TABLE III.

Influence of Ash of Milk and pH on Stability of Thiamin to Incubation at 37°C for 22 Hours.

Thiamin, μg	Treatment	Thiamin recovered, μg	% thiamin destroyed
6	In 20 cc water, pH 5	5.73	4.5
6	In 20 cc water + ash of 20 cc cow's milk	4.93	17.83
6	In 20 cc water, pH 6	5.60	6.67
6	In 20 cc water, pH 6, + ash of 20 cc cow's milk	4.87	18.83
6	In 20 cc water, pH 7	5.33	11.17
6	In 20 cc water, pH 7, + ash of 20 cc cow's milk	4.67	22.17

perature of 31°C resulted in 16% destruction. Since incubation at 37°C for 22 hours produced 46% destruction, the critical temperature of thiamin destruction in cow's milk seems to be between 31 and 37°C.

The next point of interest that seemed worthy of investigation was to determine what contribution, if any, the minerals of milk made towards the thiamin destruction at body temperature. The results of such trials are presented in Table III. It will be noted there is only a slight destruction of thiamin in aqueous solution which increases with the increase of the pH from 5 to 7. However, the ash of milk *per se* produced a destruction of 11 to 12% thiamin, regardless of the pH. In other words, the minerals of milk are responsible for about 25% of the total thiamin destruction; therefore, the character of the factor or factors which are contributory to 75% of the destruction must be organic in character and of a nature that resist heat treatment at 100°C for 10 minutes.

Summary. Incubating cow's milk for 22 hours at 37°C produces 46% destruction of thiamin but riboflavin is entirely stable under such treatment. The critical temperature for thiamin destruction appears to be between 31 and 37°C. The factors in cow's milk responsible for thiamin destruction are stable to 100°C for 10 minutes. The minerals of cow's milk are contributory to 25% of the thiamin destroyed.

14313

Effect of Certain Organic Compounds on Germicidal Efficiency of Mercuric Chloride.

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In practice disinfectants are commonly used in the presence of organic matter. The value of the results obtained by any laboratory procedure may justly be questioned if the comparisons are made in the absence of organic matter. Despite the significance of this fact, which was realized and emphasized by Chick and Martin,[1] most tests for rating germicides are still performed in the absence of organic compounds.

It is well known that the type of organic matter employed may make a big difference in the final results obtained. Some compounds are without effect whereas others influence markedly the efficiency of germicides. Unless a standard organic solution or compound is incorporated in the procedure employed for the evaluation of germicides the final results will give misleading comparisons.

The present investigation was undertaken to determine which groups of organic compounds are effective in reducing the germicidal efficiency of mercuric chloride. Definite amounts of gelatin, peptone, a number of amino acids, and several carbohydrates were employed. The concentration of mercuric chloride required to kill *Staphylococcus aureus* in the presence of definite amounts of

[1] Chick, H., and Martin, C. J., *J. Hyg.*, 1908, **8**, 654.

these organic compounds was determined and the results compared with a control series in which no organic matter was employed.

Method. To each of a series of tubes, containing 2.5 cc of organic matter adjusted to pH 6.6, was added 0.5 cc of a 24-hour broth culture of *Staphylococcus aureus*. The tubes were brought to 37°C in a water bath.

A series of aqueous dilutions of mercuric chloride were prepared and brought to 37°C. Two cc of each dilution of the germicide were pipetted into the tubes containing the mixture of bacteria and organic matter, bringing the final volume to 5 cc. The contents of the tubes were mixed thoroughly.

TABLE I.
Effect of Carbohydrates on the Germicidal Efficiency of Mercuric Chloride Against *Staphylococcus aureus*.

Dilution of $HgCl_2$	Control	Glucose	Maltose	Sucrose
1:12,500	—	—	—	—
1:25,000	+	+	+	+
1:37,500	+	+	+	+
1:50,000	+	+	+	+

— = no growth; + = growth after 96 hours at 37°C.

After 10 minutes the tubes were removed from the water bath and a 4 mm loopful transferred from each tube to broth. To eliminate a bacteriostatic effect, subcultures were made to 100-cc amounts of broth contained in flasks. The killing dilution was determined by the absence of growth in the subcultures after an incubation period of 96 hours at 37°C.

Controls were run for each series, using 2.5 cc of distilled water in place of the organic matter.

Effect of Carbohydrates. Glucose, maltose, and sucrose were selected as being representative of the carbohydrates. Each sugar was dissolved in distilled water to give a concentration of 2%. The solutions were sterilized by passage through Chamberland filters.

The results are given in Table I. The figures show that the 3 carbohydrates tested had no effect on the destruction of *Staphylococcus aureus* by mercuric chloride. The highest dilution of germicide showing no growth of the organism was 1:12,500 regardless of whether the sugars were present or absent.

Effect of Amino Acids. Glycine, cystein, aspartic acid, glutamic acid, arginine, and lysine were dissolved in distilled water to produce solutions of M/15 concentrations, then sterilized. With the exception of cystein, the acids were sterilized in the autoclave at 15 lb pressure for 30 minutes. Since cystein in the presence of free oxygen, traces of iron, and heat is rapidly converted into insoluble cystine, it was sterilized by passage through a Chamberland filter and used immediately.

The results are reported in Table II. The amino acids produced considerable inactivation of the mercuric chloride. With the exception of cystein, the acids showed the same degree of inactivation of the germicide. In the presence of the amino acids, a dilution of 1:2500 of mercuric chloride was required to kill the test organism; in the absence of the acids a dilution of 1:12,500 produced the same result. Cystein was an exception in that it showed considerably more inactivation than the other amino acids. In its presence mercuric chloride failed to kill the test organism in a dilution of 1:250, the highest concentration used.

It is generally stated that neutral salts of the heavy metals react with amino groups to form double salts of definite composition. If this is true the dibasic acids (arginine, lysine) should produce twice as much inactivation of mercuric chloride as the monobasic acids. Such was not the case, however, as all of the acids except cystein produced equal inactivation of the germicide when employed in equimolar concentrations. No explanation is advanced for this result.

On the other hand cystein contains a sulfhydryl (—SH) group that is also capable of reacting with mercury.[2] This leads to a further inactivation of mercury with the result that more mercuric chloride is required to produce the same toxic effect on the bacteria.

Effect of Aminoids, Peptone, and Gelatin. One per cent solutions were prepared in

[2] Fildes, Paul, *Brit. J. Exp. Path.*, 1940, **21**, 67.

TABLE II.

Effect of Amino Acids on the Germicidal Efficiency of Mercuric Chloride Against *Staphylococcus aureus.*

Dilution of $HgCl_2$	Control	Glycine	Aspartic acid	Glutamic acid	Arginine	Lysine	Cystein
1:250	—	—	—	—	—	—	+
1:500	—	—	—	—	—	—	+
1:1000	—	—	—	—	—	—	+
1:2500	—	—	—	—	—	—	+
1:5000	—	+	+	+	+	+	+
1:7500	—	+	+	+	+	+	+
1:12,500	—	+	+	+	+	+	+
1:25,000	+	+	+	+	+	+	+
1:37,500	+	+	+	+	+	+	+

— = no growth; + = growth after 96 hours at 37°C.

distilled water, the solutions adjusted to pH 6.6, and sterilized in the autoclave. The results are recorded in Table III. Aminoids caused more inactivation than peptone and the latter more than gelatin. In view of the fact that Aminoids contains more highly hydrolyzed protein than peptone and, therefore, possesses more reactive groups per unit weight, the results are according to expectations.

TABLE III.

Effect of Aminoids, Peptone, and Gelatin on the Germicidal Efficiency of Mercuric Chloride Against *Staphylococcus aureus.*

Dilution of $HgCl_2$	Control	Aminoids	Peptone	Gelatin
1:2500	—	—	—	—
1:5000	—	+	—	—
1:7500	—	+	+	—
1:12,500	—	+	+	+
1:25,000	+	+	+	+

— = no growth; + = growth after 96 hours at 37°C.

Summary. The carbohydrates glucose, maltose, and sucrose produced no effect on the germicidal efficiency of mercuric chloride. The concentration required to kill *Staphylococcus aureus* was the same whether the sugars were present or absent.

The amino acids glycine, aspartic acid, glutamic acid, arginine, lysine, and cystein produced considerable inactivation of mercuric chloride. With the exception of cystein the acids produced the same degree of inactivation when employed in equimolar concentrations. Cystein produced more inactivation than the other acids because of the presence of a sulfhydryl group which is also capable of reacting with mercury.

Gelatin, Aminoids, and peptone, also produced considerable inactivation of mercuric chloride. The degree of inactivation was found to parallel the number of free amino groups present. The results show that the inactivation of mercuric chloride was dependent upon the presence of proteins, protein split products, and amino acids. Carbohydrates were without effect.