soap (4) are used. Determinations are made at least in duplicate.

After incubation, the squares of calfskin are removed from the holders and counted under a dissecting microscope at a magnification of 10 diameters by employing a 2-mm × 2-mm wire grid. To facilitate viewing the colonies, a dilute solution of Neutral Red dye can be used. Every colony is counted except those

which are sometimes found in dense groups on the edges on the agar film. This occurrence, which is infrequent, is caused by a curling away of the edge of the agar from the surface of the calfskin and therefore represents uninhibited growth of cells which were not in contact with bacteriostat.

Experiments to determine the effect of soap per se on the inhibition of growth indicated that the contribution of soap was either negligible or nonexistent. Even if the effect of nonmedicated soap were considerable, comparisons of antibacterial soaps would still be valid.

The data presented below were obtained to determine the influence of the time of contact of antibacterial soap on the growth of bacteria on calfskin, i.e., the kinetics of microbiological availability. In each case the organism was *S. aureus* ATCC 6538, the concentration of soap solution was 0.5%, and each soap contained 1% by weight of germicide, i.e., 50 ppm of germicide in the solution. A soap concentration of 0.5% was chosen because, at higher levels, differences between the more active bacteriostats tended to be obscured.

Results and Discussion

The microbiological availabilities of four soap bacteriostats on calfskin for *S. aureus* ATCC 6538 as a function of time and measured by inhibition are presented in Figure 3. Whether there is a correlation between these curves, which resemble Freundlich isotherms, and the rates of adsorption of the bacteriostats (that is, their substantivities) is to be determined.

Several similarities and differences between the various materials represented in Figure 3 are apparent. First, there is the time of appearance of any inhibition whatsoever; this takes the increasing order G-11 = CF_3, TCC, TBS. Although a difference in this respect probably exists between G-11 and CF_3, it has not been practical to work at intervals less than 0.5 minute. Second, the slopes of the curves are nearly the same for hexachlorophene, the fluorinated carbanilide, and the bromated salicylanilide. Third, there is the achievement of essentially complete inhibition, which seems to be in the order G-11 = CF_3 > TBS >> TCC.

TCC occupies a surprising place in this hierarchy of microbiological availability for the slope of its curve is relatively small, and even after 12 minutes the inhibition is minimal. A number of possible explanations for this can be made. Since experiment shows that, over a fairly wide range of concentrations, the inherent effectiveness of TCC changes very slowly, increasing the amount of sorbed TCC may result in only a slight increase in effectiveness (6). TCC may be only weakly substantive; contrariwise, TCC may be very substantive to calfskin, and the rate of desorption may be quite slow. Desorbed TCC may precipitate and be relatively unavailable for exerting its inhibitory effect; also TCC may diffuse through agar only with difficulty. Evidence that one or more of the last four possibilities may be involved is given by Vinson's zone of inhibition method. Calfskin which has been steeped in soap containing TCC shows a rather diffuse zone, indicating the relative unavailability of bacteriostat as compared with the zones exhibited by hexachlorophene or TBS. Some of these possibilities are currently being investigated.

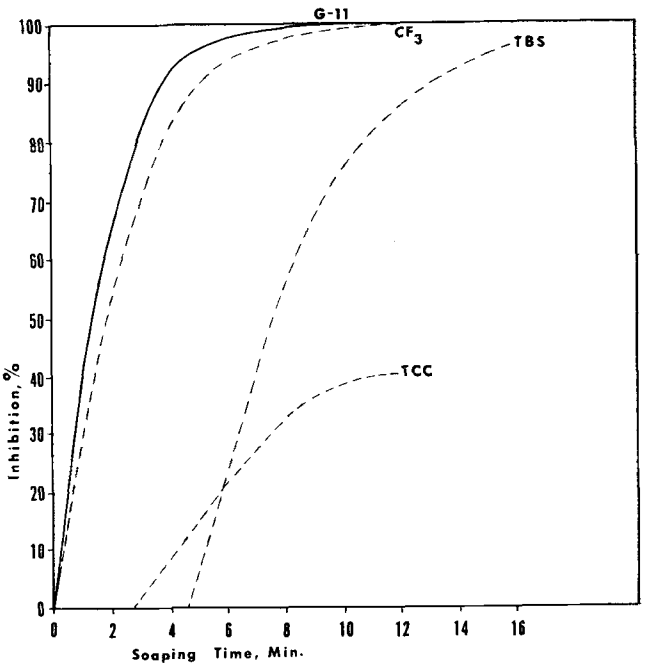


FIG. 3. Microbiological availability of soap germicides (*S. aureus* ATCC 6538).

The significance of microbiological availability becomes apparent when one compares the curves of Figure 3 with the corresponding minimum inherent effectiveness in Table I. Hexachlorophene, the MIC of which is higher than that of TCC, nevertheless is more inhibiting. 4,4′-Dichloro-3-(trifluoromethyl) carbanilide, which is much more inherently effective than hexachlorophene, is at best equivalent to the latter with respect to its ability to inhibit the growth of *S. aureus* ATCC 6538 on calfskin. These unexpected findings are understandable if one considers the interaction of inherent effectiveness and substantivity—the microbiological availability—and not either of these factors alone.

In principle, it is apparent that relatively low inherent effectiveness may be compensated for by a suitable order of substantivity, and, conversely, an inherently effective material may be only poorly inhibitory when applied from soap if its substantivity is inadequate. A neat balance of each of these properties is required, and it is precisely this balance which is the meaning of microbiological availability. Elucidation of the quantitative relationship (if such exists) between inherent effectiveness, substantivity, and microbiological availability must await the determination of exact substantivity values.

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