AN EVALUATION OF THREE ANTISEPTICS USED IN THE PRE-SURGICAL PREPARATION OF PATIENTS AT THE MICHIGAN STATE UNIVERSITY VETERINARY CLINIC

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AN EVALUATION OF THREE ANTISEPTICS USED IN THE PRE-SURGICAL PREPARATION OF PATIENTS AT THE MICHIGAN STATE UNIVERSITY VETERINARY CLINIC

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ABSTRACT

Laboratory and clinical tests were performed on three antiseptics that were being used in the presurgical preparation of patients at the Michigan State University Veterinary Clinic. Two of the antiseptics (Weladol and Triocil) were on a trial basis, while the third (Liquid Germicidal Detergent) has been in use for a number of years.

Phenol coefficients of 2.83 for Weladol and 444.4 for Liquid Germicidal Detergent were determined. Triocil did not give results from which a phenol coefficient could be established. The speed of disinfection and use dilution tests were employed to determine the per cent reduction of broth cultures of *Salmonella typhosa* and *Staphylococcus pyogenes* var. *aureus* upon exposure to the particular antiseptic. These cultures were standardized by at least three transfers at 24 hour intervals. Samples from the cultures were taken within one half hour of the 24 hour time interval so the number of organisms present would be consistent.

A swab technique was used to obtain culture material from the skin of cattle, swine, and dogs after they had been prepared for surgery. The cotton swab was placed in 10 cc of sterile saline and shaken well. Five cc of the saline were plated on five per cent blood tryptose agar plates for 48 hours, and then the number of colonies was counted.

From the results obtained by these testing methods it was determined that Liquid Germicidal Detergent was the most effective of the three antiseptics.

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I. INTRODUCTION

Aseptic technique may be symbolized as a pillar of modern successful surgery. The veterinary practitioner is often compelled to work alone and under difficult field conditions, where the opportunities for contamination are enhanced. Therefore, it is a matter of prime importance for him to know which antiseptics are most effective on the skin, and how they may best be used, so that the likelihood of infection from this source may be reduced to a minimum.

In gathering laboratory and clinical data on the efficiency of an antiseptic, one must recognize that there is not yet available a single universal method of testing which is applicable under all conditions. Efficiency under conditions of use is, in the final analysis, the important criterion.

In this work, an attempt was made to evaluate the effectiveness of three antiseptics that are being used for presurgical preparation of patients in the large and small animal clinics at Michigan State University. The effectiveness of the antiseptics was based on both *in vitro* studies with known organisms and on swab cultures obtained from the sites prepared for surgery.

II. REVIEW OF LITERATURE

A. HISTORY

The earliest precursors to present-day antiseptics and disinfectants were the preservative measures of drying, salting, freezing and the use of spices, wines, and vinegars to prevent the spoilage of food. The ancient Egyptian's art of mummification evidences the effective use of salts, spices, oils, and dehydration in the prevention of decomposition.

Many references in the Bible indicate an understanding of the effect of what is now known as bacterial infection. Such dangers were known to Aristotle (14), who advised Alexander the Great to require his armies to "boil the water and bury the dung".

At an early date, the Greek physician, Hippocrates (14), realized the possility of wound infection and demanded that attending physicians wear clean robes, keep their fingernails trimmed, and cleanse the patient's wound with wine.

During the Middle Ages, various efforts were made to check the plagues which were sweeping across all of Europe. In 1365, during the pneumonic form of the plague, John of Burgundy (7) instructed that the air in the infected rooms be purified by burning of incense to provide fumes for the patient to inhale.

It was not until the eighteenth century, however, that quantitative tests were made to determine the effectiveness of disinfectants. In 1750, Sir John Pringle (7), the founder of modern military medicine and sanitation, presented to the Royal Society some experiments comparing the preservative and disinfectant action of various substances. Although the drugs he tested are no longer of interest, his experiments have remained outstanding as the first scientific comparison of disinfectants. His procedure consisted of cutting beef into pieces of equal size, adding equal amounts of water, and then known amounts of the chemical he wished to test. The water, meat, or air provided the necessary bacteria. Taking as a standard the action of 60 grains of sea salt dissolved in two ounces of water, he was able to determine the relative value of many different substances.

One of the great problems confronting surgeons at that time was the mortality resulting from puerperal septicemia. Women confined to hospitals during childbirth were seven times as apt to die as those cared for in private homes. In 1843 Oliver Wendell Holmes published his paper on "The Contagiousness of Puerperal Fever" (14). Holmes aroused violent criticism from his colleagues by suggesting that women in childbirth should not be attended by doctors who had been performing autopsies on women dead of puerperal fever. It was not until 1847, when the Austrian, Semmelweis, discovered the cause of puerperal fever, that steps were taken to reduce hospital mortality from this cause (4). As obstetrical assistant in the Allgemines Krankenhause in Vienna, Semmelweis noticed that the mortality rate in the

First Division of the hospital was much higher. This section was utilized for the training of medical students. The rest of the hospital, which utilized nurses, had a much better record. He observed that after a colleague cut his finger while performing an autopsy, the man exhibited identical symptoms as the women dying of puerperal fever. He also observed that many of the medical students performed dissections on cadavers prior to visiting the wards. His conclusion was that the disease was transferred from the cadavers to the obstetrical patients in the hospital via the medical students. Thereafter Semmelweis insisted that his students wash their hands in a solution of chlorinated lime before attending a patient. Mortality rates dropped more then twenty per cent after this procedure was adopted. This doctrine of prophylaxis proposed by Semmelweis was not readily accepted in Vienna, and was unknown in other places in Europe. After his death it was so completely forgotten that Lister did not know of it until long after he developed his own ideas of antiseptic surgery.

Even after the introduction of anesthetics in the early nineteenth century, post-operative infection was very common. Operations were limited to surface wounds and amputations, and these were extremely hazardous because of the almost inevitable infection which resulted.

The existing conditions of that time can best be illustrated by the mortality following major amputations. In military cases it ran from 65 to 90 per cent, while civil cases ranged from 26 to 60 per cent. The medical men believed that the suppuration of wounds was due to some constituent of the air. They suggested that this could be controlled by eliminating air from the area of the incision.

It was not until 1863 that Joseph Lister, professor of surgery at Edinburgh University, familiarized himself with the researches of Pasteur. He came to the conclusion that the organisms described by Pasteur in his fermentation reactions were likewise responsible for the putrefaction and suppuration of wounds. He became convinced that it was not the oxygen in the air that caused putrefaction, but airborne microorganisms settling on the wound were causing the infection. Lister sought measures by which he could kill these organisms before they entered the open wound. His original practice was to apply crude carbolic acid to the incision or wound. He reasoned that this powerful agent would destroy each organism as it was deposited from the air. To complete the arrangement, Lister introduced the practice of spraying the air with dilute carbolic acid during an operation or when changing a dressing. Although the first attempts at antisepsis were crude, the results were apparent from the start, as mortality rates steadily decreased. Lister met with strong opposition and his practices were slow to be accepted. The germ theory on which he based his practices was new. Many debates about the value of antisepsis turned into arguments over acceptance of Pasteur's germ theory. With continual improvement in technique, antiseptic surgery gradually became an accepted practice, and at the time of Lister's death in 1912, his precepts were commonly followed.

The second period in the study of disinfection may be said to have started around 1881 with the semiquantitative studies of Koch. He tested various chemical substances in the presence of pure cultures of test organisms to determine their disinfectant properties. George Sternberg (7), a military surgeon and pioneer American bacteriologist, began his study of the comparative action of commercial disinfectants in 1878. His method was simple: to mix equal parts of his cultures of bacteria in broth with a disinfectant for two hours. He would then subculture, using the absence of growth in the subculture as the criterion for the bactericidal properties of the disinfectant. This was the beginning of a long and useful series of investigations which have led to the present methods of testing antiseptics and disinfectants.

B. ORIGIN OF LABORATORY TESTS

A number of methods have been devised for the laboratory testing of antiseptics. The results of these methods permit the comparison of one antiseptic with another in terms of their effects upon bacteria in culture media. *In vitro* tests have reached a considerable degree of standardization, while clinical tests are more difficult to perform and to evaluate.

The first method of evaluating the germicidal value of an antiseptic was developed by Robert Koch in 1881 (14). The method was based on the use of bacteria impregnated threads as the test material. In 1889, Geppert (14) proved that the antiseptic carried by the thread in Koch's method was responsible for the exceptionally high values obtained with mercuric chloride. To eliminate this factor, Kronig and Paul in 1897 employed bacteria coated garnets as test objects and in their thorough study formulated tenets which have served as a foundation for procedures subsequently devised.

In 1903 Rideal and Walker (14) devised a test tube method of examining chemicals for their killing action. In 1911, Anderson and McClintic published a procedure designed to eliminate some of the objectionable features of the Rideal-Walker method. In 1916 Shippen combined the best features of these two tests. In 1927 this was published by Reddish (14) as the Rideal-Walker method. This test, with very few changes, is known today as the Food and Drug Administration method. This method is the standard phenol coefficient test by which thousands of antiseptics and disinfectants intended for all sorts of use have been arbitrarily measured.

The most recent U. S. version is known as the "A.O.A.C. Phenol Coefficient Method". The A.O.A.C. (Association of Official Agricultural Chemists) method (9) is being used routinely by a regulatory agency operating under the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act; and to this extent it enjoys a quasi-legal status.

Anderson and Mallmann (1) showed that penetration of the bacterial cell by the antiseptic or disinfectant is essential to the ultimate destruction of the cell. They also showed that this power to penetrate rapidly is a prime requisite of all compounds designed to kill bacteria within a short period of time. They proposed the speed test as a means of measuring the penetrative power of antiseptics and disinfectants.

Mallmann and Hanes (11) presented a technique of measuring antiseptics and disinfectants under conditions of practical application. It was designed to measure and evaluate the dilution of an antiseptic that could be successfully used under field conditions. It is called the use dilution method.

C. SELECTION OF AN ANTISEPTIC

As yet, no single chemical agent has been found which embodies all the desirable characteristics of a good antiseptic, and at the same time is practical under most conditions. A generalized list of properties which such an antiseptic should possess can be made, and used as a reference when selection of an antiseptic is made. Such a list would include:

1. SPECIFICITY. An ideal antiseptic should have a wide killing range. Many of the germicides which are only slightly toxic to tissues are very specific in their actions, and are effective against only a few types of organisms. Since the chances of finding a pure culture in the area being scrubbed are slight, an antiseptic should be sufficiently broad in its action to give reasonable assurance of success.

2. EFFICIENCY IN THE PRESENCE OF ORGANIC MATERIAL. An antiseptic should not have marked affinity for the organic matter which may be present, or the antiseptic will be used to satisfy the organic material and its ability to destroy bacteria will be dissipated.

3. COEFFICIENT OF DILUTION. Antiseptics are rarely used without further dilution of the solution. A good antiseptic must, therefore, be effective not only in its original concentration, but also in the dilution existing upon application.

4. HOMOGENEITY AND STABILITY. A good antiseptic should also possess the qualities of homogeneity and stability. Homogeneity is important in that every drop should be just as effective as every other drop. Many compounds would be good antiseptics if it were not for the fact that they form emulsions in water rather than homogeneous solutions. Stability until the actual time of use is important. Many compounds deteriorate on standing, and the decomposition products which result have little or no effect.

5. TISSUE TOXICITY. The toxic effect an antiseptic has on tissue must be considered when a selection is being made. Unfortunately, the relationship between the protoplasm of bacterial cells and tissues is so close that there is no chemical known which is highly toxic to bacteria and non injurious to the body tissues. Some substances exert a specific action against certain types of organisms which they kill or inhibit in dilutions that can be tolerated by the tissues.

6. PENETRABILITY. Penetrability is also a factor to be considered when selecting an antiseptic. In order to obtain a complete kill of the bacteria the antiseptic must be able to penetrate the organic material surrounding it. If an antiseptic can penetrate organic matter, then it generally can penetrate the bacterial cell. Thus it can effect a quicker kill than antiseptics that merely coat the surface of the cell

and interfere with its metabolism. Hence the property of penetration is an important measure of the practical value of a compound and should be used as one measurement of its efficiency.

There are, in addition to the above mentioned, several other factors which may well be considered in characterizing an ideal antiseptic. Such properties as detergent action, solubility, chemical compatibility, odor, and cost are all important.

D. LITERATURE ON TRIOCIL, WELADOL, AND LIQUID GERMICIDAL DETERGENT

1. TRIOCIL. Triocil* (hexetidine) has been recommended for several uses. Kral (8) states that it has been found effective in pyogenic otitis and some types of dermatomycoses. He successfully treated superficial pyogenic dermatosis by local application without any systemic treatment. Green (6) reported on the use of Triocil in the treatment of 316 cases of dermatitis, including fungus infections, acute and chronic eczemas, and non specific dermatoses. He obtained excellent results in 211 cases, 89 were satisfactory, and in only 15 cases were results unsatisfactory.

Ripps (16) reported on the use of Triocil in the treatment of a variety of skin conditions, which included dermatoses of many types, lacerations, cuts, and otitis. A total of 64 cases were treated. Fifty five were relieved or showed marked improvement, five were improved, and four showed no improvement. Sixty two of these cases were dogs. One horse

* Warner-Chilcott Laboratories, Morris Plains, New Jersey.

and one parakeet were treated.

In a personal communication with the Company, it was stated that: "Triocil has been used with complete success as a presurgical scrub." (5) In the Company's summary of research data, clinical abstracts on the treatment of many primary and secondary infections associated with skin conditions were given, but no laboratory or clinical work was included on its use as a presurgical scrub.

2. WELADOL. Weladol* is an iodine preparation containing one per cent available iodine. Iodine is known as an active and reliable antiseptic and disinfectant. Since the latter part of the nineteenth century, much has been written extolling the virtues of iodine as an antiseptic. Iodine has been used in various ways as an antiseptic for the skin, wounds, and mucous surfaces of the body; for sterilization of the air and of inanimate objects such as catgut and surgical instruments; as a prophylactic and therapeutic agent in diseases caused by bacteria, viruses, and fungi. The factors which have often made iodine preparations unsuitable for use as a skin antiseptic are that they often stain badly, leaving a characteristic brown stain when applied to the skin, and repeated application may cause blister formation. Iodine may be irritating to the tissues and painful to the patient.

Weladol represents an attempt to retain the antibacterial properties of iodine without its toxic effects. It contains a surfactant-iodine complex, polyalkyleneglycol, usually referred to as an iodophor or "iodine carrier". This combina-

* Pitman-Moore Co. Division Allied Laboratories, Indianapolis, Indiana.

tion of iodine reduces iodine vapor pressure to a low level, reduces the toxicity of iodine, and changes certain iodine absorption equilibria and chemical rates. As a result, free iodine is liberated slowly when diluted with water and is available for antibacterial action without its characteristic toxic, irritating, corrosive, odorous, and staining properties (19).

Terry and Shelanski (20) reported results of bacteriological testing comparing iodine in Lugol's solution versus an iodine-iodophor complex against _Staphylococcus pyogenes_ var. _aureus_.

TUBE A 5.0 ml iodine (Lugol's solution, 100 ppm) plus _S. pyogenes_ var. _aureus_ (2×10^8) after 1 minute, no viable bacteria remained.

Then 4×10^8 _S. pyogenes_ var. _aureus_ were added to the same tube, and after 1 minute, 2×10^8 organisms still remained.

TUBE B 5.0 ml iodine (iodine-iodophor complex, 100 ppm) _S. pyogenes_ var. _aureus_, (2×10^8). After 1 minute no viable bacteria remained.

Then 4×10^8 _S. pyogenes_ var. _aureus_ were added and after 1 minute no viable bacteria were found.

These results indicate that Lugol's solution does not maintain its efficacy upon repeated exposures to bacteria as well as an iodine-iodophor complex. This shows that the available iodine in Lugol's solution is utilized immediately, while the iodine-iodophor complex, which is releasing iodine slowly, will maintain its germicidal properties after repeated exposures to bacteria.

Morrill (13) showed by use dilution tests that 50 ppm of aqueous iodine would give complete kill in 60 seconds.

Research data by the Pitman-Moore Co. on Weladol showed that on presurgical scrub tests of 42 human subject's hands, 10 showed 100 per cent reduction of bacteria, 18 were between 95 and 100 per cent, and 14 were below 95 per cent reduction (19). The length of time the subject's hands were exposed to the Weladol in the scrub tests was not stated.

3. LIQUID GERMICIDAL DETERGENT. Liquid Germicidal Detergent* has been in use in the field for a number of years. In 1948 Byran and associates (3) reported on studies conducted at the Michigan State College Veterinary Clinic. By plating swabs taken from the skin of dogs and horses they showed that it was a satisfactory skin cleanser and antiseptic.

Research by the Parke-Davis Company (15) showed that when Liquid Germicidal Detergent was tested against such common skin contaminants as *Staphylococcus pyogenes* var. *aureus*, *Salmonella typhosa*, a hemolytic streptococcus, *Escherichia coli*, and *Pseudomonas aeruginosa* it was effective in relatively high dilutions. The maximum dilution that was effective varied with the organism tested, ranging from 1:200 against *Pseudomonas aeruginosa* to 1:2000 against a hemolytic streptococcus.

* Parke-Davis and Co., Detroit, Michigan

III. MATERIALS AND METHODS

A. LABORATORY TESTS

Since it is impractical to test a particular antiseptic in the presence of all the organisms found in connection with the bacterial flora of the skin, two of the more representative species were used. These were Salmonella typhosa (representative of the Gram negative, non sporulating bacilli), and Staphylococcus pyogenes var. aureus (representative of the suppurative group, and a Gram positive cocci).

The cultures were supplied by the Department of Microbiology and Public Health, Michigan State University. The cultures were transfered at least three times at twenty four hour intervals. This was done so that a constant growth curve was established, and the number of bacteria present in the broth was relatively consistent. The tubes were shaken vigorously by hand for fifteen minutes prior to use so as to break up the clumps and make the culture as homogeneous as possible.

1. PHENOL COEFFICIENT TEST

The Federal Drug Administration test for the determination of phenol coefficients was used. This method was developed by Lloyd P. Shippen, George F. Reddish, C. M. Brewer, and B. L. A. Ruehl and identified as the "Food and Drug Administration Phenol Coefficient" in the U. S. Department of Agriculture Circular No. 198 (13).

The Federal Drug Administration test for the determination of phenol coefficients is conducted as follows: five milliliter amounts of a five per cent stock solution of phenol are prepared in dilutions of 1:70, 1:80, 1:90, and 1:100. Dilutions of 1:70, 1:80, and 1:90 are used for Staphylococcus pyogenes var. aureus. For Salmonella typhosa, dilutions of 1:80, 1:90, and 1:100 are used. Five milliliter amounts of the antiseptic to be tested are prepared in dilutions arranged in a series of decreasing concentrations. Both the phenol and antiseptic dilutions are placed in a water bath at 20 degrees C. The 24 hour broth cultures are shaken by hand for 15 minutes to break up the clumps. Using a sterile 2 ml pipette, add 0.5 ml of the 24 hour broth culture of the test organism to one tube of each dilution of the antiseptic at intervals of 30 seconds. At the end of five minutes from the time of each inoculation a transfer is made with a loop (4 mm inside diameter, No. 23 B and S gauge) from the proper seeding pot to a tube of nutrient broth. These operations are repeated at intervals of 10 and 15 minutes. As soon as all transfers from the seeding pots are completed, a second set of transfers from the broth tubes just seeded is made. This is called Shippen's modification (17). Four loopfuls are transfered from each tube of inoculated broth to another tube of broth. This additional transfer dilutes the antiseptic beyond the point where it will be able to exert bacteriostatic action. Thus it can be determined if negative growth in the first set of tubes indicates actual kill, or if the first set is negative, but the second is positive, it would indicate that the action of the antiseptic is bacteriostatic. All cultures are incubated at 37 degrees C. for 48 hours.

The phenol coefficient is computed by dividing the highest dilution of the disinfectant which will kill in 10 minutes but not in 5 minutes by the highest dilution of phenol which will do the same.

2. USE DILUTION TEST

Mallmann and Hanes (11) proposed this test as a means of measuring the actual dilution of an antiseptic or disinfectant that can be used for field use.

In this method a 24 hour culture of *Staphylococcus pyogenes* var. *aureus* is used. Sterile glass rods one inch in length and a quarter inch in diameter, having a loop at one end for handling, are dipped in a broth culture of the test organism and then laid in petri dishes on sterile filter paper to dry. Care is taken to avoid rolling the rods while drying. A drying period of 30 minutes is used.

A medication pot (one inch by four inches) containing 10 ml of the disinfectant in the dilution being tested, and four pots each containing 10 ml of sterile saline rinse, are placed in a 20 degree C. water bath.

At the end of the drying period, four rods are dipped simultaneously into the medication pot containing the test disinfectant. At intervals of one, five, ten, and thirty minutes, a rod is removed and immersed for one minute in a tube containing 10 ml of sterile saline. A neutralizing substance may be incorporated into the saline to control bacteriostatic action. For Liquid Germicidal Detergent the neutralizer consisted of a mixture of azolectin and Tween 80. Sodium thiosulfate was used for Weladol. Tween 80 was used to neutralize Triocil.

At the end of one minute, the rod is transfered to a tube containing 10 ml of nutrient broth. This tube is shaken vigorously to remove all remaining viable organisms adhering to the rods. One milliliter amounts of the nutrient broth are then plated in tryptose dextrose agar to measure quantitatively the extent of the kill. The tubes containing the rods, and the plates are incubated at 37 degrees C. for 48 hours. Lack of growth in both the tubes and plates at the end of this period is accepted as evidence of complete kill.

Controls are prepared by dropping a rod covered with the dried organisms into a pot containing 10 ml of sterile saline for one minute, and then into a tube containing 10 ml of broth. One milliliter of the nutrient broth is used to make dilutions of 1:100, 1:1000, and 1:10,000, which are plated in tryptose dextrose agar. The plates are incubated at 37 degrees C. for 48 hours.

The number of organisms surviving on the rods exposed to the antiseptic is determined by counting the number of colonies on the plate and multiplying by ten, as only one tenth of the nutrient broth is used.

In order to determine the control number a plate is chosen having between 30 and 300 colonies. The number of colonies is multiplied by the dilution factor of that plate, and then by ten, as one tenth of the broth is used.

3. SPEED OF DISINFECTION TEST

In order to test the speed of action of a particular disinfectant, the following procedure is followed (1). Add 0.5 ml of a 24 hour broth culture of *Staphylococcus pyogenes* var. *aureus* to a seeding pot containing 10 ml of a particular antiseptic dilution. Also place 10 ml of sterile distilled water in a seeding pot. Place seeding pots in a water bath at 20 degrees C. At intervals of 15, 30, 45, 60, 120, and 180 seconds a loop transfer is made from the seeding pot to tubes of melted agar in a 45 degree C. water bath. After thorough mixing the agar is poured into sterile Petri dishes and incubated at 37 degrees C. for 48 hours. After that time the plates are counted.

Controls are run to determine the original number of organisms present before addition of the antiseptic. Controls are made by adding 0.5 ml of the 24 hour broth culture of the organism to the seeding pot containing 10 ml of sterile distilled water. Transfer one loopful to a tube containing 10 ml of nutrient broth. One milliliter of this nutrient broth is then used to make dilutions of 1:10, 1:100, and 1:1000. These dilutions are also plated and incubated at 37 degrees C. for 48 hours.

In order to determine the control number a plate is chosen having between 30 and 300 colonies. The number of colonies is multiplied by the dilution factor of that plate, and then by ten, as one tenth of the broth is used.

The per cent of organisms remaining is determined by dividing the number of colonies found on the test plate by the control number. The per cent reduction is determined by subtracting the per cent remaining from 100 per cent.

B. CLINICAL TESTING

This phase of the testing employed animals in the Michigan State University Veterinary Clinic. These animals were hospital cases that were being prepared for surgery in the manner routinely employed. No special measures were taken, as it was desired that the samples be representative of the standard procedure used in the hospital.

A swab technique was used to obtain measurements of the number of organisms present on the skin after the presurgical scrub. It is recognized that this technique does not measure the absolute number of organisms present because the resident bacteria living deep in the skin are not reached. However, this procedure does allow the accumulation of data relative to the comparative number of transient bacteria present following the use of different antiseptic preparations.

Cotton swabs on wooden applicator sticks were used to obtain the samples. Immediately after use, they were placed in screw cap vials containing 10 ml of physiological saline and a neutralizing compound. Both the swabs and the saline had previously been sterilized by the use of the autoclave.

To prevent the possibility of bactericidal or bacteriostatic action by the antiseptic which might be carried over to the saline on the cotton, appropriate neutralizers were added to the saline. For Liquid Germicidal Detergent the neutralizer consisted of 2.22 grams of azolectin and 15.6 ml of Tween 80 added to one liter of distilled water. Then 1.25 ml of a phosphate buffer, potassium acid phosphate-disodium phosphate, were added and the solution heated until clear. After the preparation was autoclaved, it was stored until used.

To neutralize Weladol, sodium thiosulfate was used. Three hundred and twenty milligrams of sodium thiosulfate were added to one liter of distilled water, and 1.25 ml of the phosphate buffer were added. For Triocil, a one per cent solution of Tween 80 in distilled water was employed. All three preparations were sterilized in the autoclave at 248 degrees F. for 20 minutes at 15 pounds pressure. One milliliter of the appropriate neutralizer was added to the saline after the sample was obtained.

Cattle, swine, and dogs were checked at the termination of the surgical preparation. The sample was obtained from the approximate place the incision was to be made. The following procedure was used for the preparation of cattle and swine for surgery. The hair was clipped from a liberal area around the proposed incision line. Stewart Clipmaster clippers with a No. .84AU head were used. The area was wet with water from a hose. The antiseptic to be used was then liberally applied from a polyethylene squeeze bottle. The area was scrubbed vigorously with a stiff brush. The lather was washed away with tap water, and the process was repeated. Total scrubbing time ranged from four to six minutes.

The scrubbing procedure for the dog usually entailed five applications of the antiseptic to be used. The surgical area was clipped using a model A-2 Oster clipper, with a size 40 head. The antiseptic to be used was then applied from a polyethylene squeeze bottle. The scrubbing was done by hand and the lather was removed between each application with several cotton wipes. The cotton was from a roll of non-surgical bleached cotton (non-sterile). The procedures described above for cattle, swine and dogs remained the same no matter which antiseptic was employed, or what type of operation was performed.

The following procedure was used in obtaining samples: 1. The swab was removed from the glass vial that was used as a container while it was being sterilized. 2. Approximately two square inches of skin were thoroughly wiped at the termination of the presurgical preparation. The swab and applicator stick were immediately placed in the tube containing the sterile saline. The tube was shaken vigorously, and the contents plated within one-half hour after the swabs were taken. Five per cent blood tryptose agar was used as the culture medium. Plates were prepared by adding a sufficient amount of sterile bovine blood to previously prepared and sterilized tryptose agar to make a five per cent suspension. The blood agar was then poured into disposable plastic Petri dishes and five ml of the original 10 ml saline sample added. The plates were incubated for 24 hours at 37 degrees C. After that time the plates were examined for growth and hemolysis. The colony count obtained was doubled because only half the sample was used.

C. ANTISEPTICS TESTED

1. TRIOCIL

Triocil is Warner-Chilcott's trade name for hexetidine (bis-1, 3-beta-ethylhexyl-5 methyl-5 amino hexahydropyrimidine). The preparation contains 0.5 per cent of the active ingredient. It is a colorless oil that is soluble in organic solvents such as alcohol, acetone, and ether. It is soluble in water only to the extent of one part in 10,000.

The manufacturer's recommendations for its use as a presurgical scrub (15) are as follows:

a. Triocil Spray (0.5%) can be used as the sole agent cleansing the surgical area.

b. In physically dirty dogs, Triocil Solution (0.5%) with a detergent base is diluted from 1:2 to 1:5 and is used as a cleansing agent. This is removed with water and Triocil Spray is applied.

c. Triocil Spray may be used as the final step if other preparations are used initially.

d. Triocil Solution may be used initially if other products are used as a final step.

2. WELADOL

Weladol is a product of the Pitman-Moore Company. It contains a surfactant iodine complex, polyalkyleneglycol, commonly known as an iodophor or "iodine carrier". This preparation contains one per cent available iodine.

As a presurgical scrub, Weladol is used in the same manner as any other antiseptic detergent; that is, by thoroughly scrubbing the site with Weladol and warm water for an adequate length of time (19).

3. LIQUID GERMICIDAL DETERGENT

Liquid Germicidal Detergent is Parke-Davis and Co.'s trade name for a suspension of high molecular weight alkylamide hydrochlorides containing 2.5 per cent of a quaternary ammonium chloride compound (phemerol)*. It has wetting and emulsifying properties, mixing freely with water of any temperature and hardness. The product is recommended for use at either full strength, or a 1:5 dilution. Optimal scrubbing time with Liquid Germicidal Detergent will vary, depending on the character of the skin surface and the amount of gross contamination. In general, however, a period of five to ten minutes is recommended (10).

* Benzethonium chloride, Parke-Davis and Co.

IV. RESULTS

A. RESULTS OF LABORATORY TESTING ON TRIOCIL SOLUTION

1. PHENOL COEFFICIENT TEST

Table I-A shows that when Triocil was employed in the phenol coefficient test, absence of growth occurred only after 15 minutes exposure at full strength with *Salmonella typhosa* as the test organism. Against *Staphylococcus pyogenes* var. *aureus* growth occurred in all tubes (Table I-B).

2. USE DILUTION TEST

Tables II-A through II-E give the results when Triocil was employed in the use dilution test against *Staphylococcus pyogenes* var. *aureus*. At full strength a 100 per cent reduction was not obtained until five minutes exposure. In Tables II-B, II-D, and II-E complete reduction was never obtained. The greatest reduction was achieved after 30 minutes exposure. This was 99.984 per cent for the 1:2 dilution, 99.067 per cent for the 1:4 dilution, and 99.133 per cent for the 1:8 dilution.

3. SPEED OF DISINFECTION TEST

Tables III-A through III-C give the results when Triocil was used in the speed of disinfection test. When used undiluted, complete reduction was obtained in the minimum time of exposure (15 seconds). Table III-B shows that when Triocil was diluted 1:4 a reduction of 99.740 per cent was obtained after the minimum time of 15 seconds. This does not differ appreciably from the reduction obtained after the maximum exposure time of ten minutes, which was 99.917 per cent. Similarly, in Table III-C where a 1:8 dilution was used, a reduction of 99.145 per cent was obtained in the minimum time of 15 seconds, while a 99.508 per cent reduction was obtained in 10 minutes. Salmonella typhosa was the test organism used.

B. RESULTS OF LABORATORY TESTING ON WELADOL

1. PHENOL COEFFICIENT TEST

Tables IV-A and IV-B indicate the results when Weladol was used as the test antiseptic in the phenol coefficient test. A phenol coefficient of 2.83 was established, using Salmonella typhosa as the test organism. The Shippen's modification indicates that there was no bacteriostasis in the tubes showing no growth.

2. USE DILUTION TEST

When Weladol was tested by the use dilution method (Tables V-A through V-E) a 1:25 dilution gave 100 per cent reduction in the minimum time of one minute, while a 1:75 dilution gave a 100 per cent reduction in a minimum time of five minutes. Dilutions of 1:225, 1:250, and 1:300 required ten minutes to elicit a complete reduction (Tables V-C, V-D, and V-E).

3. SPEED OF DISINFECTION TEST

Weladol was used in the speed of disinfection test at dilutions ranging from 1:2 through 1:300 (Tables VI-A through VI-K). Staphylococcus pyogenes var. aureus was the test organism used. At dilutions from 1:2 through 1:32 all plates were negative (Tables VI-A through VI-E). At dilutions of 1:100, 1:150, and 1:200 complete reduction was obtained in a minimum time of 30 to 60 seconds (Tables VI-G, VI-H, and VI-I). At dilutions of 1:250 and 1:300 a 100 per cent reduction was not obtained until the culture was exposed to the antiseptic for two minutes (Tables VI-J and VI-K).

C. RESULTS OF LABORATORY TESTING ON LIQUID GERMICIDAL DETERGENT

1. PHENOL COEFFICIENT TEST

When Liquid Germicidal Detergent was used in the phenol coefficient test, with *Salmonella typhosa* as the test organism, a phenol coefficient of 444.4 was established. The Shippen's modification indicates that no bacteriostatic action occurred (Tables VII-A and VII-B).

2. USE DILUTION TEST

In the use dilution test all the plates were negative when Liquid Germicidal Detergent was used at full strength (Table VIII-A). At a 1:5 dilution a minimum time of five minutes was needed for complete reduction, while at dilutions of 1:7 and 1:9, ten minutes was needed for complete reduction (Tables VIII-B, VIII-C, and VIII-D).

3. SPEED OF DISINFECTION TEST

In the speed of disinfection test (Tables IX-A through IX-D) dilutions from full strength through 1:7 gave 100 per cent reduction on all plates. At a dilution of 1:9 a minimum time of 60 seconds was required for 100 per cent reduction (Table IX-E). When using a sample of unknown concentration taken from a clinic dispenser, 100 per cent reduction was effected in a minimum time of 15 seconds (Table IX-F).

D. CLINICAL TESTING

1. TRIOCIL

In the clinical testing of Triocil (Table X) at a dilution of 1:8 a completely negative sample was never obtained. Numbers of colonies ranged from a low of 12 (canine ovariohysterectomy) to a high of 340 (porcine umbilical hernia). Three of the plates contained over 300 colonies. Four of the plates contained hemolytic colonies, with the highest being 12 per cent.

2. WELADOL

In the clinical testing of Weladol (Table XI), used at full strength, a negative sample was never obtained. With one exception, the number of colonies ranged from 2 (canine ovariohysterectomy) to 342 (porcine inguinal hernia). One case (canine perineal hernia) had a count of 7076 colonies. Hemolytic colonies were demonstrated in eight of the samples. One plate had 50 per cent hemolytic bacteria.

3. LIQUID GERMICIDAL DETERGENT

Clinical testing of Liquid Germicidal Detergent at a 1:5 dilution (Table XII), showed that seven of the samples taken were negative. The greatest number of colonies cultured from one sample was 66. In positive samples counts ranged from a low of 2 (canine ovariohysterectomy) to a high of 66 (bovine rumenotomy). Only one sample contained bacteria that were hemolytic (bovine rumenotomy).

Table XIII lists the number of samples obtained, the average number of colonies per sample, and the number of samples that contained hemolytic colonies for the three antiseptics tested.

A. RESULTS OF LABORATORY TESTING ON TRIOCIL SOLUTION

PHENOL COEFFICIENT

Both _Salmonella typhosa_ and _Staphylococcus pyogenes_ var. _aureus_ were used as test organisms in running phenol coefficients on Triocil solution. In each case the test was repeated four times with the same results obtained.

TABLE I-A

TEST ORGANISM: _Salmonella typhosa_

DILUTION Antiseptic	TIME Nutrient Broth Tubes		
Phenol	5 min.	10 min.	15 min.
1-70	-	-	-
1-80	+*	-	-
1-90	+	+	-
Triocil			
1-200	+	+	-
1-500	+	+	+
1-1000	+	+	+
1-10,000	+	+	+
1-50,000	+	+	+
1-100,000	+	+	+

* + indicates growth; - absence of growth

TABLE I-B

PHENOL COEFFICIENT TEST ON TRIOCIL SOLUTION (cont.)

TEST ORGANISM: Staphylococcus pyogenes var. aureus

DILUTION Antiseptic	TIME Nutrient Broth Tubes		
Phenol	5 min.	10 min.	15 min.
1-70	-	-	-
1-80	+	-	-
1-90	+	+	+
Triocil			
1-200	+	+	+
1-500	+	+	+
1-1000	+	+	+
1-5000	+	+	+
1-10,000	+	+	+
1-50,000	+	+	+
1-100,000	+	+	+

TABLE II-A

USE DILUTION TEST ON TRIOCIL SOLUTION

TEST ORGANISM: <u>Staphylococcus</u> <u>pyogenes</u> var. <u>aureus</u>

DILUTION: Full strength (1-200)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	180	00.090% *	99.910%
5 minutes	negative	00.000%	100.00%
10 minutes	negative	00.000%	100.00%
30 minutes	negative	00.000%	100.00%

TABLE II-B

DILUTION: 1:2 (1-400)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	6120	3.362%	96.641%
5 minutes	520	.287%	99.713%
10 minutes	220	.120%	99.880%
30 minutes	30	.016%	99.984%

* Procedure for obtaining percentages isfound in section on Material and Methods.

TABLE II-C

USE DILUTION TEST ON TRIOCIL SOLUTION (cont.)

CONTROL (for Full and Half Strength Solutions)

DILUTION	NO. OF ORG. PER PLATE	NO. OF ORG. PER ML	NO. OF ORG. PER ROD
1-100	182	18200	182,000
1-1000	40	4000	40,000
1-10,000	19	190	19,000

TABLE II-D

DILUTION: 1:4 (1-800)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	11,170	5.319%	94.681%
5 minutes	10,670	5.083%	94.917%
10 minutes	2,180	1.076%	98.924%
30 minutes	1,960	.933%	99.067%

TABLE II-E

USE DILUTION TEST ON TRIOCIL SOLUTION (cont.)

DILUTION: 1:8 (1-1600)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	20,460	9.745%	90.255%
5 minutes	14,730	7.016%	92.984%
10 minutes	4,640	2.213%	97.787%
30 minutes	1,820	.867%	99.133%

TABLE II-F

CONTROL (for 1/4 and 1/8 Strength Solutions)

DILUTION	NO. OF ORG. PER PLATE	NO. OF ORG. PER ML	NO. OF ORG. PER ROD
1-100	210	2,100	210,000
1-1000	2	20	20,000
1-10,000	0	0	0

TABLE III-A

SPEED OF DISINFECTION TEST ON TRIOCIL SOLUTION

TEST ORGANISM: _Staphylococcus_ _pyogenes_ var. _aureus_

DILUTION: Full Strength (1-200)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE III-B

SPEED OF DISINFECTION TEST ON TRIOCIL SOLUTION (cont.)

DILUTION: 1:4 (1-800)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	784	.260%	99.740%
30 seconds	805	.264%	99.700%
45 seconds	716	.234%	99.773%
60 seconds	558	.185%	99.818%
2 minutes	493	.163%	99.837%
3 minutes	374	.124%	99.876%
4 minutes	371	.123%	99.877%
5 minutes	355	.117%	99.883%
6 minutes	314	.104%	99.896%
7 minutes	289	.096%	99.904%
8 minutes	152	.050%	99.917%
9 minutes	247	.082%	99.918%
10 minutes	151	.050%	99.917%

TABLE III-C

SPEED OF DISINFECTION TEST ON TRIOCIL SOLUTION (cont.)

DILUTION: 1:8 (1-1600)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	2684	.855%	99.145%
30 seconds	2615	.862%	99.138%
45 seconds	2598	.863%	99.137%
60 seconds	2567	.852%	99.148%
2 minutes	2463	.864%	99.136%
3 minutes	2170	.720%	99.280%
4 minutes	1116	.370%	99.630%
5 minutes	1560	.517%	99.483%
6 minutes	1426	.473%	99.527%
7 minutes	1488	.494%	99.606%
8 minutes	1520	.505%	99.495%
9 minutes	1513	.503%	99.497%
10 minutes	1482	.492%	99.508%

TABLE III-D

CONTROL

DILUTION	NO. OF ORG. PER PLATE	NO. OF ORG. PER ML	NO. OF ORG. PER LOOP
1-10	1310	13,100	131,000
1-100	301	30,100	301,000
1-1000	52	52,000	520,000

B. RESULTS OF LABORATORY TESTING ON WELADOL

TABLE IV-A

PHENOL COEFFICIENT

TEST ORGANISM: *Salmonella typhosa*

DILUTION Antiseptic	TIME Nutrient Broth Tubes 5 min.	10 min.	15 min.	SHIPPEN'S MODIFICATION Subculture Tubes 5 min.	10 min.	15 min.
Phenol	5 min.	10 min.	15 min.			
1-80	-	-	-			
1-90	+	-	-			
1-100	+	+	-			
Weladol				5 min.	10 min.	15 min.
1-100	-	-	-	-	-	-
1-1000	+	+	+	+	+	+
1-10,000	+	+	+	+	+	+
1-100,000	+	+	+	+	+	+
1-200,000	+	+	+	+	+	+
1-300,000	+	+	+	+	+	+
Phenol	5 min.	10 min.	15 min.			
1-80	-	-	-			
1-90	+	-	-			
1-100	+	+	-			
Weladol				5 min.	10 min.	15 min.
1-100	-	-	-	-	-	-
1-300	+	+	+	+	+	+
1-500	+	+	+	+	+	+
1-600	+	+	+	+	+	+
1-800	+	+	+	+	+	+
1-900	+	+	+	+	+	+
1-1000	+	+	+	+	+	+

TABLE IV-B

PHENOL COEFFICIENT TEST ON WELADOL (cont.)

DILUTION Antiseptic	TIME Nutrient Broth Tubes			SHIPPEN'S MODIFICATION Subculture Tubes		
Phenol	5 min.	10 min.	15 min.			
1-80	-	-	-			
1-90	+	-	-			
1-100	+	+	-			
Weladol				5 min.	10 min.	15 min.
1-125	-	-	-	-	-	-
1-150	-	-	-	-	-	-
1-200	-	-	-	-	-	-
1-225	+	-	-	+	-	-
1-250	+	+	+	+	+	+
1-275	+	+	+	+	+	+
1-300	+	+	+	+	+	+

$$\text{Phenol Coefficient} = \frac{225}{90} = 2.83$$

TABLE V-A

USE DILUTION TEST ON WELADOL

TEST ORGANISM: Staphylococcus pyogenes var. aureus

DILUTION: 1-25

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	negative	00.000%	100.00%
5 minutes	negative	00.000%	100.00%
10 minutes	negative	00.000%	100.00%
30 minutes	negative	00.000%	100.00%

TABLE V-B DILUTION: 1-75

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	1	00.0002%	99.998%
5 minutes	negative	00.000%	100.00%
10 minutes	negative	00.000%	100.00%
30 minutes	negative	00.000%	100.00%

TABLE V-C

USE DILUTION TEST ON WELADOL (cont.)

DILUTION: 1-225

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	15	00.0026%*	99.9973%
5 minutes	2	00.0003%	99.9997%
10 minutes	negative	00.000%	100.00%
30 minutes	negative	00.000%	100.00%

TABLE V-D DILUTION: 1-250

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	17	00.0029%	99.9971%
5 minutes	8	00.0013%	99.9987%
10 minutes	negative	00.000%	100.00%
30 minutes	negative	00.000%	100.00%

* Procedure for obtaining percentages is found in section on Materials and Methods.

TABLE V-E

USE DILUTION TEST ON WELADOL (cont.)

DILUTION: 1-300

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	22	00.0038%	99.9962%
5 minutes	12	00.0020%	99.9980%
10 minutes	negative	00.000%	100.000%
30 minutes	negative	00.000%	100.000%

TABLE V-F

CONTROL

DILUTION	NO. OF ORG. PER PLATE	NO. OF ORG. PER ML	NO. OF ORG. PER ROD
1-100	176	17,600	176,000
1-1000	58	58,000	580,000
1-10,000	4	40,000	400,000

TABLE VI-A

SPEED OF DISINFECTION ON WELADOL

TEST ORGANISM: *Staphylococcus pyogenes* var. *aureus*

DILUTION: 1-2

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-B DILUTION: 1-4

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-C

SPEED OF DISINFECTION TEST ON WELADOL (cont.)

DILUTION: 1-8

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-D DILUTION: 1-16

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-E

SPEED OF DISINFECTION TEST ON WELADOL (cont.)

DILUTION: 1-32

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-F

CONTROL

DILUTION	NO. OF ORG. PER PLATE	NO. OF OR. PER ML	NO. OF ORG. PER LOOP
1-10	1048	10,480	104,800
1-100	160	10,600	106,000
1-1000	96	96,000	960,000

TABLE VI-C

SPEED OF DISINFECTION TEST ON WELADOL (cont.)

DILUTION: 1-100

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	3	00.0031%	99.9969%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-H DILUTION: 1-150

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	2	00.0020%	99.9970%
30 seconds	1	00.0010%	99.9990%
45 seconds	1	00.0010%	99.9990%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-I

SPEED OF DISINFECTION TEST ON WELADOL (cont.)

DILUTION: 1-200

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	3	00.0031%	99.9969%
30 seconds	2	00.0020%	99.9970%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-J DILUTION: 1-250

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	3	00.0031%	99.9969%
30 seconds	3	00.0031%	99.9969%
45 seconds	2	00.0020%	99.9970%
60 seconds	1	00.0010%	99.9990%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-K

SPEED OF DISINFECTION TEST ON WELADOL (cont.)

DILUTION: 1-300

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	10	00.0103%	99.9897%
30 seconds	6	00.0062%	99.9938%
45 seconds	5	00.0052%	99.9948%
60 seconds	2	00.0020%	99.9980%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE VI-L

CONTROL

DILUTION	NO. OF ORG. PER PLATE	NO. OF ORG. PER ML	NO. OF ORG. PER LOOP
1-10	4472	44,720	447,200
1-100	832	83,200	832,000
1-1000	64	64,000	640,000

C. RESULTS OF LABORATORY TESTING ON LIQUID GERMICIDAL DETERGENT

TABLE VII-A

PHENOL COEFFICIENT

TEST ORGANISM: *Salmonella typhosa*

DILUTION Antiseptic	TIME Nutrient Broth Tubes		
Phenol	5 min.	10 min.	15 min.
1-80	-	-	-
1-90	+ *	-	-
1-100	+	+	+
L. G. D.			
1-40	-	-	-
1-80	-	-	-
1-120	-	-	-
1-400	-	-	-
1-800	-	-	-
1-16000	-	-	-

DILUTION Antiseptic	TIME Nutrient Broth Tubes		
Phenol	5 min.	10 min.	15 min.
1-80	-	-	-
1-90	+	-	-
1-100	+	+	+
L. G. D.			
1-1600	-	-	-
1-2000	-	-	-
1-2500	-	-	-
1-3000	-	-	-
1-3500	-	-	-
1-4000	-	-	-
1-8000	-	-	-

* + indicates growth in tube; - indicates no growth in tube

TABLE VII-B

PHENOL COEFFICIENT ON LIQUID GERMICIDAL DETERGENT (cont.)

DILUTION Antiseptic	TIME Nutrient Broth Tubes			SHIPPEN'S MODIFICATION Subculture Tubes		
Phenol	5 min.	10 min.	15 min.			
1-80	-	-	-			
1-90	+	-	-			
1-100	+	+	+			
L. G. D.				5 min.	10 min.	15 min.
1-1600	-	-	-	-	-	-
1-8000	-	-	-	-	-	-
1-12,000	-	-	-	-	-	-
1-16,000	-	-	-	-	-	-
1-20,000	-	-	-	-	-	-
1-24,000	-	-	-	-	-	-
1-40,000	+	-	-	+	-	-

$$\text{Phenol Coefficient} = \frac{40{,}000}{90} = 444.4$$

TABLE VIII-A

USE DILUTION TEST ON LIQUID GERMICIDAL DETERGENT

TEST ORGANISM: *Staphylococcus pyogenes* var. *aureus*

DILUTION: Full Strength

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	negative	00.000%	100.00%
5 minutes	negative	00.000%	100.00%
10 minutes	negative	00.000%	100.00%
30 minutes	negative	00.000%	100.00%

TABLE VIII-B

DILUTION: 1:5 (1-200)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	10	00.00004%	99.99996%
5 minutes	negative	00.000%	100.00%
10 minutes	negative	00.000%	100.00%
30 minutes	negative	00.000%	100.00%

TABLE VIII-C

USE DILUTION TEST ON LIQUID GERMICIDAL DETERGENT (cont.)

DILUTION: 1:7 (1-280)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	19	00.00008%	99.99992%
5 minutes	7	00.00002%	99.99998%
10 minutes	negative	00.000%	100.00%
30 minutes	negative	00.000%	100.00%

TABLE VIII-D

DILUTION: 1:9 (1-360)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
1 minute	27	00.0001%	99.9999%
5 minutes	10	00.00004%	99.99996%
10 minutes	negative	00.000%	100.00%
30 minutes	negative	00.000%	100.00%

TABLE VIII-E CONTROL

DILUTION	NO. OF ORG. PER PLATE	NO. OF ORG. PER ML	NO. OF ORG. PER ROD
1-100	1,488	148,800	1,488,000
1-1000	26	26,000	260,000
1-10,000	-	-	-

TABLE IX-A

SPEED OF DISINFECTION ON LIQUID GERMICIDAL DETERGENT

TEST ORGANISM: *Staphylococcus pyogenes* var. *aureus*

DILUTION: Full Strength (1-40)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE IX-B

SPEED OF DISINFECTION TEST ON LIQUID GERMICIDAL DETERGENT (cont.)

DILUTION: 1:3 (1-120)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE IX-C DILUTION: 1:5 (1-200)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE IX-D

SPEED OF DISINFECTION TEST ON LIQUID GERMICIDAL DETERGENT (cont.)

DILUTION: 1:7 (1-280)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE IX-E DILUTION: 1:9 (1-360)

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	11	00.0074%	99.9926%
30 seconds	7	00.0047%	99.9953%
45 seconds	3	00.0020%	99.9970%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE IX-F

SPEED OF DISINFECTION TEST ON LIQUID GERMICIDAL DETERGENT (cont.)

DILUTION: Unknown, sample taken directly from dispenser bottle in clinic.

TIME	NUMBER OF COLONIES PER PLATE	PER CENT REMAINING	PER CENT REDUCTION
15 seconds	negative	00.000%	100.00%
30 seconds	negative	00.000%	100.00%
45 seconds	negative	00.000%	100.00%
60 seconds	negative	00.000%	100.00%
2 minutes	negative	00.000%	100.00%
3 minutes	negative	00.000%	100.00%

TABLE IX-G

CONTROL

DILUTION	NO. OF ORG. PER PLATE	NO. OF ORG. PER ML	NO. OF ORG. PER LOOP
1-10	1874	18740	187,400
1-100	147	14700	147,000
1-1000	13	13000	130,000

D. CLINICAL TESTING

TABLE X

Triocil Solution at a Dilution of 1:8 was the Antiseptic

SPECIES OF ANIMAL AND OPERATION PERFORMED	TOTAL NUMBER OF COLONIES FOUND IN SAMPLE SWABS	NUMBER OF HEMOLYTIC COLONIES (PERCENTAGE BASIS)	
		NON-HEMOLYTIC COLONIES (PER CENT)	HEMOLYTIC COLONIES (PER CENT)
CANINE			
Benign tumor on skin of abdomen	16	100	0
Ovariohysterectomy	24	96	4
Ovariohysterectomy	12	100	0
Foreign body in esophagus	300	100	0
Abscessed anal gland	302	95	5
Laparotomy	60	95	5
PORCINE			
Scrotal hernia	250	88	12
Umbilical hernia	340	94	6
Tumor on rear leg	24	100	0

TABLE XI

Weladol Shampoo, 1% Available Iodine, Water Added so as to Form a Lather

SPECIES OF ANIMAL AND OPERATION PERFORMED	TOTAL NUMBER OF COLONIES FOUND IN SAMPLE SWABS	NUMBER OF HEMOLYTIC COLONIES (PERCENTAGE BASIS)	
		NON-HEMOLYTIC COLONIES (PER CENT)	HEMOLYTIC COLONIES (PER CENT)
CANINE			
Ovariohysterectomy	198	50	50
Perineal hernia (dog died post-operatively. Infection was present in surgical area)	7076	76	24
Ovariohysterectomy	4	100	0
Ovariohysterectomy	2	100	0
PORCINE			
Inguinal hernia	130	91	9
Inguinal hernia	342	92	8
Umbilical hernia	252	95	5
BOVINE			
Rumenotomy	182	98	2
Rumenotomy	210	92	8
Rumenotomy	58	97	3

TABLE XII

Liquid Germicidal Detergent Dilution 1:5

SPECIES OF ANIMAL AND OPERATION PERFORMED	TOTAL NUMBER OF COLONIES FOUND IN SAMPLE SWAB	NUMBER OF HEMOLYTIC COLONIES (PERCENTAGE BASIS)	
		NON-HEMOLYTIC COLONIES (PER CENT)	HEMOLYTIC COLONIES (PER CENT)
CANINE			
Ovariohysterectomy	negative	-	-
Ovariohysterectomy	30	100	0
Ovariohysterectomy	negative	-	-
Ovariohysterectomy	16	100	0
Ovariohysterectomy	negative	-	-
Ovariohysterectomy	2	100	0
Diaphragmatic hernia	negative	-	-
Tumor in region of vulva	10	100	0
Non-union of humerus	50	100	0
Ovariohysterectomy	negative	-	-
Ovariohysterectomy	36	100	0
Ovariohysterectomy	12	100	0

TABLE XII (cont.)

Liquid Germicidal Detergent Dilution 1:5

SPECIES OF ANIMAL AND OPERATION PERFORMED	TOTAL NUMBER OF COLONIES FOUND IN SAMPLE SWABS	NUMBER OF HEMOLYTIC COLONIES (PERCENTAGE BASIS)	
		NON-HEMOLYTIC COLONIES (PER CENT)	HEMOLYTIC COLONIES (PER CENT)
BOVINE			
Claw amputation	20	100	0
Rumenotomy	66	100	0
Rumenotomy	10	100	0
Prolapsed vagina	negative	-	-
Rumenotomy	50	100	0
Rumenotomy	12	100	0
Laparotomy	negative	100	0
Rumenotomy	10	100	0
Umbilical hernia	26	100	0
Rumenotomy	48	80	20

TABLE XIII

Comparison of Cultures of Clinical Samples

ANTISEPTIC	NUMBER OF SAMPLES OBTAINED	AVERAGE NUMBER OF COLONIES PER SAMPLE	NUMBER OF SAMPLES THAT CONTAINED HEMOLYTIC COLONIES
Triocil	9	215	5
Weladol	10	150	8
Liquid Germicidal Detergent	22	16	1

V. DISCUSSION

A. TRIOCIL

In this work, phenol coefficients were run on Triocil using both test organisms (Tables I-A and I-B). Since growth occurred in all of the tubes, the tests were repeated four times with each culture. To exclude the possibility of the positive growth in the tubes being due to contamination from faulty technique, a control was run whereby the standard procedure was followed except the tubes were not seeded with the bacteria. All tubes in this procedure were negative, indicating contamination was not responsible for the growth. A phenol coefficient could not be established because results were never obtained which would satisfy the criteria for a phenol coefficient (the dilution of Triocil which will kill in 10 minutes but not in five, divided by the dilution of phenol that will kill in 10 minutes, but not in five).

It is interesting to note that in the use dilution test and the speed of disinfection test, satisfactory results, that is, 100 per cent reduction, were obtained at full strength, but the efficiency dropped off as soon as it was diluted. One factor that must be considered here is bacteriostasis. When undiluted, more of the antiseptic is transferred on the loop or rod, thus increasing the possibility of bacteriostatic action. Another factor to consider is Triocil's low solubility in water (1-10,000). All antiseptics are diluted with water in the scrubbing process. If a compound cannot maintain its effectiveness when diluted with water, its desirability as an antiseptic is lowered.

Since the laboratory testing showed that Triocil's efficiency was lowered when it was diluted, it would follow that in the clinical testing, where it is used in a dilution of 1:8 the samples obtained would not be totally free from bacteria. This was borne out in the clinical testing, where no negative samples were obtained.

In the speed of disinfection test when Triocil was diluted, the time interval was extended to 10 minutes to see if the longer exposure to the antiseptic would give a complete reduction. Although the reduction was high, some viable organisms still remained.

B. WELADOL

Work carried out by the author established a phenol coefficient of 2.83 for Weladol (Tables IV-A and IV-B). The test was first run at a wide range of dilutions, and then in increasingly narrower ranges to establish this figure. *Salmonella typhosa* was the test organism. This compares with a phenol coefficient of 5.8 for Lugol's iodine against the same organism (9). Since it was at dilutions of 1:225 to 1:250 that growth started to occur in this test, the use dilution test and the speed of disinfection test were run through a slightly higher dilution (1:300).

As regards the use dilution test, it was found that a higher dilution of Weladol was needed to give the results Morrill obtained with an aqueous iodine. This difference in concentration probably is due to the relative availability of iodine. Aqueous iodine is available immediately, while the iodophor form is released slowly over a period of time.

The speed of disinfection test showed that Weladol was effective in a short period of time at relatively high dilutions.

In the clinical testing (Table XI), with one exception, the average number of colonies per plate was 150. In the one exception, a count of over 7000 was obtained. The case was a dog with a bilateral perineal hernia which had been present for two years. Surgical repair was attempted. The bladder was displaced into the hernia and necrosis of the perineal fat was found. The dog died postoperatively. Upon post mortem examination a hemorrhagic cystitis involving both the serosal and mucosal surfaces was present. An ascending infection was traced through the ureters to the kidney. A diagnosis of nephritis was made. This operation was performed in an area where contamination could occur despite all precautions. The fault could lie with the antiseptic, or with insufficient preparation. Since this one case differs so markedly from other surgical cases where Weladol was employed, it would seem that the antiseptic itself would not be entirely at fault. In subsequent cases where Weladol was employed, no postoperative complications were encountered.

C. LIQUID GERMICIDAL DETERGENT

A phenol coefficient of 444.4 was established for Liquid Germicidal Detergent against *Salmonella typhosa*. This figure compares with a range of 300-400 given for most quaternary ammonium compounds. Work by the Parke-Davis Co. showed Liquid Germicidal Detergent to be germicidal in 5 minutes at dilutions ranging from 1:120 to 1:250 against certain common skin contaminants. In this work, in the same time interval Liquid Germicidal Detergent was germicidal in a dilution of 1:200 against *Staphylococcus pyogenes* var. *aureus*.

The speed of disinfection test showed that Liquid Germicidal Detergent was efficient in 15 seconds up to a dilution of 1:7.

The clinical testing was consistent with the *in vitro* testing. Often an antiseptic that works well in laboratory tests does not function as well when used on the skin. This work indicates that Liquid Germicidal Detergent will function as well on the skin as in laboratory tests.

D. COMPARATIVE EVALUATION OF ANTISEPTICS TESTED

It is difficult to make a direct comparison of the laboratory testing. Evaluation of the results obtained, particularly in the case of phenol coefficients, depends largely on the nature of the antiseptic tested, that is, its chemical composition, mode of action, killing range, affinity for organic matter, homogeneity, toxicity, and penetrability.

In view of these limitations, although Liquid Germicidal Detergent has a much higher phenol coefficient than Weladol, this does not necessarily give a correct indication of their relative effectiveness.

Similarly in comparing these antiseptics on the basis of the use dilution test, the above mentioned factors apply. The per cent of active ingredients also varies with each antiseptic, hence there is no common denominator for effective dilutions. However, in comparing the effective dilution against the dilutions recommended by the manufacturer, Liquid Germicidal Detergent and Weladol were effective at dilutions higher than those recommended, while Triocil lost its effectiveness when diluted.

The speed of disinfection test gives an indication of the rapidity of action of a compound and its penetrative powers. The factors which limit the value of comparisons mentioned previously apply to this test also. Triocil did not give a complete reduction when it was diluted. Weladol and Liquid Germicidal Detergent gave quite similar results in this test. Weladol gave a reduction of 99.996% at a dilution of 1:250 in 15 seconds while Liquid Germicidal Detergent gave 100% reduction at a dilution of 1:280 in the same time interval.

In the clinical testing there was a sharp distinction in the incidence of hemolytic colonies. Of the nine samples taken when Triocil was used, five contained hemolytic colonies, while of the ten Weladol samples, eight showed hemolysis. Of the twenty-two samples obtained when Liquid Germicidal Detergent was used, one sample contained hemolytic colonies.

VI. CONCLUSIONS

1. From the results of the testing performed the use of Triocil as a presurgical scrub is not indicated.
2. The testing of Weladol indicated that it was more effective *in vitro* than *in vivo*. Clinical testing indicated that more evidence is needed before it is accepted as a presurgical scrubbing agent.
3. Liquid Germicidal Detergent consistently gave the best results in the tests carried out in this work. On the basis of these tests Liquid Germicidal Detergent is recommended as the most satisfactory and reliable of the antiseptics tested.

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