of Richter and collaborators the area of low resistance in the skin is related to sympathetic innervation and to the distribution of sweat glands. These areas expand in conditions associated with increased sympathetic activity such as muscular exercise and emotional excitement, and decrease in sleep. Cold and warm tub baths respectively decrease and increase the size of the low resistance area. In the light of these observations the reversible enlargement of the areas of low resistance under the influence of muscle pain indicates that the latter increases sympathetic discharges. This effect is likewise observable under conditions of cutaneous pain. There is, however, another interpretation possible which could point to the relationship between these effects of pain and emotion and attribute to the emotional disturbances induced by pain the changes described in this paper. This objection is held invalid for two reasons: first, the persons on whose experimental records this investigation is based were selected from a larger group because of their emotional stability and low sympathetic excitability as indicated by the size of the low resistance area of the skin. Richter mentions that the low resistance area comprises the whole volar surface of the skin. We find this to be true only for rather excitable persons who were excluded from this series for this reason. Secondly, the experiments were performed repeatedly on the same subjects with very similar results although emotional disturbances would be apt to decline on repetition.

Richter points out that the distribution of the low resistance area in the skin does not reflect either the distribution of peripheral nerves or that of spinal dermatomes, but rather that of cortical patterns of autonomic innervation. This statement seems to be valid for the changes seen as a result of muscle or cutaneous pain. It is of interest to mention in this connection an earlier investigation which demonstrated an increased responsiveness of the motor cortex to electrical stimulation as a result of muscle pain.[1]

Summary. Muscle or cutaneous pain induced by movements of muscles in ischemia or immersion of ischemic fingers in hot water increases the area of low resistance of hand, forearm, and face reversibly. The effects indicate increased sympathetic discharges. The pattern suggests the involvement of supraspinal mechanisms.

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Increased Germicidal Efficiency of Iodine in the Presence of an Oxidation-Reduction System.

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In a previous communication[1] it was shown that inorganic salts of iron, manganese, and tin, when tested individually, exhibited very little or no germicidal activity against the test organism *Staphylococcus aureus*. However, if an oxidized and a reduced salt, such as ferric and ferrous sulfates, were dissolved in water to form an oxidation-reduction system, the solution exhibited a pronounced germicidal action. The phenomenon was shown to be a function of the positive metallic ions; the negative ions apparently played no part in the reaction. For example, a mixture of sodium sulfate and sodium sulfite exhibited no increase in germicidal activity over that of the same salts tested individually.

The salts must be combined in certain definite proportions for maximum germicidal activity to occur. In the case of the chlorides of iron, the most effective germicidal activity occurred when the salts were mixed in the proportion of two moles of ferric chloride and one

[1] Guest, Howard L., and Salle, A. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 272.

mole of ferrous chloride. The sulfates of iron behaved in a similar manner, the most effective combination being in the proportion of one mole of each salt. Stannous and stannic chlorides also functioned most effectively in the proportion of one mole of each.

The germicidal effect was not limited to combinations having a common metallic ion. Metallic salts of different cations and anions were crossed with the same general effect. The important consideration was to have an oxidation-reduction system for increased germicidal activity to occur.

In a later communication[2] it was shown that the addition of an appropriate metallic salt to an organic germicide, to produce an oxidation-reduction system, resulted in a great increase in the efficiency of the germicide. For example, the addition of ferric sulfate to phenol increased the germicidal potency of the latter 18 times. The addition of another oxidation-reduction system, composed of a mixture of ferrous and ferric chlorides, to the ferric sulfate-phenol combination resulted in a further increase in the potency of the latter approximately 45 times.

Experimental. The oxidation-reduction (o-r) system used was composed of equimolar quantities of manganous sulfate ($MnSO_4 \cdot H_2O$) and ferric sulfate ($Fe_2(SO_4)_3 \cdot 6H_2O$). These two salts produced a stable o-r solution. The solution was observed for over a year without any change in color, appearance, and strength. The solution was prepared by dissolving 50 g of the combined salts (12.5 g $MnSO_4 \cdot H_2O$ + 37.5 g $Fe_2(SO_4)_3 \cdot 6H_2O$) in sufficient distilled water to make 1000 cc. This was considered a 1:20 dilution.

A stock solution of iodine was prepared by dissolving 5 g of iodine and 10 g potassium iodide in sufficient distilled water to make 1000 cc. This was considered a 1:200 dilution.

The 24-hour cultures of *Staphylococcus aureus* and *Eberthella typhosa* were standardized in a photoelectric colorimeter before use. Turbidity standards of barium sulfate were prepared according to the McFarland nephelometric method. The cultures were diluted with broth until they gave the same reading as a No. 7 standard.

TABLE I.
Toxicity of Iodine and the Oxidation-Reduction Solution for Embryonic Chick Heart Tissue Fragments.

Solution	Killing dilution for tissue
O-R solution alone	1:2000
Iodine + 1:3000 o-r	1:3750 iodine

All bactericidal and tissue toxicity tests were carried out in a shaking water bath at a temperature of 37°C and the exposure period was 10 minutes.

The effect of the o-r solution alone and with iodine on embryonic chick heart tissue fragments was determined by the tissue culture technic.[3] A dilution of 1:2000 o-r solution killed the tissue fragments. In order to evaluate the killing dilution of iodine in combination with the o-r system, it was necessary to use a more dilute solution of the metal salts. Accordingly, a 1:3000 dilution of the o-r solution was employed in the test. Iodine in combination with a 1:3000 dilution of the o-r solution killed chick heart tissue fragments in a dilution of 1:3750 of iodine. The results are given in Table I.

The killing dilutions of iodine for *Staph. aureus* and *E. typhosa*, in the absence of organic matter, were 1:20,000 and 1:17,500 respectively. In the presence of organic matter the dilutions were reduced to 1:2000 for *Staph. aureus* and 1:1750 for *E. typhosa*. The killing dilutions of the o-r solution for *Staph. aureus* and *E. typhosa* in the absence of organic matter were 1:75 and 1:1000 respectively. In the presence of organic matter these figures became 1:55 for *Staph. aureus* and 1:250 for *E. typhosa*. Since a 1:75 dilution of the o-r solution killed *Staph. aureus* it was necessary to combine a more dilute solution with iodine to make it possible to find the killing dilution of the combination. Iodine combined with a 1:100 o-r solution killed *Staph. aureus* in a dilution of 1:450,000 of iodine in the absence of organic matter, and 1:30,000 in its presence. Iodine combined with a 1:3000 o-r solution (the same strength as used in the tissue toxicity tests) killed

[2] Salle, A. J., and Guest, Howard L., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 26.

[3] Salle, A. J., McOmie, W. A., Shoehmeister, I. L., and Foord, D. C., *J. Bact.*, 1939, **37**, 639.

TABLE II.
Killing Dilution of Iodine Alone and in Combination with an Oxidation-Reduction System.

Solution	Killing dilution: Without organic matter	Killing dilution: With organic matter*
Staphylococcus aureus		
Iodine alone	1:20,000	1:2,000
O-R solution alone	1:75	1:55
Iodine + 1:3,000 o-r	1:80,000 I	1:5,000 I
Iodine + 1:100 o-r	1:450,000 I	1:30,000 I
Eberthella typhosa		
Iodine alone	1:17,500	1:1,750
O-R solution alone	1:1,000	1:250
Iodine + 1:3,000 o-r	1:60,000 I	1:4,500 I

* The organic matter consisted of a mixture of 3 parts embryonic extract and 1 part defibrinated horse serum.

The embryonic extract was prepared by mincing 12-days-old chick embryos, diluting the pulp to 5 times its volume with Tyrode's solution, and centrifugating the mixture. The clear supernatant liquid is the extract.

Staph. aureus in a dilution of 1:80,000 and *E. typhosa* in a dilution of 1:60,000, both in the absence of organic matter. In the presence of organic matter *Staph. aureus* was killed in a dilution of 1:5000 and *E. typhosa* in a dilution of 1:4500 of iodine. The results are recorded in Table II.

Toxicity indexes may be calculated from the results of the bactericidal and tissue toxicity tests. The Toxicity Index may be defined as the ratio of the highest dilution of germicide required to kill embryonic chick heart tissue fragments in 10 minutes at 37°C to the highest dilution required to kill the test organism under the same conditions. Theoretically, an index less than one means that the germicide is more toxic to bacteria than to tissue; an index greater than one means that the germicide is more toxic to tissue than to bacteria. The smaller the Toxicity Index the more nearly perfect the germicide. The results are recorded in Table III.

It may be seen that although the toxicity indexes of the o-r solution are higher than those of iodine alone[4] the toxicity indexes of the combinations of iodine and o-r solution are considerably lower than those of the two components tested individually. The figures show that iodine with the o-r solution is 4 times more efficient than iodine alone.

This observation possesses considerable importance. It means that less iodine is required to produce an effective germicidal solution. A 1:500 solution of iodine with a proportionate amount of the o-r salts should eliminate entirely any tendency of iodine to burn or irritate and still be a powerful germicide. Because of its very low tissue toxicity, freedom from bacteriostasis and cumulative action,[5] such a solution should prove of great value for clinical application as well as for general use.

After this work was completed it was noted that the mixture of iodine and the o-r salts produced a slight precipitate on long standing. By using an o-r solution composed of the chlorides of manganese and iron instead of the sulfates precipitation did not occur.

The o-r solution now recommended is prepared by dissolving 50 g of combined salts (13.4 g $MnCl_2 \cdot 4H_2O$ + 36.6 g $FeCl_3 \cdot 6H_2O$) in sufficient distilled water to make 1000 cc. This was considered a 1:20 dilution.

Iodine in combination with a 1:3000 dilution of the new o-r solution (o-r II) killed *Staph. aureus* in a dilution of 1:120,000 and *E. typhosa* in a dilution of 1:50,000 of iodine. Iodine in combination with a 1:3000 dilution of the o-r II solution killed chick heart tissue fragments in a dilution of 1:5000 of iodine. The results are recorded in Table IV.

The figures show that the o-r II system in combination with iodine gave a smaller Toxicity Index against *Staph. aureus* and a larger one against *E. typhosa*. Since *Staph. aureus* is considered a more important organism for the evaluation of germicides it may be concluded that the new o-r solution not only produced a stable solution with iodine but also improved the efficiency of the latter.

Conclusions. In the absence of organic matter, iodine killed *Staphylococcus aureus* in a dilution of 1:20,000 and *Eberthella typhosa* in a dilution of 1:17,500 in 10 minutes at 37°C. In the presence of organic matter the dilutions were reduced to 1:2000 for *Staph. aureus* and 1:1750 for *E. typhosa.*

Iodine dissolved in a 1:3000 dilution of an oxidation-reduction solution (composed of a

[4] Salle, A. J., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 141.

[5] Salle, A. J., *J. Pharm. and Exp. Therap.*, 1943, **79**, 271.

TABLE III.
Toxicity Indexes of Iodine Alone and in Combination with an Oxidation-Reduction System.

Solution	Killing dilution			Toxicity index	
	Tissue (A)	*Staph. aureus* (B)	*Eberthella typhosa* (C)	*Staph. aureus* A/B	*Eberthella typhosa* A/C
Iodine alone	1:4,000	1:20,000	1:17,500	0.20	0.23
O-R solution alone	1:2,000	1:75	1:1,000	26.60	2.00
Iodine + 1,3000 o-r	1:3,750 I	1:80,000 I	1:60,000 I	0.05	0.06

TABLE IV.
Toxicity Indexes of Iodine in Combination with an Oxidation-Reduction System (O-R II).

Solution	Killing dilution			Toxicity index	
	Tissue (A)	*Staph. aureus* (B)	*Eberthella typhosa* (C)	*Staph. aureus* A/B	*Eberthella typhosa* A/C
Iodine + 1:3,000 o-r II	1:5,000	1:120,000	1:50,000	0.042	0.1

mixture of one mole each of manganous sulfate and ferric sulfate) killed *Staph. aureus* in a dilution of 1:80,000 and *E. typhosa* in a dilution of 1:60,000, both in the absence of organic matter. In the presence of organic matter the killing dilutions were 1:5000 and 1:4500 respectively.

A dilution of 1:2000 of the o-r solution killed embryonic chick heart tissue fragments in 10 minutes at 37°C. Iodine dissolved in a 1:3000 o-r solution killed the tissue fragments in a dilution of 1:3750 of iodine.

Toxicity indexes may be calculated by dividing the highest dilution of iodine required to kill the tissue by the highest dilution required to kill the test organism under the same conditions. The Toxicity Index of iodine + 1:3000 o-r solution was 0.05 for *Staph. aureus* and 0.06 for *E. typhosa*. The Toxicity Index of iodine alone was 0.2 for *Staph. aureus* and 0.23 for *E. typhosa*. Theoretically, an index less than one means that the germicide is more toxic to bacteria than to tissue; an index greater than one means that the germicide is more toxic to tissue than to bacteria. The smaller the index the more nearly perfect the germicide.

When iodine was dissolved in a 1:3000 o-r solution composed of manganous and ferric chlorides instead of the sulfates, the Toxicity Indexes were 0.042 for *Staph. aureus* and 0.1 for *E. typhosa*.

The importance of this observation is discussed.

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Attempts to Find Poliomyelitis Virus in Fish.*

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Each year since 1932 we have searched in vain for poliomyelitis virus in the gastrointestinal contents of fish caught in waters near epidemic areas with consistently negative results. There might have been several reasons for our failures. (1) The gastrointestinal contents used in our experiments

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