Prussian blue granules anywhere in the tissues. At 8 hours masses of blue granules are still to be seen tangled in mucus over the mucosa. Some absorption is still occurring through the olfactory cells and, very occasionally, between the cells. In the submucosa a very few granules can be seen in the tissue spaces or within lymphatics subjacent to areas of mucosa where absorption is still occurring. Along one or two branches of the olfactory nerve, small clumps of granules appear within the perineural sheath and similar clumps appear within the subarachnoid space. A few scattered granules occur in the pia-arachnoid over the olfactory bulb.

Absorption, therefore, occurs chiefly by way of the olfactory sensory cells and appears at its maximum in mice killed at 2 minutes. Passing chiefly along the nerve fibres, it has reached the subarachnoid space and the pia-arachnoid membrane within this time.

8626 C

Comparison of Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. VIII. Mercuric Chloride.

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The use of mercuric chloride as a disinfectant dates from the time of Robert Koch.[1] It has long been recognized as one of the most powerful disinfectants known. The germicidal power is exceedingly high in aqueous solution but enormously reduced in the presence of organic matter and some inorganic ions. It is precipitated by proteins and other organic compounds, hard water, alkalies, numerous salts, metals and sulfides.

It appears to be definitely established that mercuric chloride owes its germicidal properties to the positive or mercuric ion. This means that the compound is effective only in the dissociated state. Krönig and Paul[2] found that a 1-500 solution of the mercurial salt in water is much less than twice as active as a 1-1,000 dilution. The

1 Koch, R., Ueber Desinfection, Mittheil, Kaiserl. Gesendheitsant, Vol. 1, quoted from "A Handbook on Antiseptics," by H. D. Dakin, New York, The Macmillan Co., 1917.

2 Krönig, B., and Paul, Th., *Z. f. Hygiene*, 1897, **25**, 1.

addition of any substance suppressing ionization causes a decrease in its germicidal efficiency. Sodium or ammonium chloride is sometimes added to increase the stability of the compound, but the germicidal power is greatly reduced due to a decrease in ionization. It is not applicable to the disinfection of sputum, excreta, and the like due to the formation of a coagulum which prevents further penetration.

In previous papers of this series[3-9] comparisons were made of the resistance of *Staphylococcus aureus* and embryonic chick heart tissue to phenol, Merthiolate, Metaphen, Mercurochrome, Hexylresorcinol, iodine, iodine tri-chloride and potassium mercuric iodide. Indices of toxicity were determined by dividing the highest dilution of the germicide that killed the tissue by the highest dilution of the chemical preventing growth of the test-organism. Theoretically the smaller the index the more nearly perfect the chemotherapeutic agent.

The methods employed were the same as those given in the first paper of this series. Widely spaced dilutions were first prepared to determine approximately the least concentration of the germicide required to destroy the bacteria in 10 minutes but not in 5 minutes. Usually one such preliminary series was sufficient. Then a series of dilutions covering a narrow range was prepared to determine more accurately the least concentration necessary to kill the bacteria in the specified period of time. In every case the results were checked a second time. If the second series failed to check, the first tests were repeated until checks were obtained. The same procedure was followed to determine the last concentration of the germicide required to kill the living embryonic tissue, except that a period of 48 hours was used instead of 10 minutes.

A *S. aureus* phenol-coefficient was first determined for mercuric chloride by the method of Reddish.[10] Phenol killed *S. aureus* in a dilution of 1-65 in 10 minutes but not in 5 minutes. The highest dilution of mercuric chloride required to kill the test organism

[3] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 665.

[4] *Ibid.*, 937.

[5] *Ibid.*, 1057.

[6] *Ibid.*, 1119.

[7] *Ibid.*, 1481.

[8] *Ibid.*, **33**, 8.

[9] *Ibid.*, 393.

[10] Reddish, G. F., The Newer Knowledge of Bacteriology and Immunology, E. O. Jordan and I. S. Falk, University of Chicago Press, 1928.

under the same conditions was found to be 1-16,000. Therefore, the *S. aureus* phenol-coefficient was 246.

A considerable literature has been built up on the bactericidal efficiency of mercuric chloride. Since so much of it is contradictory no attempt will be made to review the results in this paper.

Cultures were prepared from chick heart tissue obtained from 9-day-old embryos. The fragments of tissue were embedded in guinea pig plasma in Carrel flasks. The various dilutions of phenol and mercuric chloride were made in dilute chick embryonic fluid. The plasma, after coagulation, was washed with Tyrode solution to remove the uncoagulable constituents, after which were added the various dilutions of germicide in embryonic fluid. Final observations were made at the end of 48 hours.

The results are summarized in Table I.

TABLE I.
Toxicity of Phenol and Mercuric Chloride to Chick Heart Tissue and Bacteria.

	Highest dilution showing no tissue growth = A	Highest dilution showing no growth of *S. aureus* = B	Toxicity index = A/B	*S. aureus*-phenol Coefficient
Phenol	1-840	1-65	12.9	
Mercuric chloride	1-45,000	1-16,000	2.8	246

Lambert[11, 12] employed human tissue from spleen and lymph glands embedded in a mixture of chick plasma and human serum. He found that the explants were killed by a 1-80,000 dilution of mercuric chloride while a concentration of 1-40,000 was required to kill *S. aureus*.

Later Lambert and Meyer[13] employed rabbit's splenic tissue embedded in homologous plasma. They found that splenic cells were more resistant to the action of the disinfectant than bacteria, the concentrations required being 1-2,500 and 1-10,000 respectively. They concluded that some of the classic antiseptics, including mercuric chloride, approached nearer the "ideal" than certain of the newer preparations.

The results given in Table I show that mercuric chloride is almost 3 times more toxic to tissues than to bacteria. However, it rates next to the iodine compounds and considerably superior to the newer organic mercurial preparation so far studied

[11] Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

[12] Lambert, R. A., *J. Am. Med. Assn.*, 1916, **67**, 1300.

[13] Lambert, R. A., and Meyer, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 429.

when tested by the tissue-culture method. The germicides may now be placed in the following order on the basis of their toxicity indices: iodine 0.09; iodine trichloride 0.40; mercuric chloride 2.8; Hexylresorcinol 3.0; Metaphen 12.7; phenol 12.9; potassium mercuric iodide 13.3; Merthiolate 35.3; Mercurochrome 262.0.

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Cysteine in Jensen's Sarcoma.

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With the stimulating effect of cysteine and other sulphydryl compounds upon tissue growth in mind, it was felt that dilutions of these compounds injected into rapidly growing neoplastic tissue might serve as a sufficient stimulation to accelerate maturity and subsequent death of cells. To evaluate the effect of sulphydryl radicals upon the growth of tumor-cells *in vivo*, a group of 6 rats was chosen which had been inoculated with Jensen's sarcoma from 10 to 21 days previously. These tumors varied in size from 4 to 9 cm. in diameter and all were in a normal state of growth, none showing necrosis or evidence of regression. Twenty mg. of a freshly prepared solution of cysteine hydrochloride was injected into the center of each of 5 of these tumors at 2-day intervals for 4 doses. The sixth was kept as a control. Forty-eight hours after the first dose, some tenseness and edema was observed with a subsequent and rather rapid necrosis of tissue in the center of the tumor in each case which subsequently progressed to the periphery. In every instance, all of the tumor-tissue died as the necrotic tissue was extruded, after which the skin healed normally over the ulcerated area. Six months have elapsed since the initial injections and there is no sign of tumor-recurrence.

This experiment has been repeated on 4 groups of animals with the same result following injection of sarcomatous tumor-tissue with cysteine hydrochloride. In each group, except the first, 5 rats were injected and 2 animals maintained as controls. In each case, the control-group showed the usual rate of tumor-growth with no evidence of regression and gradual, progressive enlargement of the tumors until the animals died from them, while the injected tumors regressed and disappeared. No reasonable explanation for this regressive action is at hand.