SCIENTIFIC SECTION

THE APPLICATION OF THE U. S. P. X YEAST FERMENTATION TEST TO COLLOIDAL SILVER COMPOUNDS.*

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The author applied the U. S. P. X yeast test to various commercial silver preparations and classification was made according to this test. The minimum dose required to inhibit yeast growth was determined and these doses compared with the phenol coefficients and germicidal values of the preparations in question. Results show that the inhibition of yeast growth measures only approximate silver-ion concentration and gives no indication of the germicidal value of these preparations.

The U. S. P. X (1), under tests for identity of strong and mild protargin, gives a test in which the inhibitory action of the silver compound upon a yeast-sucrose solution is used as a distinction between mild and strong preparations. This test is objectionable in that it requires considerable time and apparatus. In the light of work published by Pilcher and Sollmann (2) it is also very easy to misinterpret this test, or modifications of the test, as being indicative of the germicidal efficacy of the preparation in question. Accordingly the present work was undertaken in order to determine whether or not there is any direct quantitative relation between the inhibitory action of colloidal silver compounds on yeast growth and germicidal action. It was also desirable to determine the accuracy of the test as a measure of silver-ion concentration.

While silver nitrate has very high antiseptic and germicidal power its use is limited by its side reactions, such as irritation and pain, astringency and corrosion. Consequently colloidal silver preparations, which combine in many instances a high degree of antiseptic action with a much smaller degree or entire absence of irritant side actions, have come into common use. The irritant and antiseptic actions of silver nitrate have been correctly attributed to the free silver ions.

The antiseptic action of the colloidal silver preparations has also been attributed by some to the presence and liberation of low concentrations of silver ions, the concentration having been claimed to be so low as to be practically non-irritant, but still sufficient to be more or less antiseptic. Dreser (3) has shown that the antiseptic action of the commercial "colloidal metallic silver" preparations is destroyed if they are treated with agents that reduce silver ions to metallic silver (zinc dust, hydroquinones, pyrogallol); or by agents that bind silver ions (sodium thiosulphate, potassium cyanide or sodium chloride). Gros (4) concludes that the colloidal silver preparations, notwithstanding their low concentration of silver ions, may be more efficiently antiseptic in the presence of sodium chloride, than is silver nitrate; because the silver chloride from colloidal silver forms a finer precipitate and therefore redissolves more readily than when it is precipitated from silver nitrate (except in very dilute solutions). Pilcher and Sollman (2) have also attributed the antiseptic action of such preparations to the presence of silver ions. But are we correct in assuming that the antiseptic action is due entirely to silver ions? It is not the purpose of this paper to explain the antiseptic action

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of colloidal silver preparations but rather to show that there are other factors than silver-ion concentration. What these factors are will be brought out in subsequent investigations.

The numerous commercial brands of colloidal silver preparations may be grouped under a limited number of types (5). Clinical experience has shown that these types differ in irritation and in antiseptic efficiency, so that their therapeutic field is not quite identical; but no exact comparisons have been made, for want of suitable quantitative methods. Even the classification of individual products, which should reflect the therapeutic grouping, was sometimes doubtful. Experiments on the restraint of bacterial growth have been made under various conditions by many observers but the results have been contradictory, probably because of technical difficulties, so that they have but little value as a guide either to therapeutics or classification. Dreser (3) drew from his observations the conclusion that the growth of ordinary yeast furnishes an accurate and convenient index of silver ions, of antiseptic efficiency and of irritation. He applied the method to "colloidal metallic silver" preparations and suggested that it might be useful for quantitative comparisons of organic silver compounds with silver nitrate; but did not elaborate further. Pilcher and Sollman (2) have modified this method and incorrectly claim that it furnishes reliable criteria for the comparative antiseptic efficiency of silver compounds, inorganic, protein and colloidal. Their modification—the Pilcher-Sollman-Dreser method—briefly is as follows:

The minimum inhibitory dose of the compound in question required to prevent gas formation in ten cubic centimeters of a 10% sucrose-4% yeast (Fleischmann's) solution for one hour at 38° C. is determined. A control of silver nitrate is used in order to correct for the variation in strength of the yeast. This method differs from that of the U. S. P. X in that it determines the minimum inhibitory dose of each preparation, whereas the U. S. P. X method uses a definite dosage and observes whether or not the growth of yeast is inhibited.

Pilcher and Sollman examined some thirty-four samples representing various commercial brands and their results show that, according to the total activity, as determined by their method, the silver compounds arrange themselves into five distinct groups, separated by relatively wide gaps. The groups correspond very satisfactorily to the generally accepted clinical types. They conclude that the fermentative activity of yeast cells offers a convenient and sufficiently accurate measure of the concentration of the silver ions in solutions of the so-called "colloid" and "protein silver compounds" as well as in pure salts, that the content of silver ions is not only responsible for the irritant action, but is also responsible for all or nearly all of the antiseptic action on yeast and that in so far as the antibacterial efficiency of silver compounds is *due to silver ions* this would also be faithfully reflected by the yeast method; or if silver compound X has a hundred times the efficiency of silver compound Y on yeast, it will also have approximately a hundred times the efficiency of compound Y on any bacterium. They qualify the above statement by saying that the yeast method might not be applicable for making deductions if the antibacterial action were due to constituents other than silver ions, to which it might be argued, bacteria are much more susceptible than yeast. They further say that since the methods of estimating antibacterial efficiency on bacteria directly do not appear to give concordant results, there is at

present no good evidence that may be used either for or against this rather vague assumption or *ex parte* argument. Thus it will be seen that the applicability of the yeast fermentation method for determining antiseptic efficacy depends upon the assumption that the antiseptic efficacy of colloidal silver compounds is due entirely to silver ions. This assumption is incorrect and without experimental basis, in fact results reported later in this paper show that antiseptic efficacy of colloidal silver compounds is due not only to silver ions but to a large extent to other factors which are yet to be determined. Consequently the yeast fermentation method, while correctly reflecting the approximate silver-ion concentration, cannot be used to determine antiseptic efficacy.

EXPERIMENTAL.

Various commercial silver preparations were first subjected to the U. S. P. X yeast test as prescribed on page 63 of the U. S. P. X with the following results.

Protargentum Protargol	classed as "strong"	
		Collargolum Argyrol Solargentum
Proganol—intermediate between "mild" and "strong" but most probably "strong"		Silvol Neo-Silvol Cargentos Novargentum

"mild"

In order to determine if the inhibition of yeast growth is directly related to germicidal efficacy the minimum doses of the before-mentioned compounds required to inhibit yeast growth was determined and compared with the phenol coefficients and Squibb Germicidal Values. The method used was a combination of the Pilcher-Sollmann-Dreser method and the U. S. P. X method. A 10% aqueous sucrose solution is prepared and a 4% suspension of yeast (Fleischmann's) made with this solution. Twenty cc. of the yeast-sucrose mixture is then placed in fermentation tubes containing varying doses of the silver preparation being tested and incubated at 38° C. for one hour. The dose which will just inhibit gas formation for one hour is recorded as the minimum inhibitory dose. In order to have a control of the strength of the yeast silver nitrate is used. Since 0.50 mg. of silver nitrate will inhibit gas formation for one hour in almost all cases, this amount of silver nitrate was taken as a standard. A control was run in each case and where the strength of the yeast was stronger than the standard the dose may be reduced to standard conditions by using the Pilcher-Sollman formula:

$$\frac{\text{True inhibitory dose of the compound}}{\text{Determined inhibitory dose of the compound}} = \frac{0.50}{\text{Determined inhibitory dose of } AgNO_3}$$

In order to avoid undue dilution where more than one cc. of the silver solution has to be added to the yeast sucrose mixture a 20% sucrose, 8% yeast solution is used and after adding the required amount of silver solution, the whole is diluted to 20 cc. with distilled water. Chloride free water should be used in all cases and readings of the gas formation should be made immediately upon removal from the incubator.

The phenol coefficients were determined by the method given in "Disinfectant Testing by the Hygienic Laboratory Method," *Public Health Reports* 36, pp. 1559–64 (July 8, 1921). While the preparations under test are in no way

related to phenolic compounds, their phenol coefficients were determined solely for comparative values.

The method which gives most reliable results is that known as the Squibb Germicidal Test, which has been developed in the Squibb Biological Laboratories. (6)

A staphylococcus aureus culture is grown for 24 hours at 37° C. on beef extract bouillon.

Various dilutions of the material to be tested are made with sterile distilled water, and 5 cc. of each solution placed in a test-tube. To each dilution of 5 cc. is added 0.1 cc. of the standard culture emulsion and thoroughly mixed. After standing at room temperature the time indicated in the test table, one loopful from each tube is planted in 10 cc. of beef extract bouillon. The bouillon tubes are incubated for 48 hours at 37° C. and readings made. All tests are made in duplicate and all duplicate tubes read exactly the same. The time and dilution required to kill are recorded.

All glassware, apparatus and media are prepared as for the official phenol coefficient test which is described in the reference given above.

All samples of silver preparations were purchased in the open market except Novargentum which was prepared in this laboratory. The phenol coefficients, Squibb Germicidal Values and minimum inhibitory doses against yeast were determined by the above methods and are given in the following table:

	Yeast Fermentation Test. Inhibiting dose. Range. mg./20 cc.	Average. mg./20 cc.	Concn. Average. 1:	Squibb Germicidal Values. Dilution required to kill Staphylococcus. Aureus in minutes. 1 1:	5 1:	10 1:	15 1:	20 1:	30 1:	Phenol coefficient.
Protargol	3.88–4.4	4.13	4842	No effect	75	100	100	150	150	7.92
Protargentum	4.44–5.51	4.79	4175	600	600	800	900	1000	1000	9.29
Proganol	10–10.71	10.35	1932	No effect	75	100	125	125	125	3.65
Collargolum	20.7–21.6	21.15	945	No effect	75	75	100	100	125	1.39
Cargentos	46.25–46.66	46.45	430	No effect	100	125	125	150	150	1.47
Solargentum	57.14–59.89	58.25	343	No effect	100	125	125	150	200	1.57
Argyrol	73.57–75.0	74.13	269	No effect	75	75	100	125	125	1.57
Novargentum	1228–1271	1250	16	300	400	400	500	...	...	4.71
Neo-Silvol	....	>8000	<2.5	No effect	75	100	125	150	200	1.57
Silver Nitrate	0.50	0.50	40000							

From the above table it will be readily seen that those compounds which are classed as "strong," namely, Protargol, Protargentum and Proganol, show the greatest activity against yeast. Two of these compounds, Protargol and Protargentum, also show high phenol coefficients, while that of Proganol is relatively low. On the other hand Protargentum is extremely active against *staphylococcus aureus*, one part in six hundred killing in one minute, while Protargol and Proganol are comparatively weak against *staphylococcus aureus*, one part in seventy-five being required to kill in five minutes.

Among the "mild" preparations, Novargentum shows a very low activity against yeast as against a very high activity against *staphylococcus aureus* and a comparatively high phenol coefficient. Collargolum, Cargentos, Solargentum and Argyrol all show decidedly less action against *staphylococcus aureus* and have lower phenol coefficients than Novargentum and yet they are much more active

against yeast. Neo-Silvol shows no inhibition of yeast growth at all, yet it has a germicidal value against *staphylococcus aureus* fully equal to Argyrol.

If the preparations are listed in order of their strength against the three tests, series will be obtained which will readily show that no direct relation exists between the inhibition of yeast growth and germicidal value.

The results of the Squibb Germicidal Test determinations are interesting in that they show some preparations to be very active within a short space of time, one minute, a point which should be of interest therapeutically. The phenol coefficient does not give this information.

From the results obtained the following conclusions are drawn:

The yeast test measures only the approximate silver-ion concentration and is of value only in distinguishing between "mild" and "strong" silver preparations. It is not absolutely reliable in distinguishing between preparations of the same group.

Silver-ion concentration is not directly related to the germicidal efficacy of colloidal silver preparations and consequently the yeast test cannot be used as an index to germicidal value.

In view of the cumbersomeness of the yeast test and the length of time required to perform it, it can well be replaced by the test recently developed by Keelan (7) in which an aqueous solution of the silver preparation is coagulated by exsiccated magnesium sulphate and the clear filtrate treated with hydrochloric acid. Strong preparations give a turbidity whereas mild preparations remain clear. This test requires very little apparatus and can be carried out in about five minutes.

I wish to express my appreciation to Dr. George F. Leonard of the New Brunswick Biological Laboratories, E. R. Squibb & Sons, for his determinations of the phenol coefficients and Squibb Germicidal Values reported herein.

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THE VOLATILE OIL OF HYPERICUM PERFORATUM.*

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The genus hypericum contains about two hundred species belonging in the plant family *Hyperaceæ*, a group of beautiful flowering plants. A number of these species are mildly medicinal and some have been extensively used.

The species *perforatum* is commonly known as St. John's Wort. It is a native of Europe and Asia and was introduced into the United States as a garden flower.

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