samples to the same end-point, other factors must help to explain the deviations in menthol percentages.

From the data listed in Table II, it would seem that the time of acetylation may vary within broad limits, while the time of saponification had best be varied between 45 minutes and 60 minutes to give results which do not deviate abnormally from the mean. It has been observed in earlier work (1) that at 50° C., acetylated peppermint oil is 80% hydrolyzed in 50 minutes and 84% hydrolyzed in 60 minutes. At steam-bath temperatures it is reasonable to assume that the reaction would go almost to completion in the same length of time. It will be observed that in those cases where the oil was saponified for periods shorter than 45 minutes the deviation was usually negative, indicating incomplete saponification, and that in the case of those samples heated for more than 60 minutes the deviation was positive, indicating, it is believed, that side reactions involving potassium hydroxide take place, thereby showing in the final calculation an erroneous per cent of menthol. While experimental evidence is not yet available to prove the contention, it is believed that resinification or polymerization of certain constituents, evidenced by a darkening of the reaction mixture, is induced by prolonged heating with potassium hydroxide, and that in the reaction some base is used up, thereby leading to erroneous results.

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A COMPARATIVE STUDY OF THE ANTISEPTIC PROPERTIES OF OFFICIAL PREPARATIONS. A MODIFICATION OF THE REDDISH CUP METHOD FOR VOLATILE SUBSTANCES.*

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The purpose of this investigation is to study the inhibitory properties of several official preparations using Reddish's original method (1).

EXPERIMENTAL.

Staphylococcus aureus was grown at 37° C. in broth containing 1% Armour's Peptone, 0.5% Leibig's Beef Extract and 0.5% sodium chloride, the p_H being adjusted to 6.8. 0.1 ml. of a 24 hour old culture was thoroughly mixed with 15 to 20 ml. of nutrient agar (composed of 1% Armour's Peptone, 0.5% Leibig's Beef Extract, 0.5% sodium chloride and 1.5% bacto-agar, the p_H adjusted to 7.2–7.4) at 45° C., the mixture poured into a sterile petri dish, and allowed to harden. A disk was cut out in the agar by means of a sterile cork borer, 1.5 cm. in diameter. The disk was removed with sterile forceps and any cracks or crevices sealed with one or two drops of melted agar. After the agar cup was prepared six drops of the liquid antiseptic to be tested were placed in the cup and the plate incubated under an unglazed porcelain top for 24 to 48 hours at 37° C. Ointments, pastes and salves were melted. Enough of the melt was used to obtain a complete peripheral contact.

If the preparation is antiseptic or inhibitory a circular zone of clear agar surrounds the cup; the unaffected organisms continue to grow outside this zone. The distance from the edge of the cup to the edge of the zone is read in mm. This distance is a measure of the inhibitory (or penetra-

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tion) value of the antiseptic. A reading of zero indicates no penetration or no antiseptic value; 35 mm. or more signifies that the entire plate is clear of any organisms. This is the optimal penetration value. If the antiseptic to be tested is recommended for cuts or open wounds 1 mm. of 10% beef blood serum is added to the agar.

The following groups of compounds were tested (1) acids and bases, (2) inorganic compounds, (3) organic compounds, (4) mercury, silver and iodine compounds, (5) essential oils, (6) miscellaneous.

TABLE I.

Acids and Bases.	Penetration or Clear Area in Mm.
Acetic acid, 5%	20
Acetic acid, glacial, U. S. P. XI	35
Aqua regia, N. F. VI	35
Aqua regia, dilute, N. F. VI	25
Ammonium hydroxide, 25%	35
Ammonium hydroxide, 10%	25
Boric acid, 1%	0
Boric acid, 1/2 sat.	3
Boric acid, sat. 5%	6
Hydrochloric acid, conc.	35
Hydrochloric acid, 10%	24
Hydrochloric acid, 2%	11
Lactic acid, 85%	28
Lactic acid, 10%	14
Nitric acid, conc.	35
Nitric acid, 10%	21
Phosphoric acid, 85%	35
Potassium hydroxide, 10%	11
Sodium hydroxide, 10%	11
Sulfuric acid, 10%	29
Inorganic Preparations.	
Solution of aluminum chloride, N. F. VI	14
Solution of aluminum acetate, N. F. VI	3
Solution of aluminum subacetate, N. F. VI	11
Calamine ointment, N. F. VI	0
Solution of ferric chloride, U. S. P. XI	12
Solution of ferric subsulfate, N. F. VI	17
Hydrogen peroxide, 1%	7
Hydrogen peroxide, U. S. P. XI	11
Potassium chlorate, 6%	0
Potassium dichromate, 1%	12
Potassium dichromate, 5%	15
Potassium nitrate, 12%	1
Potassium permanganate, 1:2000	5
Sodium carbonate, 10%	0
Sodium chloride, 10%	0
Sodium chloride, 25%	0
Sodium perborate, 2%	5
Zinc sulfate, 1%	3
Organic Preparations.	
Phenol, 5%	5
Formaldehyde, 5%	35
Ethyl alcohol, 70%	35
Basic fuchsin, 3% in 10% alcohol	5
Safranine, 3% in 10% alcohol	12
Acriflavine, 3% in 10% alcohol	8

Thymol, 1% in 50% alcohol	35
Picric acid, 5% in 95% alcohol	5
Picric acid, 5% in 50% alcohol	6
Salicylic acid, sat. aq. sol.	0
Tannic acid, 5%	3
Trichloracetic acid, 5%	8
Trichloracetic acid, 10%	12
Essential Oils, Fixed Oils and Oleoresins.	
Oil of Anise	5
Oil of Bitter Almonds	35
Oil of Caraway	10
Cod Liver Oil	6
Castor Oil	0
Oil of Chaulmoogra	0
Oil of Cinnamon	15
Oil of Coriander	7
Croton Oil	1
Oil of Clove	6
Eucalyptol	15
Oil of Eucalyptus	7
Oil of Fennel	7
Oil of Juniper	7
Oil of Lavender	7
Oil of Lemon	7
Oil of Myristica	6
Rectified Oil of Tar	6
Oil of Peppermint	6
Oil of Pimenta	10
Olive Oil	0
Oil of Rosemary	10
Oil of Sassafras	2
Oil of Santal	6
Oil of Spearmint	11
Oil of Turpentine	11
Oil of Thyme	0
Oil of Wintergreen	0
Oleoresin of Capsicum	0
Oleoresin of Cubeb	1
Oleoresin of Ginger	0
Oleoresin of Malefern	7
Essential Oil of Mustard	35
Miscellaneous Preparations.	
Tincture of Iodine, U. S. P. XI	35
Mild Tincture of Iodine, U. S. P. XI	23
Stronger Tincture of Iodine, N. F. VI	35
Iodoform	5
Silver nitrate, 1%	8
Mild protein silver, 10%	7
Strong protein silver, 5%	5
Harrington's solution	35
Mercuric chloride, 1–5000	9
Black wash, N. F. VI	7
Yellow wash, N. F. VI	7
Ammoniated mercury ointment, U. S. P. XI	8
Mild mercurial ointment, U. S. P. XI	5

Compound Ointment of Benzoic Acid, N. F. VI	6
Ointment of Ichthammol, N. F. VI	1
Antiseptic solution, N. F. VI	4
Aromatic sodium perborate, N. F. VI	15
Boric acid, powder	7
Compound Tincture of Green Soap, N. F. VI	7
Compound Powder of Zinc Sulfate	12
Dakin's Solutions, U. S. P. XI	5
Dental Liniment, N. F. VI	11
Dobell's solution, N. F. VI	3
Solution of methyl-rosaniline	9

EFFECT OF A SPORE PRODUCING ORGANISM ON THE PENETRATION VALUE.

A further study was made to test the penetration value of a group of compounds using *Bacillus subtilis*, a spore producing organism. The procedure and media used with *B. subtilis* were identical to those described for *S. aureus*. In general, if a preparation showed any penetration with *S. aureus* as the test organism, the penetration increased when *B. subtilis* was substituted.

TABLE II.

Compound.	Penetration in Mm. *S. Aureus.*	*B. Subtilis.*
Antiseptic soln., N. F. IV	4	12
Alkaline aromatic soln., N. F. IV	0	5
Alkaline aromatic soln., N. F. VI	0	3
Calamine ointment, N. F. VI	2	20
Comp. Tinct. of Green Soap, N. F. VI	7	10
Burow's soln., N. F. VI	6	13
Solution of ferric chloride, U. S. P. XI	12	16
Solution of ferric subsulfate, N. F. VI	17	20
Tannic acid, 5%	3	4
Yellow wash, N. F. VI	7	14
Black wash, N. F. VI	7	12
Hydrochloric acid, 2%	11	15
Comp. Coal Tar Ointment, N. F. VI	0	0
Comp. ointment of Benzoic Acid, N. F. VI	6	10

EFFECT OF SEROUS MATERIAL ON THE PENETRATION VALUE OF ANTISEPTICS.

Preparations recommended for use on open wounds or cuts will be effective only if they exhibit activity in the presence of serous fluids. The penetration value of an antiseptic may increase or decrease in the presence of blood serum. A group of skin antiseptics was tested with and without beef blood serum using *S. aureus* as the test organism. Noteworthy is 7% Tincture of Iodine, which shows no decrease in penetration value in the presence of blood serum.

TABLE III.

Compound.	No Serum Added.	Serum Added.	Observation.
Tinct. of Iodine, U. S. P. XI	35	35	No change
Tinct. of Iodine, mild, U. S. P. XI	21	23	Increase
Picric acid, 5% in 50% alcohol	6	6	No change
Harrington solution	35	30	Decrease
Burow's soln., N. F. VI	6	4	Decrease
Thymol, 1% in 50% alcohol	35	11	Decrease
Solution of ferric chloride, U. S. P. XI	12	7	Decrease

EFFECT OF VOLATILE CONSTITUENTS OF ANTISEPTICS ON THEIR PENETRATION VALUES.

Many antiseptics have constituents that are highly volatile. Ammonia, formaldehyde alcohol, iodine and the essential oils are a few that may be mentioned. These substances may

volatilize easily when placed in the incubator for a period of 24 hours. The volatile gas may pass through the unglazed porcelain top of the petri dish or may be absorbed into the surface of the agar. Thus the penetration value of the antiseptic will be interpreted erroneously. To determine whether a volatile antiseptic acts (1) as a vapor (by surface adsorption on the agar), or (2) by penetration only through the agar or (3) a combination of both, three agar plates were prepared as usual. A cup (1.2 cm. in diameter) was cut from the first plate, the agar removed, and into this hole was placed a thin glass thimble (1.1 cm. in diameter and 0.5 cm. high). Six drops of antiseptic were placed in the glass thimble and the covered petri dish incubated. A cup (1.2 cm. in diameter) was cut from the second plate. Six drops of antiseptic were placed in the cup and the cup sealed with a glass cover slip (2.3 cm. square). This petri dish was covered and incubated. The third plate was prepared in the usual manner, six drops of antiseptic were used and the plate incubated.

TABLE IV.

a—Antiseptic placed in cup.
b—Antiseptic placed in glass thimble.
c—Antiseptic placed in cup and covered with glass slide.

Compound.	Method.	Penetration in Mm.
Formaldehyde 5%	a	35
Formaldehyde 5%	b	35
Formaldehyde 5%	c	35
7% Tincture of Iodine	a	35
7% Tincture of Iodine	b	35
7% Tincture of Iodine	c	20
70% Ethyl alcohol	a	35
70% Ethyl alcohol	b	35
70% Ethyl alcohol	c	35
Essential Oil of Mustard	a	35
Essential Oil of Mustard	b	35
Essential Oil of Mustard	c	35
Oil of Bitter Almonds	a	35
Oil of Bitter Almonds	b	35
Oil of Bitter Almonds	c	0

From this table it will be noted that (*a*) formaldehyde, 70% alcohol, and Essential Oil of Mustard act as vapors; as penetrants; or by a combination of both, with equal efficiency; (*b*) Tincture of Iodine acts best as a vapor and least by penetration (*c*) Oil of Bitter Almonds acts only as a vapor (by surface adsorption) and exhibits no penetration.

SUMMARY.

1. The inorganic acids and bases, in general, exhibit high penetration. The organic acids; picric, salicylic, benzoic, tannic and trichloracetic show slight penetration values when compared with the common inorganic acids and bases.

2. The heavy metal salts of aluminum and iron show medium penetration values. The sodium and potassium salts show very small penetration values. Potassium dichromate is an exception.

3. The organic preparations have variable penetration values. Five per cent phenol shows a penetration value of 5 mm. Of the dyes studied, acriflavine, methylrosaniline (gentian violet) and safranine show small penetration values. The ointments in general, show very slight penetration values. Two exceptions are Ointment of Ichythol and Compound Ointment of Benzoic Acid. Seventy per cent ethyl alcohol, formaldehyde and 1% thymol show excellent results.

4. The best results are exhibited with iodine tinctures. Mild Tincture of Iodine gave very good results. Aqueous iodine solutions, in concentration up to

7% show no inhibitory properties. A notable exception which gives values equal to 7% Tincture of Iodine is an aqueous solution of 7% I_2, 14% KI, 5% benzene. The iodine ointments show little or no values. Silver compounds, with or without protein, give results equal to phenol. The mercury compounds give good results.

5. The essential oils exhibit a wide variation of values. Oil of Bitter Almonds, Essential Oil of Mustard show the highest penetration values; oils of cinnamon, turpentine and thyme, and eucalyptol, show medium penetration values; oils of clove, eucalyptus, lavender, lemon, caraway, pimenta, rosemary, indicate values somewhat greater than 5% phenol.

6. In studying the effect of the spore producing organism, *B. subtilis*, on the penetration value, a group of 60 antiseptics of which 14 are listed, were selected at random. It was found, in general, that if the antiseptic showed any penetration value when *S. aureus* was used, the penetration value increased when *B. subtilis* was substituted. However, no direct relationship could be established.

7. The effect of serous material on the penetration value of an antiseptic has been investigated. Mild Tincture of Iodine shows a small increase in value. Seven per cent Tincture of Iodine, and picric acid (5% in 50% alcohol) show no change, and all others studied show a decrease in value.

8. A modified procedure for the determination of the penetration values of volatile antiseptics is introduced. This procedure shows that the volatile antiseptics, formaldehyde, 70% alcohol and Essential Oil of Mustard may act as vapors, or by penetration through the agar with equal efficiency; that 7% Tincture of Iodine acts more efficiently as a vapor than by penetration; and that Oil of Bitter Almonds acts only as a vapor and shows no penetrative action.

CONCLUSION.

1. A study of the penetrative value of many official antiseptics was made using Reddish's cup plate method.

2. The effect of the penetration value of a group of antiseptics using a spore producing organism was studied.

3. The effect of serous material on the penetration values of some skin antiseptics was noted.

4. A study was made on the mode of action of volatile antiseptics.

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THE REHABILITATION COMMITTEE OF THE AMERICAN LEGION.

National Commander Chadwick has appointed Secretary Kelly of the A. PH. A. as a member of the Advisory Board to the Rehabilitation Committee. The Board membership consists of representatives from the groups directly interested in public health. The Rehabilitation Committee has charge of the first and most important of the eight major programs which the American Legion carries on for its members. The Committee arranged for a National Rehabilitation Conference in Washington on January 26th and 27th, which was attended by representatives from every state in the Union and the National officers, and plans were made to continue and to extend the services which the Legion offers to disabled veterans.