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ANTISEPTICS: A COMPARATIVE STUDY OF LABORATORY AND PRACTICAL TESTS.*

BY GEORGE F. REDDISH.[1]

Antiseptic substances were used for the preservation of food long before putrefaction and decay were shown to be caused by microörganisms. When Pasteur demonstrated the microbic origin of fermentation, he not only solved the problem of food spoilage but also took the first step in the scientific study of the cause of disease. The discovery of the cause of fermentation and disease led immediately to a study of the methods for preventing microbic activity pertaining to each. While Pasteur at first directed his efforts toward methods for controlling fermentation, a young English surgeon, Joseph Lister, made the first attempts to control the cause of infection.

Remembering the speculation of Robert Boyle two centuries earlier that the discovery of the cause of fermentation would lead to explanation of the cause of disease, Lister began his epochal studies on infection shortly after Pasteur announced results of his brilliant studies on fermentation. Without actually isolating the bacteria causing "hospital gangrene," Lister assumed that some kind of microorganism was the cause of these infections and in his effort to prevent post-operative "gangrene" he used a chemical which was known to be effective for preventing putrefaction. Carbolic acid in concentrations of 1–20 and 1–40 were employed for this purpose. After using this germicide for disinfecting surgical instruments, dressings, bandages and the operative field, infection following operations was greatly reduced. The use of this germicide in surgery formed the basis of Lister's system of antiseptic surgery.

From that day to this, antiseptics have been employed for two purposes (1) to preserve food by preventing the growth of bacteria causing putrefaction and decay, and (2) to kill or inhibit the bacteria which cause infections. This double meaning of the word "antiseptic" is still recognized. While the inhibitory meaning is emphasized in bacteriology textbooks, the germicidal meaning is most common among the medical profession and laity.

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Following the announcement of Lister's system of antiseptic surgery in 1867, efforts to discover the cause of diseases and infections generally were intensified with the result that the cause of suppuration—"hospital gangrene"—was soon discovered. *Staphylococcus aureus* and *albus* and the streptococci were early found to be the principal causes of wound infections. Carbolic acid in the dilutions employed by Lister were found by practical experience and later by laboratory test to be effective in killing these suppurative microörganisms.

In the early laboratory methods for testing the effectiveness of germicides, none of the suppurative bacteria were employed as test organisms. The anthrax bacillus, *B. coli* and *B. typhosus* were the principal ones employed in testing the value of germicides. When new germicides were developed during the succeeding years, their germicidal activity was measured by what is known as the phenol coefficient test, with *B. typhosus* as the test organism. This condition obtained for almost thirty years up until 1924.

While bacteriologist in charge of testing disinfectants in the U. S. Food and Drug Administration, I began, in 1924, the study of methods of testing antiseptics. Since *Staphylococcus aureus* is the most common cause of suppuration, as well as the most resistant of the non-sporing disease-producing bacteria, it was naturally selected as the principal test organism. After studying twenty-five freshly isolated strains of *Staphylococcus aureus*, a phenol standard of resistance was established and at the same time a method for testing the germicidal activity of soluble liquid antiseptics was described (1). A little later methods for testing the various kinds of antiseptic preparations, for germicidal as well as bacteriostatic activity, were published (2); these procedures were subsequently adopted as standard methods for testing antiseptics by the U. S. Food and Drug Administration (3).

In each of these methods for testing antiseptics a considerable margin of safety is established so that the public will be assured of the maximum protection. For example, excessive numbers of the most resistant of the non-sporing pathogens, *Staphylococcus aureus*, are used in the various tests. Although an average of but 40 bacteria are found per square centimeter on the human skin, and although only approximately 15,000,000 bacteria of all kinds are found per cubic centimeter of rinsings of the mouth surface, approximately 350,000,000 of the most resistant of all the disease-producing bacteria found on the skin and mucuos membranes are employed in the principal germicidal test. This is a far greater number than is found even in an equal quantity of pus. This is a severe test, but being so, it serves to weed out ineffective preparations which claim to be antiseptic.

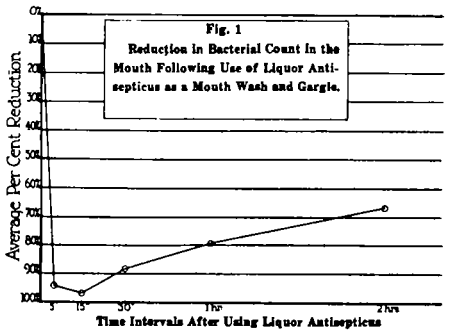
Since 1925 the Food and Drug Administration has required liquid antiseptics which are recommended for short time application to kill approximately 350,000,000 *Staphylococcus aureus* by this standard test within five minutes (0.5 cc. of broth culture of *Staphylococcus aureus* in 5 cc. of antiseptic at 37° C.). Liquor Antisepticus N. F. IV,[1] which was employed in the present study, not only passes this test, but does so within a much shorter time than is required. Instead of requiring five

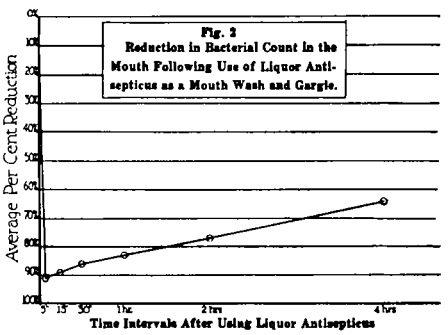
[1] Liquor Antisepticus N. F. IV was selected for this study because the N. F. V formula was changed in a way that so reduced the germicidal efficiency that it no longer passed the standard Food and Drug Administration test for liquid antiseptics. (This work was done before publication of National Formulary VI. Liquor antisepticus N. F. VI, however, possesses the same germicidal efficiency as Liquor Antisepticus N. F. IV).

minutes to kill *Staphylococcus aureus* by this test, this antiseptic has been shown to pass this severe test in from thirty to ninety seconds.

Experience has shown that germicides which pass this test—which kill approximately 350,000,000 *Staphylococcus aureus* within 5 minutes or less—also kill the other pyogenic organisms in even larger numbers. Some germicides, however, are specific in their activity toward certain classes of microörganisms, especially as between Gram positive and Gram negative bacteria, in which cases the test must be varied accordingly. A few germicides are highly bacteriostatic, necessitating additional transfers in broth in order to overcome this factor (4). Liquor antisepticus N. F. IV, however, is not selective in its activity and it is not bacteriostatic in even low dilution. It is even more effective against other disease germs than it is against *Staphylococcus aureus*. According to the standard Food and Drug Administration Method, therefore, Liquor Antisepticus N. F. IV is shown to be an effective antiseptic.

It is of interest to know just what such an antiseptic will do when used in practice. In other words, how effective will an antiseptic which passes the government





standard test be in killing bacteria under practical conditions of use? Since the oral cavity harbors millions of bacteria and since suitable methods are available for determining the reduction of bacterial count in the mouth and throat, the efficiency of this antiseptic in killing bacteria in the oral cavity is selected for this comparison. Using a method which was developed in Johns Hopkins University (5), bacterial counts were made of the oral cavity before and after using this antiseptic and the reduction in numbers of bacteria computed.

This method, which is simple, gives results which are consistent and which are easily interpreted in terms of practical value. A slight modification of the Feirer and Leonard test (5) was employed in these studies. The mouth and throat are first rinsed thoroughly for 30 seconds with 20 cc. of sterile water. This rinsing is immediately plated in proper dilution in nutrient agar. After a two-hour wait for the bacterial count of the mouth and throat to return to normal, it is rinsed in a similar manner with 20 cc. of Liquor Antisepticus N. F. IV for 30 seconds. This is discarded, and the mouth and throat are then rinsed with 20 cc. of sterile water for 30 seconds at intervals of 5 minutes, 15 minutes, 30 minutes, 1 hour and 2 hours, after the use of Liquor antisepticus. These rinsings are immediately plated in proper dilutions in nutrient agar. All plates are incubated at 37° C. for 48 hours. On the following day a control test is made in which sterile water is used instead of Liquor Antisepticus N. F. IV, the rinsings being made at the same time periods, that is 5, 15 and 30 minutes, and 1 and 2 hours after the first control rinsing. At the end of 48 hours, the bacteria are counted and the percentage reduction is calculated.

It was found that following the use of Liquor Antisepticus, the numbers of bacteria in the mouth and throat were reduced as much as 98.7%, with an average of 96.7%. These reductions were observed fifteen minutes after the antiseptic has been used as a mouth wash and gargle. Reduction in bacterial numbers was not temporary, but lasted for two to four hours or more. The following average reductions in bacterial count were obtained after this antiseptic had been used as a mouth wash and throat gargle (thirty tests): 5 minutes, 94.6%; 15 minutes, 96.7%; 30 minutes, 87.0%; 1 hour, 79.5%; and 2 hours, 66.3% (see Table I and Fig. 1). In another series of 152 tests with Liquor Antisepticus N. F. IV, the reduction in bacterial count in the oral cavity four hours after such use showed an average reduction of 64% (see Fig. 2). The water control, on the other hand (the same quantity of water used as a mouth wash and throat gargle in place of the antiseptic), mechanically removed mouth bacteria to the extent of only 35% (see Fig. 3). It is obvious that the difference between 35% and 96.7% is due to the germicidal activity of the antiseptic.

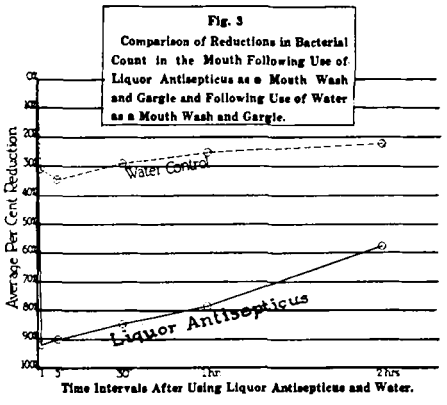


Fig. 3
Comparison of Reductions in Bacterial Count in the Mouth Following Use of Liquor Antisepticus as a Mouth Wash and Gargle and Following Use of Water as a Mouth Wash and Gargle.

TABLE I.—BACTERIAL REDUCTION ON SURFACE OF MOUTH FOLLOWING USE OF LIQUOR ANTISEPTICUS N. F. IV AS A MOUTH WASH AND GARGLE.

Subject.	Test.	Bacterial Count.	Per Cent Reduction.	Subject.	Test.	Bacterial Count.	Per Cent Reduction.
"A"	Control	1,550,000		"F"	Control	14,654,000	
	5 min.	75,000	95.2%		5 min.	361,000	97.5%
	15 min.	32,000	97.9		15 min.	512,000	96.5
	30 min.	217,000	86.0		30 min.	1,223,000	91.7
	1 hour	321,000	79.3		1 hour	1,895,000	87.1
	2 hours	412,000	73.4		2 hours	3,554,000	75.7
"B"	Control	1,730,000		"G"	Control	2,520,000	
	5 min.	92,000	94.7%		5 min.	250,000	90.1%
	15 min.	46,000	97.3		15 min.	175,000	93.1
	30 min.	269,000	84.5		30 min.	825,000	67.3
	1 hour	488,000	71.8		1 hour	950,000	62.3
	2 hours	492,000	71.6		2 hours	1,725,000	31.5
"C"	Control	1,321,000		"H"	Control	1,980,000	
	5 min.	85,000	93.6%		5 min.	96,000	95.2%
	15 min.	29,000	97.8		15 min.	51,000	97.4
	30 min.	142,000	89.3		30 min.	195,000	90.2
	1 hour	398,000	69.8		1 hour	278,000	86.0
	2 hours	561,000	57.5		2 hours	495,000	75.0
"D"	Control	2,462,000		"I"	Control	7,620,000	
	5 min.	123,000	95.0%		5 min.	325,000	95.7%
	15 min.	62,000	97.5		15 min.	428,000	94.4
	30 min.	439,000	82.2		30 min.	659,000	91.4
	1 hour	517,000	79.0		1 hour	980,000	87.1
	2 hours	624,000	74.7		2 hours	2,760,000	63.8
"E"	Control	5,651,000		"J"	Control	3,680,000	
	5 min.	276,000	95.1%		5 min.	210,000	94.3%
	15 min.	73,000	98.7		15 min.	120,000	96.7
	30 min.	293,000	94.8		30 min.	256,000	93.0
	1 hour	915,000	83.8		1 hour	572,000	84.5
	2 hours	1,849,000	67.3		2 hours	1,020,000	72.3

Average Reduction in Bacterial Counts:				
5 min.	15 min.	30 min.	1 hour	2 hours
94.6%	96.7%	87.0%	79.5%	66.3%

Five laboratories took part in these investigations and have proved conclusively that Liquor Antisepticus is effective in killing very large numbers of bacteria when used under practical conditions. It is true that all of the bacteria in the mouth and throat are not killed, but it has been proved that their numbers are reduced to a significant degree, and this is all that is expected of any antiseptic under conditions of practical use. No germicide will completely sterilize the skin, mucous membranes or infected tissue without doing serious damage to the tissue. By reducing the bacterial numbers to a significant degree, the desired purpose is accomplished. Liquor Antisepticus, which passes our standard germicidal test for liquid antiseptics, and meets government requirements as to germicidal efficiency as gaged by this test, is found by a large number of practical tests to be effective in killing significant numbers of bacteria when used under practical conditions of use.

Reduction of the bacterial count on skin and mucous membranes is of great importance because the number of disease-producing bacteria present on any body tissue has a direct relation to the possibility of infection. If large numbers of bacteria are present, the danger of infection is greater; if the numbers of bacteria are reduced, the danger of infection is lessened. Since this is true, it is to be expected that the clinical use of an effective antiseptic will in fact reduce the incidence of infection.

These extensive practical studies prove conclusively that antiseptics when used under practical conditions kill significant numbers of disease-producing bacteria and by so doing aid materially in preventing infection and in mitigating disease. These investigations also prove the adequacy of our present standard method of testing the germicidal efficiency of liquid antiseptics. Liquor Antisepticus N. F. IV passes the standard method for testing antiseptics used for short time contact and meets the requirements of the Food and Drug Administration for this class of drugs, and under practical conditions of use this antiseptic kills very large numbers of bacteria, reducing them to a significant degree.

SUMMARY.

Liquor Antisepticus N. F. IV passes the Food and Drug Administration test for soluble liquid antiseptics and it is shown to be effective in killing bacteria under practical conditions of use. Under the conditions of use in the oral cavity it reduces the bacterial count to a significant degree, an average reduction of 96.7% being demonstrated.

These findings give us added assurance that the standard Food and Drug Administration method of testing liquid antiseptics is satisfactory as a means of estimating the effectiveness of such antiseptics for practical use.

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The Kingsway Pharmacy, Toronto, Canada.

The Kingsway Pharmacy in Toronto features modern drug store ideas with the professional pharmacy, both of a high order. The pharmacy has been given the important place. A consulting room is adjacent to the dispensary and the Baby Room; in the latter are infant foods and related preparations. The consulting room provides the opportunity for physicians to speak with their patients. Ideas in the management of the professional side are incorporated from the suggestions of Professor Hogstad. The dispensary is in full view and of open construction; characteristic of the pharmacy in embossed letters of gold is the statement "In accordance with the fine art of the apothecary."

The building and interior are specially designed for this establishment. The sections devoted to other than professional service maintain dignity—there is a section devoted exclusively to toiletries, and while items carried in the modern store are displayed and sold extensively, these goods do not detract from the pharmacy, because of the arrangement. The soda fountain and cigars are important divisions and sources of income.

The opening of Kingsway Pharmacy was an event in Toronto on November 12th—it was estimated that more than five thousand people visited the store. The booklet given out was well designed and contained the story of pharmacy and described some of the features of the establishment and its various services.

The following are shown in the picture—the proprietors of the pharmacy—I. S. Wolfe and J. Senelnick, standing; seated are: Mr. Wolfe, a relative of the owners; Dean Charles F. Heebner, Dr. R. B. J. Stanbury, Secretary of Canadian Pharmaceutical Association; Prof. Anton Hogstad, Jr., chairman of the National Pharmacy Week Committee.