THE COMPARATIVE GERMICIDAL EFFICIENCY OF PHENOL, MERCURIC CHLORIDE AND POTASSIUM MERCURIC IODIDE.*

BY JOSEPH G. CADORA.

The value of any substance as a disinfectant, particularly of one to be employed under conditions where its behavior toward the tissues of the human body must be considered, depends upon the correlation of four principal factors, namely, the power to kill promptly and surely all forms of bacterial life; relative freedom from poisonous properties in dilutions germicidally efficient; the absence of any marked precipitating action on proteins; and the cost.

The application of biologic methods to the testing of disinfectants has made it possible to determine accurately not only the killing power of any substance upon bacteria but also to measure its toxicity and its precipitating action and, therefore, from this data to calculate its actual final efficiency as a germicide.

In these various determinations phenol is taken as the standard of comparison, and all values found are expressed as relative coefficients of the phenol values. For measuring the germicidal action of any substance under controlled conditions the method of determining the phenol coefficient as advocated by the United States Hygienic Laboratory[1] is applicable and in the hands of experienced workers yields reliable information. The actual, or relative, toxicity may be ascertained by the method described by Hale,[2] while the precipitating or coagulating action on proteins may be measured by any of several methods.

The present study was undertaken with the purpose of obtaining definite information concerning the germicidal value of potassium mercuric iodide, especially as compared with the more commonly used mercuric chloride and phenol. This double salt of mercury and iodine has long been used in the treatment of various affections of infectious origin, but not until comparatively recent years has it been employed to any wide extent as a general disinfectant. The work of Macfarlan,[3] Watson,[4] McKenna and Fisher,[5] and others would seem to show that potassium mercuric iodide possesses to a marked degree the essential properties of a highly efficient germicide, and that, on account of these properties, it is singularly well suited to a variety of applications in medical and surgical practice.

On account of the promise held out by the work of these authors it seemed that it would be of value to make a quantitative study of the relative germicidal power, coagulating action and toxicity of this mercury salt as compared with similar properties of the somewhat related mercuric chloride, with phenol as the standard of comparison.

EXPERIMENTAL.

1. Preparation and Assay of Solutions.—A. Chemicals: The phenol and mercuric chloride were of reagent grade, as were also the potassium iodide and mercuric iodide employed in the preparation of the potassium mercuric iodide. These chemicals were tested by the usual chemical assays for determining chemical purity, and were found to be of the desired standard.

B. The Solutions: Phenol, 5 Per cent. A five per cent. phenol solution was prepared by making a slightly overstrength solution, titrating by the U. S. P. IX

* An investigation made at the Massachusetts College of Pharmacy, under the direction of Benjamin White, Ph.D., Associate Professor of Biologic Assaying and Immunology.

method, adjusting to the desired point and retitrating. The assay of the final standard solution as used showed it to contain 5.002 per cent. phenol.

Mercuric Chloride, 1 Per cent. The same procedure was followed in preparing this solution and its final assay showed it to contain 1.001 per cent. mercuric chloride.

Potassium Mercuric Iodide, 1 Per.cent. This solution was prepared by dissolving 5.77 grams red mercuric iodide in 1000 cc water containing 8.65 grams potassium iodide. The assay was performed according to the U. S. P. IX method for the determination of mercury and mercuric iodide in Donovans' solution, and showed it to contain 0.979 per cent. potassium mercuric iodide.

All solutions were made from distilled water redistilled from glass and were stored in the dark in Pyrex flasks with air-tight stoppers.

2. *Phenol Coefficient.*—The phenol coefficient of the two mercury salts was not personally determined by the author, because the figures from a recent test on similarly assayed solutions competently performed by one experienced in this determination were available.* The protocol follows:

PHENOL COEFFICIENT WITHOUT ORGANIC MATTER.

1. *Phenol:* Time	$2^1/_2$	5	$7^1/_2$	10	$12^1/_2$	15 (minutes)
1–80	...	...	...	...	...	...
1–90	x	x	...	...	...	...
1–100	x	x	x	x	...	...
2. *Potassium Mercuric Iodide:*						
1–40,000 = 80,000	...	...	...	...	...	...
1–42,000 = 84,000	x	...	...	...	...	...
1–44,000 = 88,000	x	...	...	...	...	...
1–46,000 = 92,000	x	x	...	...	...	...
1–48,000 = 96,000	x	x	x	...	...	...
1–50,000 = 100,000	x	x	x	...	...	...
1–52,000 = 104,000	x	x	x	...	...	...
1–54,000 = 108,000	x	x	x	x	...	...
1–56,000 = 112,000	x	x	x	x	...	...
1–58,000 = 116,000	x	x	x	x	...	...
1–60,000 = 120,000	x	x	x	x	x	...
3. *Mercuric Chloride:*						
1–35,000	...	...	...	...	...	...
1–38,000	x	...	...	...	...	...
1–40,000	x	x	...	...	...	...
1–46,000	x	x	x	...	...	...
1–50,000	x	x	x	x	...	...
1–54,000	x	x	x	x	x	x

Potassium Mercuric Iodide $\frac{80{,}000}{80} \quad \frac{120{,}000}{100} = \frac{1000 + 1200}{2} = \frac{2200}{2} = 1100$

Mercuric Chloride $\frac{35{,}000}{80} \quad \frac{50{,}000}{100} = \frac{435.5 + 500}{2} = 468.7$

Taking the germicidal power of phenol as one, the coefficient for mercuric chloride, according to this determination, was 468.7, and for potassium mercuric iodide it was 1100, or more than twice that of the bichloride.

* The protocol was kindly placed at the writer's disposal by Dr. R. W. Lamson of the Harvard Medical School.

3. *Toxicity Coefficient.*—The method used was that of Hale.[2] White mice, weighing between 15 and 20 grams, were used, animals of varying weights being equally allocated in the three series. The solutions were so diluted (using precision pipettes and calibrated burettes) that the total dose never exceeds 0.8 cc per mouse. All doses were calculated in grams per gram of body-weight. The injections were made subcutaneously on the dorsal surface at the root of the tail, and hypodermic needles of 27 gauge were used to lessen the liability to leakage and to avoid trauma. The animals were kept under the most sanitary conditions in glass jars and observed for a maximum period of fourteen days. The results are shown in the following table:

Phenol.		Mercuric Chloride.		Potassium Mercuric Iodide.	
Dose—Grams per Gm. body wt.	Result.	Dose—Grams per Gm. body wt.	Result.	Dose—Grams per Gm. body wt.	Result.
0.00028	S	0.00001	S	0.00002	S
0.00028	S	0.000011	S	0.00002	S
0.00029	S	0.000011	S	0.000022	S
0.0003	S	0.000011	S	0.000022	D—10 days
0.0003	S	0.000011	D— 4 days	0.000023	S
0.00031	S	0.000011	D— 4 days	0.000023	D— 4 days
0.00032	S	0.000011	D— 4 days	0.000024	D—10 days
0.00035	D— 1 hr.	0.000012	S	0.000024	S
0.00035	D— 1 hr. 40 min.	0.000012	S	0.000025	S
0.00035	D—14 hrs.*	0.000012	D— 3 days	0.000025	S
0.00036	D— 1 hr.	0.000012	D— 3 days	0.000025	S
0.00036	D— 1 hr.	0.000012	D—10 days	0.000026	D— 4 days
0.00037	D— 1 hr.	0.000012	D—10 days	0.000026	D— 4 days
0.00037	D—14 hrs.*	0.000013	D— 2 days	0.000026	D—11 days
0.00038	D— 1 hr. 17 min.	0.000013	D— 4 days	0.000027	D— 4 days
0.00038	D— 1 hr. 17 min.	0.000013	D— 4 days	0.000027	D— 4 days
0.0004	D—14 hrs.	0.000013	D—10 days	0.000028	D— 8 days
		0.000014	D— 2 days	0.000029	D— 3 days
		0.000014	D— 4 days	0.00003	D— 8 days
		0 000014	D— 4 days		
M. L. D. 0.00035 Gm. per Gm. mouse		M. L. D. 0.000012 Gm. per Gm. mouse		M. L. D. 0.000026 Gm. per Gm. mouse	

D = Died.
S = Survived.

Therefore taking the toxicity of phenol as one, the coefficient of mercuric chloride is 29.2 and that of potassium mercuric iodide is 13.4, or slightly less than one-half that of mercuric chloride.

4. *Coagulation Coefficient.*—The method described by Schneider[6] was used for the first determination. This method consists in taking as a standard the degree of turbidity produced by adding 5 cc of the 5 per cent. phenol solution to 5 cc of specially prepared and assayed 1 per cent. egg albumin solution. An equal volume of varying dilutions of the mercuric chloride solution was then added to 5 cc of the standard albumin solution until a dilution was found which gave the same degree of turbidity as that produced under identical conditions by the phenol solution. This dilution was 1:1100. Therefore it is evident that if 5 cc of a 1:1100

* Between 2 and 14 hours—No observations made in interval.

solution of mercuric chloride will produce a degree of albumin coagulation equal to that produced by 5 cc of a 5 per cent. solution of phenol, the proportionate coagulating effect is as 1100 is to 20, or 55.

With the 1 per cent. potassium mercuric iodide solution no turbidity was produced when it was added to the egg albumin solution, and, furthermore, no turbidity could be produced with larger volumes of more concentrated solutions of the double mercury salt.

The results obtained by this method were checked by tests in which horse serum was substituted for the egg albumin. This modification was made because it seemed that inasmuch as it is the coagulating or precipitating effect of a germicide on serum proteins that is of importance, a fairer measure of this action could be obtained by using the horse serum instead of egg albumin. Analysis showed that the horse serum used contained approximately 8 per cent. serum protein. The horse serum was employed undiluted, and for the standard, one cc of the 5 per cent. phenol was added and the mixture made up to 10 cc with water. It required 5 cc of a 1:65 dilution of the 1 per cent. mercuric chloride solution to produce the standard degree of turbidity, which gives a coagulation coefficient for mercuric chloride by this method of 65.

The one per cent. potassium mercuric iodide solution failed to produce any turbidity in the horse serum, and it was astonishing to find on repeated trials that this double iodide as the pure salt was entirely soluble in horse serum in the proportion of one gram of salt per cubic centimeter of serum, and that with this concentration no visible coagulation of the serum proteins took place. This character of potassium mercuric iodide, namely, that of forming no insoluble combination with proteins, has been previously mentioned in the literature, but so far as the author is aware, no reports of quantitative or comparative determinations of this character have been published.

5. *Comparative Costs.*—At the time this work was completed the costs for the three germicides used (in C. P. grades) were as follows:

Phenol	$1.65 per kilo	= 1
Mercuric Chloride	4.44 per kilo	= 2.69
Potassium Mercuric Iodide*	18.07 per kilo	= 10.95

7. *Efficiency Value.*—Substituting the figures obtained in this investigation for the factors in the formula:

$$\text{Efficiency Value} = \frac{\text{Phenol Coefficient}}{\text{Toxicity Coefficient} + \text{Coag. Coef.} + \text{Cost}}$$

we obtain for

1. Phenol $\frac{1}{1+1+1} = \frac{1}{3}$ corrected (x 3) = 1 or standard.

2. Mercuric Chloride $\frac{468.7}{29+65+2.69} = 5$, corrected (x 3) = 15.

3. Potassium Mercuric Iodide $\frac{1100}{13.4+95+10.0} = 45$, corrected (x 3) = 135.

* Based on the cost of mercuric iodide and potassium iodide in the proportions used.

While it is obviously impossible to reduce such values to a strict mathematical equation, this formula seems to afford a comparative estimate of the efficiency value of these three germicides.

SUMMARY.

In this study of the comparative germicidal values of phenol, mercuric chloride and potassium mercuric iodide, comprising determinations of the phenol coefficient, the toxicity coefficient, coagulation coefficient and the cost, it was found that potassium mercuric iodide, because of its remarkably high germicidal coefficient, its relatively low toxicity as compared to mercuric chloride, and its freedom from any coagulating or precipitating action on proteins, is far more efficient as a germicide than either phenol or mercuric chloride, and, notwithstanding its greater cost, its efficiency value is fifteen times that of mercuric chloride and is approximately one hundred and fifty times that of phenol.

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THE PREPARATION OF ACID-FAST CARAMELS.*

BY GEORGE D. BEAL AND DONALD F. BOWEY.

This paper deals with the preparation of a caramel which will fully comply with the specifications used by up-to-date bottlers and others using the color and which will be sufficiently economical in preparation to compete with those already on the market. In 1913 it was pointed out by Brant that a desirable compound to be used in the coloring of beers should not suffer any change in tint when treated with a 0.5% solution of tartaric acid. Some varieties of caramel will retain their color indefinitely when used in the coloring of mildly acid beverages; others while withstanding the action of the acid will become discolored if the beverage is carbonated. Recently the demand for caramel to be used in the coloring of soft drinks has grown enormously.

An eastern manufacturer has succeeded by a secret process in preparing caramel which has been advertised as being acid-fast, and has been so successful in capitalizing this property that the majority of caramel users in the country demand an acid-fast caramel.

The specifications commonly adopted are as follows: Sixty grains of the concentrated caramel are dissolved in eight ounces of hot water. If one-half of this

* Contribution from the Chemical Laboratory of the University of Illinois. Read at the meeting of the American Pharmaceutical Association in Cleveland, Ohio.