one of these, the minimum and maximum ephedrine content of monthly composite samples during the period August to December was 0.96 and 1.30 per cent, respectively, while in the other it was 0.99 and 1.31 per cent. Composite samples from the large plots of the species from which these originally were selected contained only 0.571 and 0.672 per cent, respectively, during the same period.

Two hybrids are of special interest. The seedlings grown from these have in both cases shown a wide variation in ephedrine content, the minimum and maximum being 0.24 and 1.28 per cent in the seedlings of the one and 0.07 and 1.25 per cent in those of the other. One species from Morocco, not yet definitely named though presumed to be *E. alenda*, is unique in that it contains pseudo-ephedrine almost exclusively, only very small amounts of ephedrine having at times been found in it. This is a fast growing species that would probably produce much more tonnage of herb than any of the others and may be useful for crossing with others less vigorous but with a high ephedrine content.

The few results mentioned are examples of the kind of information that may reasonably be expected from an investigation of this sort. Data on ephedrine content under various conditions are most important, but probably no more so than information concerning the cultural requirements, yields, cost of production, etc., of these plants, all of which are factors that in the end will determine whether or not Ephedra will be grown in this country.

AN EXPERIMENTAL STUDY OF FACTORS INVOLVED IN THE USE OF SURFACE ANTISEPTICS.*

BY LEO T. SAMUELS.[1]

INTRODUCTION.

The evaluation of an antiseptic designed for surface application involves many factors which have been only imperfectly correlated.

The phenol coefficient alone has long been recognized as an imperfect gage to the value of such a preparation. The presence of body fluids greatly affects bactericidal potency. A correction for this has been introduced by adding such substances as blood serum to the dilution medium in the phenol coefficient test. In recent years the importance of the toxicity of the material to body cells in comparison with its toxicity to bacteria has been recognized. But the relative values of common antiseptics obtained by methods designed to measure such a relation do not, in many instances, coincide with clinical experience.

Irritant action is an important variable among the common surface antiseptics. This factor does not bear a simple relation to the toxicity toward body tissues; many substances are highly toxic which are not among the most irritant. It has been thought that the inflammatory process generated by a substance such as iodine may be a valuable factor in promoting healing and in certain chronic lesions this seems to be true. Since healing is desired in many uses of surface antiseptics the rôles of inflammatory reactions and other factors in this process need study.

---

* From the Laboratories of Surgident, Ltd., and the Department of Pharmacology, University of Southern California.

[1] Professor, University of Minnesota, Medical School, Minneapolis, Minn.

Duration of antiseptic potency is also a factor of practical importance which has usually been neglected in antiseptic studies. The prevention of reinfection is often as important as the primary disinfection.

The following series of experiments were carried out in an attempt to correlate some of these factors in relation to the healing rate, and to measure others not directly involved in this process.

Methods.—All of the antiseptics studied were used in the forms ordinarily applied in clinical practice, or in dilutions of such solutions. Comparisons have been made on this basis since it is the relative value of available preparations which is of practical significance. Where the active ingredient can be considered separately, this information has also been included. The following antiseptics have been studied in one or more connections:

Iodine, Tincture, U. S. P.
Iodine, Compound Solution, U. S. P.
Phenol, aqueous solution
Metaphen, 1:500 aqueous
Merthiolate, 1:1000 aqueous
Igol, glycerine solution
Igol, 50% alcohol solution.

Those solutions studied in all connections were Tr. Iodine, U. S. P.; phenol; metaphen; and Igol, glycerine solution.

Igol solutions are prepared by heating phenol, iodine, tannic acid and glycerine together at a temperature of 130° C. in a closed retort. The resulting material is a clear dark brown solution apparently containing some unstable iodine compound in equilibrium with free iodine. The alcoholic solution is made by dissolving the concentrated preparation in 50% alcohol. The glycerine solution on the market is originally prepared with an excess of glycerine present. The supplies of both forms were taken from stocks made available for clinical research by the manufacturing company.

The tincture and the compound solution of iodine were prepared according to the U. S. Pharmacopœia. The phenol used was recrystallized and the solutions freshly prepared according to the methods of the U. S. Food and Drugs Administration for use in phenol coefficient studies. Metaphen and merthiolate solutions were purchased from wholesale druggists. Both were from recently received shipments.

The following determinations were made:

1 Toxicity to bacteria
2 Toxicity to tissue cells
3 Irritant action
4 Maintenance of bactericidal action after application
5 Healing rate.

Toxicity to Bacteria.—The procedure used was that recommended by the U. S. Food and Drugs Administration (1) for the determination of the phenol coefficient. The results have been expressed in somewhat different form however to make them comparable with the tissue toxicity method. A number of experiments was carried out with each antiseptic. The maximum dilutions destroying the bacteria in 5, 10 and 15 minutes were determined. The mean of these three values was then taken as a measure of the toxicity of the antiseptic for bacteria.

Both *E. typhi* and *S. aureus* isolated from human material[1] were used in the studies but only the values on *E. typhi* were compared with the tissue values described in the following section.

Toxicity to Tissue Cells.—None of the methods for testing tissue cell toxicity utilize cells which are the exact equivalent of those acted upon in therapeutic use. Tissue cultures of mammalian cells are either of embryonic type or too difficult to grow to be used for routine testing. In

[1] The organisms were obtained from the Department of Bacteriology of the University of Southern California.

the published methods they are also exposed to the antiseptic in a different manner from the bacteria. In this study a modification of the frog pharyngeal epithelium method of Bädertscher (2) was adopted. While the tissue is not mammalian, it is adult epithelial tissue; and cessation of a normal function, the movement of the cilia, can be used as a gage of the toxic action of an antiseptic. Since the ciliated cells form a surface layer, they will all be immediately reached by solutions of the drug in which the piece of tissue is suspended. This enables one to use the same type of solution for bacteria and tissues, and to use similar short periods of exposure.

The mucous membrane of the pharynx and esophagus of a five-inch bullfrog was removed and stretched into a rectangular strip. The area of active ciliary motion was then determined by sprinkling charcoal over the surface. The active area was split into strips 3 mm. by 15 mm. Each strip was then threaded with white cotton thread at one end and suspended in frog Ringer's solution. A sufficient number of strips were thus obtained to enable those from one frog to be distributed through a full series of dilutions. The antiseptics were then diluted with frog Ringer's solution to give 100 cc. of the same dilutions as used in the preliminary phenol coefficient tests. The large volumes precluded the possibility of metabolic products of tissue strips affecting the result. Into each dilution a number of tissue strips from different animals were dropped, and the activity of the cilia determined 5, 10 and 15 minutes after immersion. The ability of the cilia to move particles of charcoal was determined after the layer of mucus which accumulated had been wiped away by passing the round shaft of a dissecting needle over the surface of the tissue.

For each time period the percentages of non-motile strips were plotted against the dilutions of an antiseptic and the dilution inhibiting ciliary motion in 50% calculated from the graph. The mean of the values for 5, 10 and 15 minutes for each antiseptic was compared with the similar mean for toxicity to bacteria. This ratio, called an "Antiseptic Coefficient," increased in magnitude with a relative increase in greater toxicity to bacteria as compared to tissue cells. By comparing the "Antiseptic Toxicity Coefficient" of each antiseptic with that determined simultaneously for phenol a more duplicable value could be obtained which was called the "phenol coefficient of relative toxicity."

Irritant Action.—The irritant action of the various antiseptics was determined by estimation of the histological changes produced in two tissues: the uterus and the skin of the rabbit. In both cases parallel determinations with more than one antiseptic were made on the same animal.

For determination of the irritant action on the uterus, virgin female rabbits six months old were anesthetized and the uterus exposed under aseptic conditions. The two horns of the uterus were each divided into two equal segments, the uterine wall being severed but the large blood vessels being left intact. After this 0.2 cc. of the commonly used dilution of an antiseptic was introduced into the lumen of a uterine segment by means of a blunt hypodermic needle and a tight suture placed about both ends. This amount of antiseptic was not sufficient to cause abnormal distention of the segments. Three different antiseptics were introduced into three of the four segments in each animal; the fourth was treated with saline as a control. The uterus was then returned to the normal position and the abdomen closed. After intervals of two, three, four, five and six days the treated sections were removed, fixed in 4% formaldehyde and sections examined from the mid-region of each segment. The sections were then ranked on the basis of leucocytic infiltration and necrosis.

Skin irritation was determined in much the same manner. Large areas of skin were shaved on both sides of a rabbit. The animal was then left alone for a period of two days to let irritation due to shaving subside. Two equal areas were then marked off with india ink on each side of the animal, and to each of three of the four areas an antiseptic was applied daily for six days by means of a cotton swab. On the eighth day pieces of skin were snipped from the center of each area, the untreated fourth area serving as a control. The pieces of skin were fixed in Bouin's solution, and paraffin sections prepared for histological examination. The basis of evaluation was the same as for the uterus.

Length of Antiseptic Action on Skin Surface.—Rabbits were used for this study. The back of a large rabbit was shaved 24 hours before the experiment was to begin. On the day of the experiment the shaved area was scrubbed with alcohol and ether and rubbed dry with a sterile towel. Using sterile india ink and a special instrument a row of 6 to 8 squares of 1 sq. cm. area was laid

out on each side of the backbone, each square being about 2 cm. from its neighbors. The whole region was then covered with a sterile towel.

One hour later, a strip 3 cm. wide was painted with antiseptic over each row of squares including all except the most anterior square. Each square was then covered with a specially designed sterile gauze cap. The time of painting on the antiseptic was taken as the starting time of the experiment, and at definite intervals thereafter as shown in Tables III, IV and V one of the squares would be inoculated by rubbing a standard loopful of a pure culture of the organism to be studied over the surface. Five minutes later the block of skin was removed with sterile instruments, the tissue being excised to the subcutaneous areolar layer. It was then dropped into 10 cc. of sterile broth containing glass beads and vigorously shaken for three minutes. From the resulting suspension 1 cc. was pipetted into a petri dish and bacterial counts made in the usual manner. The balance of the solution together with the tissue fragment was dumped into a flask containing 100 cc. of sterile broth and incubated to determine absolute sterility.

By a series of such experiments one could determine the average length of time after application that a given antiseptic would destroy specific organisms.

Healing Rate.—The important composite result of the effects of an antiseptic on the tissues is the rate at which healing occurs in its presence. The animals from which the squares of skin had been removed in the previous experiments furnished excellent subjects for such a study. The areas were painted with antiseptic every other day until complete healing had occurred. Three antiseptics were used on each animal, three to four squares being treated with each antiseptic and the remainder serving as controls.

The healing time was taken to be the interval required for epithelium to completely cover the wound.

RESULTS.

Toxicity to Bacteria.—The values for the average of the minimum lethal concentrations of antiseptic after 5, 10 and 15 minutes exposure using *E. typhi* are given in the first two columns of Table I.

TABLE I.—RELATIVE BACTERICIDAL AND TISSUE TOXICITY OF ANTISEPTICS.

Antiseptic.	I. *E. Typhi*[1] Commercial Product. Gm./Cc.	II. *E. Typhi*[2] Pure Substance. Gm./Cc.	III. Cilia[3] Commercial Product. Gm./Cc.	IV. Toxicity[4] Coefficient. Column 1 / Column 3	V. Phenol Coefficient[5] Relative Toxicity.
Phenol	0.0116	0.0116	0.0073	0.6	1.0
Merthiolate (1:1000)	0.31	0.00031	....	...	...
Metaphen (1:500)	0.10	0.00021	0.053	0.5	0.8
Iodine, U. S. P. Tr.	0.0011	0.00008	0.0028	2.4	3.8
Iodine, U. S. P., compound sol.	0.0013	0.000065	....	...	...
Igol, glycerine sol.	0.016	.....	0.015	0.9	1.5
Igol, alcoholic sol.	0.0019	.....	0.0036	1.9	3.0

[1] *In vitro* average concentration of commercial products fatal to *E. typhi*, as calculated from 5-, 10- and 15-minute tests.

[2] *In vitro* average concentration of pure substances fatal to *E. typhi*, as calculated from 5-, 10- and 15-minute tests.

[3] *In vitro* average concentration of commercial products inhibiting cilia of 50 per cent of strips of frog pharyngeal mucous membrane as calculated from 5-, 10- and 15-minute tests.

[4] Coefficient of Relative Toxicity of the commercial products expressed as the ratio of Column 1 to Column 3 of the table.

[5] Phenol Coefficient of Relative Toxicity of the commercial products. (See text for explanation.)

Since the measurements of bactericidal power were made on the solutions as marketed, the values were low and not sharply defined for those substances which are used only in very dilute form, such as metaphen and merthiolate.

From the standpoint of the amount of dilution which can occur with the commercial products and still maintain bactericidal potency the first column of Table I is more important. This

gives the lethal values in terms of the concentration of commercial product. From the standpoint of the absolute activity the second column is significant since the values are here expressed in terms of the active constituent. Since in the case of Igol the activity is dependent on the free iodine present, which in turn is in equilibrium with an unstable iodine compound of unknown constitution no value can be given for this antiseptic in the second column.

The third column shows the average dilutions fatal to 50% of the segments of frog epithelium under comparable circumstances.

Coefficients of Relative Toxicity.—The fourth column gives the ratio of tissue toxicity to bacterial toxicity based on Column 3. This ratio would of course be the same if based on the active constituents. The phenol coefficients of relative toxicity are given in Column 5. It will be noticed that the organic mercurials show the highest toxicity to frog epithelial cells as compared to bactericidal action. If the toxic dilutions were expressed in terms of the crystalline agent, as is usual in the case of the bactericidal phenol coefficients mentioned in the advertising of these products, the concentrations would be extremely small: for metaphen 1:9500. Phenol stands intermediate between the mercurials and the iodine antiseptics, of which U. S. P. tincture of iodine is superior to the others. In so far as similar products have been compared, the relative order is the same as that found by Salle and Lazarus (3) in their experiments comparing the action of antiseptics on chick heart cultures with that on bacteria. The absolute values naturally differ from theirs, due to differences in technique.

Maintenance of Bactericidal Action After Application to the Skin.—In the experiments three bacterial species were used: a strain of *Staphylococcus aureus* isolated from a human abcess, a hemolytic streptococcus also from a human source and a culture of *B. subtilis* so treated that most of the organisms were in the spore stage. It was felt that this would cover those organisms which would show the widest variations in susceptibility, and that all of the important pathogens would fall within this range, most of them resembling the staphylococci and streptococci.

In Table II the results are given for *Staphylococcus aureus*. While the organic mercurials resembled each other in the *in vitro* tests they showed a wide variation in their ability to maintain

TABLE II.—MAINTENANCE OF BACTERICIDAL POTENCY ON SKIN.[1]

	S. aureus.																	
	Metaphen.			Merthiolate.			Tr. Iodine.				Igol, Glycerol sol.				Igol, Alc. Sol.			
	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	IV. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	IV. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	IV. Pos. >10 Col.
							Not Faded.			Faded.	Not Faded.			Faded.	Not Faded.			Faded.
0	..	..	..	1	1	0	..	..	..	..	..	..	..	..	..	..	..	..
1 Min.	..	..	..	0	0	3	..	..	..	..	..	..	..	..	..	..	..	..
5 "	..	..	..	0	0	5	..	..	..	..	..	..	..	..	..	..	..	..
10 "	..	..	..	0	1	4	..	..	..	..	..	..	..	..	..	..	..	..
15 "	3	1	1	0	0	3	2	3	0	..	2	1	0	..	5	0	0	..
30 "	5	1	0	0	0	2	4	2	0	..	4	0	0	1	4	0	0	..
45 "	3	0	0	0	0	3	4	0	0	..	3	0	0	..	4	0	1	..
60 "	5	1	0	0	0	3	4	2	0	1	4	1	0	2	5	0	0	..
90 "	5	0	0	..	..	..	5	1	0	..	2	2	0	3	4	1	0	..
120 "	7	0	0	..	..	..	8	1	0	..	4	2	0	3	5	1	0	1
150 "	5	0	1	..	..	..	6	1	0	..	3	3	1	1	3	1	0	..
180 "	4	1	0	0	0	1	6	1	0	..	6	1	0	1	3	0	0	1
210 "	1	1	1	..	..	..	7	0	0	..	2	0	2	3	1	1	2	..
240 "	2	1	0	..	..	..	4	1	0	..	0	0	1	3	1	0	1	..
270 "	1	0	1	..	..	..	2	1	0	..	0	0	1	3	1	0	1	..
300 "	..	..	..	..	..	..	..	..	..	..	0	0	1	1	..	..	..	..
435 "	..	..	..	..	..	..	0	0	1	..	..	..	..	..	..	..	..	..

[1] Sections classed as faded showed no obvious yellow color on gross inspection.

Column I. No bacterial growth on plating.
Column II. Less than 10 colonies on plating.
Columns III and IV. More than 10 colonies on plating.

their antiseptic activity after application to the skin. Both metaphen and merthiolate would destroy the organisms when the latter were present at the time of painting with the antiseptic but the merthiolate rapidly lost its bactericidal power after application. Numbers of bacteria would survive if applied to the skin as early as one minute after the antiseptic and all survived if the merthiolate had been on the skin for ten minutes. On the other hand the aqueous solution of metaphen was still active on these organisms three and one half hours after its application.

The iodine antiseptics depended for their activity on the presence of free iodine. Whenever the iodine color had disappeared the bactericidal action was gone; as long as it remained some bactericidal action was present. The U. S. P. tincture showed the best maintenance of bactericidal action. High bactericidal potency remained four and one half hours after application, and in one area tested seven and one half hours after painting with iodine most of the applied bacteria were destroyed. The two preparations of Igol were somewhat less potent, the 50% alcohol solution maintaining good activity for four and one half hours and high activity for three hours. The Igol prepared in glycerine solution faded much more rapidly than either of the other two iodine preparations. In one case the iodine color faded within thirty minutes. A majority of the areas retained the brown color of the iodine for as long as three hours, and where this occurred maintained their bactericidal potency likewise. Beyond three hours the bactericidal potency seemed lost, even though a brown iodine color remained.

In Table III the results with *Streptococcus pyogenes* are summarized. The most marked change is that seen with aqueous metaphen. Whereas it was effective against *S. aureus* over a long period it very rapidly lost its potency toward *Strep. pyogenes*. This was a very surprising result, as the mercurials seem to be particularly toxic to streptococci *in vitro*. Whereas a marked difference existed between metaphen and tincture of merthiolate in the previous comparison, neither of them was able to destroy the strain of *Strep. pyogenes* which we used for any long period after contact with the skin.

TABLE III.—MAINTENANCE OF BACTERICIDAL POTENCY ON SKIN.[1]

	Strep. pyogenes.																	
	Metaphen.			Merthiolate.			Tr. Iodine.				Igol, Glycerol Sol.				Igol, Alc. Sol.			
	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	IV. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	IV. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	IV. Pos. >10 Col.
							Not Faded.			Faded.	Not Faded.			Faded.	Not Faded.			Faded.
0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
15 Min.	1	0	2	0	0	2	5	0	0	..	5	0	0	..	5	0	0	..
30 "	1	0	2	0	0	2	5	0	0	..	4	0	0	1	3	1	0	..
45 "	0	0	3	0	0	2	4	1	0	..	2	0	0	3	5	0	0	..
60 "	0	0	3	0	0	2	4	1	0	..	1	0	1	3	5	0	0	..
90 "	0	0	3	0	0	2	4	1	0	..	2	0	0	3	5	0	0	..
120 "	0	0	3	0	0	2	5	0	0	..	3	0	0	2	4	1	0	..
150 "	1	1	2	..	..	..	3	0	0	..	3	0	0	2	4	1	0	..
180 "	2	1	1	..	..	..	3	0	0	..	3	1	0	1	3	0	0	1
210 "	0	2	2	..	..	..	3	0	0	..	2	0	0	3	2	1	1	1
240 "	0	1	3	..	..	..	2	1	0	..	2	0	0	3	5	0	0	..
270 "	0	0	4	..	..	..	0	2	0	1	0	0	1	4	5	0	0	..
300 "	0	0	3	..	..	..	3	0	0	..	0	0	2	3	4	0	0	..

[1] Sections classed as faded showed no obvious yellow color on gross inspection.

Column I. No bacterial growth on plating.
Column II. Less than 10 colonies on plating.
Columns III and IV. More than 10 colonies on plating.

With the iodine antiseptics the picture was very similar to the previous finding. Both tincture of iodine and the alcoholic solution of Igol maintained bactericidal potency for 5 hours except in the few instances where fading occurred. The glycerine preparation of Igol maintained its potency for 4 hours in those cases where the free iodine did not disappear. Fading, however, frequently occurred after short intervals.

The last organism to be compared in this respect was *Bacillus subtilis*. Table IV presents the experimental results. While the iodine antiseptics were superior to the mercurials in the other tests, it seems that they too soon lost their ability to destroy these strongly resistant forms. When applied to the spores which had been previously spread upon the skin, the antiseptics were effective, but although plenty of free iodine still remained on the skin fifteen minutes after painting, the spores applied at this time were not destroyed by five minutes exposure.

TABLE IV.—MAINTENANCE OF BACTERICIDAL POTENCY ON SKIN.[1]

	B. Subtilis.																	
	Metaphen.			Merthiolate.			Tr. Iodine.				Igol, Glycerol Sol.				Igol, Alc. Sol.			
	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	IV. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	IV. Pos. >10 Col.	I. Neg.	II. Pos. <10 Col.	III. Pos. >10 Col.	IV. Pos. >10 Col.
							Not Faded.			Faded.	Not Faded.			Faded.	Not Faded.			Faded.
0	1	0	0	..	..	..	1	0	0	..	1	0	0	..	1	0	0	..
5 Min.	1	0	0	0	0	1	..	..	..	..	0	0	1	..	0	0	1	..
15 "	1	1	0	0	0	1	0	0	3	..	0	0	2	1	0	0	3	..
30 "	1	1	0	0	0	1	0	0	2	1	0	0	..	3	0	0	1	2
45 "	1	0	1	0	0	1	0	0	2	..	0	0	..	2	0	0	..	2
60 "	1	0	1	0	0	1	0	0	3	..	0	0	1	2	0	0	..	3
90 "	1	1	0	0	0	1	0	0	3	..	0	0	1	2	0	0	..	3
120 "	0	0	1	0	0	1	0	0	1	..	0	0	1	1	0	0	1	..
150 "	0	1	0	..	..	..	0	0	1	..	0	0	1	..	0	0	1	..
180 "	0	0	1	..	..	..	1	0	0	..	0	0	1	..	0	0	1	..
210 "	0	0	1	..	..	..	0	0	1	1	0	0	..	1	1	0	0	..
240 "	..	..	..	..	..	..	..	..	..	..	0	0	1	..	..	..	..	..
270 "	0	1	0	..	..	..	0	0	..	1	0	0	..	1	0	0	1	..
300 "	0	0	1	..	..	..	0	0	..	1	0	0	1	..	0	0	1	..

[1] Sections classed as faded showed no obvious yellow color on gross inspection.

Column I. No bacterial growth on plating.
Column II. Less than 10 colonies on plating.
Columns III and IV. More than 10 colonies on plating.

Summarizing, metaphen on the skin proved very long acting against *S. aureus* but only immediately effective against *Strep. pyogenes* and the spores of *B. subtilis*. Merthiolate had only an immediate action against any of the organisms. All of the iodine antiseptics lost activity if the brown color faded. U. S. P. Tr. Iodine maintained its action for long periods against the nonsporulating organisms but was only immediately effective against *B. subtilis* in spore culture. Alcoholic Igol was only slightly less effective than U. S. P. tincture, while the glycerine solution was similar in its relative action but less long acting and more apt to fade.

Irritation.—Table V gives the rankings of the various antiseptics based on histological evidence of irritation. While the organic mercurials were inferior to the iodine antiseptics in the *in vitro* tissue toxicity studies, they produced little irritation. Igol in glycerine was somewhat more irritant than the mercurials but much less so than either tincture of iodine or Lugol's solution, both of which caused severe necrosis in the uterus. The ranking was the same both on the skin and uterus, although the greater sensitiveness of the latter tissue made the differences wider.

TABLE V.—INFLAMMATORY REACTION TO ANTISEPTICS.

	None.	Slight.	Moderate.	Severe.
	Skin.			
Metaphen	5	1	..	..
Merthiolate	4	1	1	..
Tr. Iodine	..	..	2	3
Igol, glyc. sol.	3	2	2	..
Igol, alcohol	..	..	1	3
Control	12	2	1	..

	Uterus.			
Tr. Iodine	..	..	2	14
Igol, glyc. sol.	3	5	4	3
Igol, alcohol	..	..	..	..
Control	12	4	2	..

Healing Rates.—The average length of time required for the complete healing of standard 1 sq. cm. cutaneous wounds is shown in Table VI. First inspection showed that the differences were small, but when treated statistically they were found to be significant. The various antiseptics fell into two distinct classifications: those which had practically the same healing time as the untreated controls (14.8 days), and those which showed a definite beneficial influence, the healing time being from 12.8–13.6 days. In the first group were tincture of iodine and alcoholic solution of Igol. In the second group were merthiolate, metaphen and the glycerine solution of Igol. These appeared to be beneficial when applied every other day to healing wounds.

TABLE VI.—TIME REQUIRED FOR COMPLETE HEALING OF CUTANEOUS WOUNDS OF STANDARD SIZE WHEN PAINTED ON ALTERNATE DAYS WITH ANTISEPTIC.

Antiseptic.	Phenol Coefficients of Relative Toxicity.	No. of Wounds.	Time Required for Healing in Days.	Significance of Differences from Controls. Diff. of Means, Days.	P. E.[1] Diff.	Diff. / P. E. Diff.
Tr. Iodine	3.8	50	14.7 ± 0.47	0.1	0.57	0.18
Metaphen	0.8	52	13.6 ± 0.19	1.2	0.37	3.24
Igol, alcohol	3.0	70	15.1 ± 0.23	0.3	0.40	0.75
Igol, glycerine	1.5	86	13.0 ± 0.20	1.8	0.39	4.61
Tr. Metaphen	...	31	14.6 ± 0.24	0.2	0.41	0.49
Controls	...	29	14.8 ± 0.32	...	...	...
Merthiolate	...	23	13.0 ± 0.21	1.8	0.38	4.74

[1] Probable error of the difference of the means.

The healing rates do not seem to have a high direct correlation with the "Antiseptic Coefficients" determined *in vitro*. Since the findings of Salle and Lazarus (3) on tissue cultures of mammalian cells parallel our *in vitro* findings, the discrepency cannot be due to a particular sensitivity of the frog epithelium; the organic mercurials seem to be generally toxic to tissue cells in greater dilution than the iodine antiseptics. The explanation may lie in the different ways by which the two types of compounds cause the death of the cell. The organic mercurials cause the death of the cells by precipitation or by combination with cellular elements in such a way as to stop living processes but they do not actively bring about disintegration in the cell walls. This is shown by the negligible amount of irritant or necrotic reaction which they produce, even when present in much higher concentrations than are required for cessation of ciliary motion. Iodine acts by a different means. It is primarily dependent for its tissue action on the oxidative power of its molecule. Thus it is not toxic in such high dilutions as the mercurials but when present in excess as in the irritation experiments with the tincture and Lugol's solution it causes severe inflammatory reactions and disintegration of tissue. To evaluate the influence of an antiseptic on healing processes we must not only take into consideration the relative dilutions at which bacteria and tissue cells are effected but the manner by which they are affected in the presence of the excess of the material which is ordinarily used.

This is well illustrated in the case of the glycerine solution of Igol. It, like the other iodine products was non-toxic to tissue cells in greater concentrations relative to bactericidal action than the organic mercurials. There was sufficient iodine to be slightly irritant when present as the concentrated solution, but since most of the iodine was in the form of an unstable compound in equilibrium with a low amount of free iodine, caustic action characteristic of the other iodine preparations was prevented. Thus being slightly more irritant in concentrated form but considerably less toxic to tissue cells when diluted than the organic mercurials, the advantage in one direction offset the disadvantage in the other, and this antiseptic showed a beneficial influence on the rate of healing equal to that of metaphen and merthiolate.

The correspondence between the length of time the antiseptics will maintain a sterile field and the "Antiseptic Coefficient" may or may not be fortuitous. The shorter action of the organic mercurials may be due to more rapid combination with tissue cells constituents. On the other hand this does not explain the long maintained action of metaphen against staphylococci. It may be that some substance formed when metaphen comes in contact with skin constituents remains toxic to staphylococci but non-toxic to streptococci. Certainly there seems to be something much more selective in the action of the organic mercurial antiseptics than with the iodine products.

CONCLUSIONS.

It seems that no one antiseptic is outstanding in all respects. Where maintenance of a sterile area after one application of an antiseptic is the most important factor, as in preoperative preparation of surgical fields, tincture of iodine is still the best of the group studied here. Where irritation is to be avoided, and either immediate sterilization is all that is desired, or a moist pack or repeated application can be used, the organic mercurials seem preferable. As a general antiseptic which will preserve a sterile field for some time, which produces some irritation but does not delay healing, the glycerine solution of Igol appears most valuable. It seems, however, that it will still be necessary for a physician to use intelligence and not assume that any one product known as an antiseptic is all he need prescribe in every case.

ACKNOWLEDGMENT.

The author wishes to acknowledge the constant guidance of Professor C. H. Thienes of the Department of Pharmacology, University of Southern California, during the progress of the experiments.

BIBLIOGRAPHY.

(1) Bull. U. S. Food and Drug Administration No. 198.
(2) Bädertscher, V. A., *Proc. Soc. Exptl. Biol. Med.*, 30, 441 (1933).
(3) Salle, A. J., and Lazarus, A. S., *Ibid.*, 34, 371 (1936).

RED CROSS.

"We grow, as we share." By this is meant not only as "we share" through our actual help to unfortunate neighbors, but also as "we share" through giving our memberships to keep the Red Cross prepared and fully armed to give this help. We must recognize the necessity for maintaining the Red Cross in strong financial position to meet any great disaster relief calls. We do not know now and cannot forsee what tremendous tasks may await us in this forthcoming year. We do know that the world has not faced such an unsettled future in two decades.

The Fiftieth International Congress for the Unity of Science will be held at Harvard University, September 5th to 10th, 1939. The theme of the congress is "The Logic of Science;" attention will be given to general problems connected with the unification of science, and there will be a number of special sessions and symposia. The congress is sponsored by the International Committee of the Congresses for the Unity of Science, by the International Institute for the Unity of Science, and in America, by the American Association for the Advancement of Science, the Philosophy of Science Association, the Association for Symbolic Logic and the American Philosophical Association. A series of twenty monographs, entitled "Foundations of the Unity of Science" is now being issued by the University of Chicago Press.