independent of the quantity of the drug administered.

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The Preparation and Study of Silver Antiseptics with and without Ephedrine*

By A. Slesser† and C. B. Jordan‡

The therapeutic effect of silver has been known for many years. Silver in protein combinations finds wide use in modern therapy in the treatment of infections of the eye, nose and urinary tract. These commercial preparations of silver are made in protein combination in order to obtain stable preparations with the desired disinfecting properties of the silver salts but without their irritant action. Most of the preparations are inferior to silver nitrate so far as germicidal activity is concerned.

The mild and strong protein silver preparations of the U. S. P. XI are examples of typical protein silver combinations. However, they, along with practically all other similar preparations, possess certain characteristics which are undesirable. The most important of these are: (1) their physical appearance, (2) their staining effect on the skin and clothing, (3) their instability and (4) their incompatibility with the alkaloid, ephedrine.

Solutions of mild and strong protein silver should be freshly prepared before use because, according to certain investigators, they lose some of their antiseptic activity and sometimes become irritant on standing.

At times physicians desire to prescribe for use in the nasal passages a mixture containing both silver and ephedrine, the former for its antiseptic effect and the latter for its shrinking action on the mucous lining of the nose. Combinations of alkaloids with silver in solution are unstable, particularly if the solution tends toward alkalinity, the silver being reduced. Hence the protein silver preparations, which are slightly alkaline in reaction, cannot be dispensed in combination with ephedrine.

It was for the purpose of preparing an effective silver antiseptic which would be free from the objectionable features of the protein silver medicaments and of the nitrate that this investigation was undertaken.

A portion of the research was concerned with the testing of the antiseptic potency of the silver medicaments prepared. The test employed was one used by the Food and Drug Administration, namely, the agar plate penetration method, in which the zone of inhibition of growth of organisms (from a standard culture of *Staphylococcus aureus*) immediately surrounding the antiseptic on a seeded agar plate is measured. The sodium chloride normally added to the nutrient agar medium was omitted when tests were performed on the organic silver preparations which yielded ionic silver in solution, in order to prevent the formation of slightly ionized silver chloride.

EXPERIMENTAL

PART ONE

Hydrosols of silver were prepared by four different methods and penetration tests performed. The results were not particularly significant because of variance in size of the particles.

Fine gelatin-protected dispersions of the following silver salts were prepared and incorporated into ointment form:

1. Silver chloride
2. Silver chromate
3. Silver thiocyanate
4. Silver iodide

* An abstract of a thesis submitted to the Faculty of Purdue University by Abraham Slesser in partial fulfilment of the requirements for the degree of Doctor of Philosophy, August 1939. An extensive bibliography accompanies the original thesis.

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5. Silver bromide
6. Silver oxide
7. Silver phosphate

The method of preparation was similar to that reported by Vicher, Snyder and Gathercoal (JOUR. A. PH. A., 26 (1937), 1241) for the preparation of finely dispersed calomel. Precautions were taken throughout the process to protect the suspensions from light. The zones yielded by each preparation are given in Table I. Ten plates were run on each sample and the average zone is given for each.

Table I

Ointment	Width of Zone of Penetration
Silver chloride	6.0 mm.
Silver bromide	4.0 mm.
Silver iodide	4.0 mm.
Silver chromate	15.0 mm.
Silver thiocyanate	12.0 mm.
Silver phosphate	6.0 mm.
Silver oxide	6.0 mm.

A study of the solubility product constants of the above silver salts revealed the fact that the larger zones were yielded by the salts having the larger constants, that is, by the more soluble salts.

PART TWO

The object of this portion of the experimental work was the preparation of silver salts with fatty acids in an attempt to obtain emulsifiable forms of silver which might prove therapeutically useful.

The silver salts of oleic, palmitic and stearic acids were prepared after the method of Whitmore and Lauro (1).

Emulsification was attempted using olive oil and water but the emulsions formed were dirty, greyish, viscid preparations which "broke" when a slight excess of water was added. According to Finkle, Draper and Hildebrand (2), emulsions of silver soaps are of the unstable water-in-oil type.

Emulsification was, however, accomplished in the following way: To 1 Gm. of the silver soap were added 15 cc. of oleic acid and the mixture heated, with stirring, to 90° C. at which temperature solution resulted. On cooling, the solution became a homogeneous pasty mass. It was transferred to a mortar, 3 cc. of triethanolamine were added and thoroughly incorporated into the paste. Water was then added in 10-cc. portions, with emulsification after each addition. The resulting grey-white emulsions became sufficiently fluid to pour after about 150 cc. of water had been added.

It was found that emulsification could also be effected by using 1% ephedrine sulfate solution instead of water as the dispersion medium; solutions of ephedrine alkaloid itself could not be used, however, because a slippery mixture which could not be rubbed against the sides of the mortar resulted.

Silver oxalate and silver succinate, prepared by treatment of the sodium salts of the acids with silver nitrate, were also emulsified with water and with 1% ephedrine solution in the same manner. These salts did not dissolve in the hot oleic acid but were mixed with the acid before emulsification.

The results of penetration tests performed on the emulsions were given in Table II. The zones were the same for the aqueous as well as the ephedrine emulsions. The figures are the average of ten separate tests.

Table II

Preparation	Zone of Penetration
Silver oleate emulsion	5.0 mm.
Silver palmitate emulsion	5.0 mm.
Silver stearate emulsion	5.0 mm.
Silver oxalate emulsion	6.0 mm.
Silver succinate emulsion	6.0 mm.
Oleic acid	0.0 mm.
Ephedrine sulfate 1%	0.0 mm.
Triethanolamine	0.0 mm.

Argyrol and protargol solutions containing 1% and 2% *silver* concentrations all gave zones of 12.0 mm. Potentiometric determination of silver ion concentration in the emulsions and in the protein silver preparations failed to establish correlation between the concentration of silver ions present and the widths of the zone of penetration in each case.

Each emulsion was divided into three parts, and each part was stored under different conditions as a

Table III

Emulsion	Diffuse Light — Days 1 2 3 4 5 6 7 8	Daylight — Days 1 2 3 4 5 6 7 8	Amber Bottle — Days 1 2 3 4 5 6 7 8
Silver oleate	0 0 0 sl. → dk. darkening	sl. → dk. darkening	0 0 0 0 0 0 0 0
Silver stearate	0 0 0 sl. → dk. darkening	sl. → dk. darkening	0 0 0 0 0 0 0 0
Silver palmitate	0 0 0 0 sl. → dk.	sl. → dk. darkening	0 0 0 0 0 0 0 0
Silver oxalate	0 0 0 0 0 sl. → dk.	sl. → dk.	0 0 0 0 0 0 0 0
Silver succinate	0 0 0 0 0 sl. → dk.	sl. → dk.	0 0 0 0 0 0 0 0

Key: 0 indicates slight change. dk. indicates darkening. sl. indicates "slight."

test for stability. One part was exposed to daylight in ordinary glass bottles, another in amber bottles and the third in the diffuse light of the laboratory. The preparations were examined daily and any changes in color noted. Table III lists the changes on standing of the emulsions prepared with water as the dispersing medium.

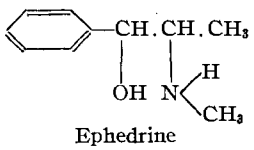
The emulsions prepared with ephedrine sulfate as the dispersing phase behaved in a similar fashion except that they began to darken about a day sooner than those prepared with water.

The emulsions stored in amber bottles began to darken after four months, gradually becoming deep brown in color.

PART THREE

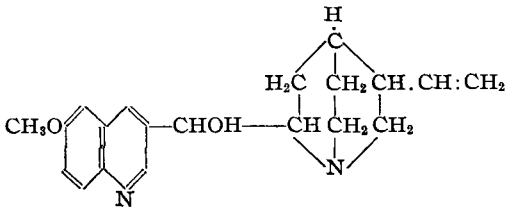
The third portion of the experimental investigation was confined to attempts to prepare stable water or oil-soluble combinations of ephedrine and silver which would possess antiseptic properties.

It has long been known that silver will form complex ions with certain reagents, one of these being ammonia. Since ephedrine contains a secondary amino group the nitrogen of which is trivalent and basic as in ammonia (see formula), it was hoped that a combination of the type $Ag(ephedrine)_2Cl$ could be prepared in the same way that $Ag(NH_3)_2Cl$ is prepared.



Ephedrine

Because ephedrine is rather expensive, quinine alkaloid, which possesses two basic nitrogen atoms in the molecule (see formula), was selected as the compound to be refluxed with some silver salts in an attempt to combine the metal with the organic molecule. It was hoped that should a combination with this alkaloid be secured, the same reaction might be applicable using ephedrine.



Quinine

Refluxing freshly prepared silver chloride and quinine together in methanol, ethanol, ether or chloroform gave no combination of the two substances.

A combination of quinine and silver was secured by refluxing 2 Gm. of silver oleate with 4 Gm. of quinine in 50 cc. of methanol. The dried reaction product was a yellowish, hydroscopic powder, which gave on analysis 8.6–9.4% silver and which had the odor of oleic acid. This quinine silver oleate, as the substance was called, was soluble to some extent in oleic acid, but insoluble in ethyl oleate and in water.

No silver-containing preparation could be obtained when the experiment was repeated using silver stearate or silver palmitate instead of the oleate.

It was hoped that, since the oleate had reacted with quinine, it might do so with ephedrine. Accordingly, 2 Gm. of ephedrine and 2 Gm. of silver oleate were refluxed together in 50 cc. of methanol for 15 minutes, at the end of which time practically all of the oleate had dissolved. The solution was filtered and set aside to evaporate in the dark. Darkening of the solution occurred on standing. Drying of the darkened concentrate was attempted by vacuum desiccation, but a dark brown, sticky residue which could not be dried resulted. Change of solvent did not alter the results.

The stearate, palmitate and lactate of silver reacted with ephedrine when refluxed with the alkaloid in methanol to yield silver-containing residues on evaporation of the solvent. The following silver salts, however, did not combine with ephedrine under similar conditions:

1. Silver citrate
2. Silver tartrate
3. Silver oxalate
4. Silver succinate
5. Silver acetate
6. Silver mandelate
7. Hexamethylenetetramine silver nitrate

PART FOUR

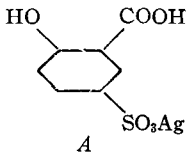
An attempt was made to prepare a water-soluble, antiseptic silver compound of such a nature that it could be chemically combined with ephedrine.

It has been mentioned before that silver nitrate reacts with the alkali salts of organic acids to yield the corresponding silver salt of the acid. With almost no exceptions these silver salts are quite insoluble in water as well as in the common organic solvents. A study of the solubilities of some silver salts of aromatic acids revealed, for example, that whereas silver benzoate is insoluble in water, silver salicylate, which can be prepared quite readily from sodium salicylate, is more soluble; the substitution of a polar (hydroxyl) group for a hydrogen atom in the atomatic nucleus had caused an increase in solubility. However, silver salicylate was not as soluble as we wished our silver salt to be; moreover, it did not contain active groups which would permit chemical linkage with ephedrine.

In order to increase the solubility as well as to introduce a group which could combine chemically with ephedrine, it was decided to resort to sulfonation. The problem was to secure a sulfonated aromatic acid, attach silver to either the sulfo or carboxyl group, leaving the remaining acid group free to react with the basic alkaloid, ephedrine, to form a salt.

Some method of introducing silver into only one of two acid groups attached to an aromatic nucleus had to be found. Whereas difficulties might be encountered if the ordinary procedure of reacting the sulfonated acid with the proper silver salt were followed, it occurred to us that these might be avoided *if we could sulfonate the silver salt itself.* Search of the literature failed to disclose accounts of any attempts to sulfonate metallic salts of aromatic acids. Obviously, any method used must involve conditions that would prevent splitting off the metallic element during the sulfonation. For this reason common sulfonating agents such as oleum (fuming sulfuric acid) and concentrated sulfuric acid were eliminated. A non-ionizing medium was indicated.

Passage of sulfur trioxide gas into stirred chloroformic and ethereal suspensions of silver benzoate did not effect sulfonation. Sulfonation was achieved, however, by the action of chlorosulfonic acid on silver salicylate suspended in dry chloroform. The yield was poor, so the silver sulfo compound was prepared indirectly by first sulfonating salicylic acid with chlorosulfonic acid and then saturating an aqueous solution of the sulfonated salicylic acid with freshly precipitated silver carbonate. The solution was filtered and set aside to crystallize. At the end of 24 hours, large prismatic crystals were discernible. When the liquid had evaporated to small volume, the colorless crystals were removed and placed on filter paper in order to remove the adhering liquid. The crystals on analysis showed a silver content of 33%. The formula of the silver sulfosalicylate would probably be represented by *A*.

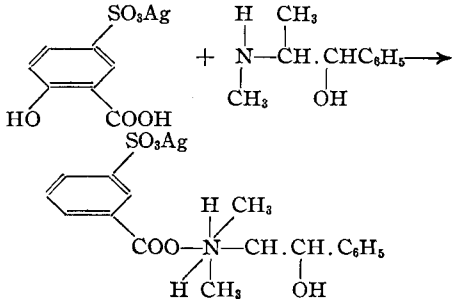


A

The silver salt showed the following properties:

1. Efflorescent in the air—becoming white in color.
2. Freely soluble in water, hot glycerin and glycol solutions.
3. Insoluble in alcohol, ether, chloroform, acetone, mineral oil, ethyl acetate, benzene and nitrobenzene.

Attempts to Form the Salt of Ephedrine with Silver Sulfosalicylate.—Solutions of silver sulfosalicylate are strongly acidic, a 5% solution having a p_H of 1.75. For this reason we hoped to be able to combine the compound with ephedrine alkaloid in accordance with the following reaction:



No well-defined salt could be obtained from solutions containing equi-molal amounts of the two salts. Reduction occurred in every case.

Mixtures of silver sulfosalicylate and ephedrine were prepared and investigated with respect to stability, p_H and penetrating power in order to determine their suitability for therapeutic use. The mixtures were prepared by adding measured amounts of 1% ephedrine solution to known amounts of 1% silver sulfosalicylate solution. Determinations of p_H were made by means of a "Beckman p_H meter." Table IV gives the composition, p_H and zone of penetration of some of the mixtures.

Table IV

Mixture	Cc. of 1% Silver Sulfosalicylate	Cc. of 1% Ephedrine	p_H
AI	5.0	25.0	10.2
BI	10.0	20.0	9.5
CI	15.0	15.0	8.3
DI	20.0	10.0	4.4
EI	25.0	5.0	2.55
FI	30.0	..	2.0

The solutions were allowed to stand at room temperature exposed to artifical laboratory illumination and the changes on standing noted. These changes are given in Table V.

Solutions *AI*, *BI* and *CI* showed silver mirror deposits after 4–5 days' standing. After three weeks, solution *DI* had acquired a pink color and solutions *EI* and *FI* showed a slight amount of dark sediment.

Table V

Mixture	After 24 Hours	After 48 Hours	After 72 Hours
AI	Slight dark sediment	Increase in sediment	Silver deposited on sides of bottle
VI	Slight light-colored sediment	Distinct dark sediment	Silver deposited on sides of bottle
CI	Slight light-colored sediment	Increase in sediment	Silver deposited on sides of bottle
DI	Slight white sediment	Little increase in sediment; solution faintly yellow	No change
EI	Slight white sediment	No change	No change
FI	No change	No change	No change

The great difference in p_H shown by solutions *AI* and *DI* was rather puzzling; however, the sudden jump in p_H may have been due to the fact that the end-point of neutralization of the acidic silver salt with the base had been exceeded in solution *AI*.

Of the solutions prepared, only *DI* had a p_H which would permit of its safe use in the nasal passage. This preparation showed little change of color and only slight sedimentation after three weeks' standing.

The Preparation of Stabilized Mixtures of Silver Sulfosalicylate with Ephedrine.—With the object in view of finding a substance which would contribute to the stability of solutions containing silver sulfosalicylate and ephedrine, the following materials were added to solutions of the silver salt and ephedrine: gelatin, acacia, dextrin, glucose, sucrose and urea. The solutions were prepared so that the p_H would range from a p_H of approximately 4.5 to 7.0. Tables 10 to 18 of the thesis indicate the compositions and p_H of the various mixtures, and Table 19 the changes on standing.

The silver salts of ortho and para aminobenzoic acids were prepared similarly to the silver sulfosalicylate. Lack of time prevented stability tests on solutions of these salts.

SUMMARY AND CONCLUSIONS

1. The zones of penetration of several insoluble silver salts in finely dispersed form have been determined.

2. Emulsions of silver oleate, palmitate, stearate, oxalate and succinate with and without ephedrine have been prepared and found to display reasonably good zones of penetration. It was found that storage in amber bottles greatly delayed the time of darkening of the emulsions.

3. A combination of silver oleate with quinine was secured by refluxing the two materials in methanol; the stearate and palmitate of silver did not react in a similar manner with quinine.

4. The reaction product of silver oleate and ephedrine in methanol darkens and decomposes when attempts are made to recover it from the filtrate; the addition of antioxidants such as phenol or benzaldehyde does not prevent the decomposition.

5. Three new soluble silver salts having antiseptic properties have been prepared.

6. Sulfonation of a silver salt of an aromatic acid has been accomplished.

7. Combination of silver sulfosalicylate with ephedrine alkaloid in the form of a salt is difficult because of the reducing effect of the alkaloid on the silver ion. This effect is more marked in solutions which tend toward alkalinity.

8. The stability of mixtures containing ephedrine and silver may be increased by the addition of a third substance. Gelatin, acacia, sucrose, urea or combinations of any of those may be used. The effect seems to be retardation rather than prevention of the reduction of the silver.

9. The addition of a reducing substance (antioxidant) as a stabilizing agent is unsatisfactory because reduction of the silver takes place more rapidly; however, the addition of an oxidizing agent, *e. g.*, quinone, also seems to hasten reduction of the silver.

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(1) Whitmore, W. F., and Lauro, M., *Ind. Eng. Chem.*, 22 (1930), 646.

(2) Finkle, F., Draper, H. D., and Hildebrand, J. H., *J. Am. Chem. Soc.*, 45 (1923), 2780.

Book Review

Standard Methods of the N. Y. State Department of Health, by AUGUSTUS B. WADSWORTH. 2nd Edition. xxiv + 681 pages, 6 x 9. 1939. Baltimore: Williams and Wilkins. $7.50.

This volume is a compilation of the laboratory methods of the Division of Laboratories and Research of the N. Y. State Department of Health. It contains all of the general procedures found useful in a large clinical laboratory and covers general bacteriological technique, the use of test animals, p_H determinations, collection of cultures, chemical methods used in clinical work, preparation and sterilization of media and glassware, methods used for the examination of various types of specimens for pathologic abnormalities and methods used for the examination of water, milk, cream and sewage. Of particular interest to the pharmacist is the section devoted to the preparation of antitoxins, serums, etc., with notes on the care and breeding of laboratory horses, sheep and other animals. The book is believed to possess value for biological manufacturers as well as clinical laboratories.—A. G. D.

CORRECTION

In column one, page 396 of the September issue, change last sentence to read: "All readings were made at 25° C."