TABLE II.
% O_2 Saturation Venous Blood in Arteriosclerotic Subjects with Intermittent Claudication.

Cases	Rest Period		Pain Period	
	Saphenous %	Popliteal %	Saphenous %	Popliteal %
1	75	51	44	42
2	71	38	88	25
3	80	42	72	35
4	55	46	80	35
5	72	35	65	32
6	82	56	67	63
7	38	32	32	35
8	86	89	78	74
9	68	49	68	44
Average	69.6	49.7	66	62

to 69.6% in arteriosclerotic subjects, and after exercise was 54%, as opposed to 66.6% in arteriosclerotic subjects. In normal subjects the average percentage of oxygen saturation of the popliteal venous blood during rest was 40.6%, as opposed to 49.7% in arteriosclerotic subjects, and after exercise was 33.3%, as opposed to 42% in arteriosclerotic subjects.

Conclusions. Determinations of the oxygen saturation of the venous blood reveal a lower oxygen content in a series of normal individuals after exercise than in a series of arteriosclerotic subjects with intermittent claudication. If intermittent claudication in arteriosclerotic subjects is produced by a tissue anoxemia, it cannot be demonstrated by determinations of the oxygen content of the superficial and deep venous blood.

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An Improved Method for the Evaluation of Germicidal Substances.

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In previous papers[1,2] a new method was described for evaluating the efficiency of germicides by testing them for their effect on the

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

[2] Salle, A. J., McOmie, W. A., and Shechmeister, I. L., *J. Bact.*, 1937, **34**, 267.

growth of living embryonic tissue and on bacteria. A number known as the toxicity-index was determined which was defined as the ratio of the highest dilution of disinfectant that showed no growth of embryonic tissue in 48 hours to the highest dilution required to kill the test-organism in 10 minutes. Theoretically the smaller the index the more nearly perfect the germicide.

The present method attempts to control several variables which were not considered in the previous work. These are: (1) performing all tests at 37°C. instead of at 25°C.; (2) keeping the time of action of germicide on tissue and bacteria the same, *viz.*, 10 minutes; (3) conducting all tests in the presence of a definite amount of organic matter.

Effect of Germicide on Tissue. Chick hearts are removed from 10-12-day-old embryos and minced into fragments about 0.5 mm. in diameter. The fragments are suspended in Tyrode's solution, about 100 pieces per cc. One-half cc. of suspension is pipetted into each of a series of tubes containing 2.5 cc. of a mixture of 3 parts embryonic extract* and 1 part defibrinated horse serum. The tubes are placed in a 37°C. waterbath.

A series of aqueous dilutions of the germicide is prepared and heated to 37°C. Two cc. of each dilution are transferred to the tubes containing the tissue-serum-extract mixture.† Final volume, 5 cc.

After 10 minutes the tubes are removed from the bath and the germicidal dilutions aspirated from the sedimented fragments. The fragments are resuspended in Tyrode's solution and the supernatant fluid again aspirated. Finally 1 cc. of Tyrode's solution is added to each tube and the fragments embedded in plasma in Carrel flasks.[1] The killing dilution is determined by the absence of proliferating cells after 72 hours or longer.

Effect of Germicide on Bacteria. One-half cc. of a 22- to 26-hr. broth culture of the test-organism is pipetted into each of a series of tubes containing 2.5 cc. of a mixture of 3 parts embryonic extract and one part horse serum. The tubes are brought to 37°C. in a waterbath.

* The extract is prepared by mincing 12-day embryos, diluting to 5 times its volume with Tyrode's solution, and centrifugating. The clear supernatant liquid is the extract.

† The germicide must be added to the mixture last since this is the order of action in clinical practice. Most germicides are considerably inactivated in the presence of organic matter. If the germicide is added to the fragments first, and then the organic material, no inactivation of the germicide will occur with the result that a smaller concentration of the chemical will produce the same effect.

A series of aqueous dilutions of germicide in tubes is also brought to 37°C. Two cc. of each dilution of germicide are transferred to the tubes containing the bacteria-serum-extract mixture. Final volume, 5 cc.

After 10 minutes the tubes are removed from the bath and a 4-mm. loopful transferred from each tube to broth. To eliminate any bacteriostatic effect subcultures are made to either 100 cc. amounts of broth or to a 15-cc. tube of broth followed by transfer of 4 loopfuls from this tube to a second one. The killing dilution is determined by the absence of growth in broth subcultures after 72 hours or longer.

Phenol, iodine, and hexylresorcinol were free from any appreciable bacteriostatic effect while the mercurial compounds metaphen, mercurochrome, and merthiolate showed a considerable effect (Table I).

TABLE I.
Bacteriostatic Effect of Some Mercurial Compounds.

Organism	Germicide	Killing concentration in 10 minutes at 37°C + organic matter		
		First transfer 15 cc. broth	Second transfer 15 cc. broth	100 cc. broth
Staph. aureus	Metaphen	1795	1450	1370
" "	Merthiolate	1040	25*	83
" "	Mercurochrome	13	—	13*
E. typhosa	Metaphen	6730	—	5665
" "	Merthiolate	3980	2660	2830
" "	Mercurochrome	175	156	140

*This concentration failed to kill all of the organisms.

Assuming that the volume of a 4-mm. loop is 0.005 cc., the final dilution of a 1:100 solution of a germicide is as follows:

First transfer—15/0.005 × 100 = 1:300,000
Second transfer—15/0.02 × 300,000 = 1:225,000,000
100 cc. broth—100/0.005 = 1:2,000,000

Although the secondary transfer method gives the greatest dilution, it is subject to chance when a large percentage of the organisms are killed. Assuming 5×10^8 bacteria are added, when the final dilution is made 5×10^7 organisms would be exposed to the germicide (bacterial suspension diluted 10 times). If 99% of the organisms are killed 5×10^5 bacteria would still be viable. One loopful would contain $0.005 \times 5 \times 10^5$ or 2500 bacteria per cc. This would be added to 15 cc. of broth giving 2500/15 or 166 bacteria per cc. Four loopfuls from the first to the second tube would transfer $4 \times 0.005 \times 166$ or 3.2 bacteria. In many instances it is quite likely that no bacteria would be transferred.

In carrying out tests for germicidal activity it is necessary to distinguish between bactericidal action and bacteriostasis. The second-transfer method is more convenient to use but in the presence of a few viable cells it is not always certain that the transfer of organisms has occurred. The flasks appear to be more reliable and the dilution obtained should be sufficient to overcome any bacteriostasis.

All germicides were tested in aqueous solutions. This eliminated any germicidal effect of alcohol or other toxic solvent.

The killing concentrations of various germicidal preparations for bacteria and tissue and their toxicity-indices are given in Table II.

The order of the toxicity-indices differs with the 2 organisms, but agrees very closely with the ratings obtained by the original method.[2]

TABLE II.
Killing Concentrations of Germicides for Tissue and Bacteria and Their Toxicity Indices in 10 Minutes at 37°C.†

Germicide	Tissue (A)	*Staph. aureus* (B)	T.I. (A/B)	*E. typhosa* (B)	T.I. (A/B)
Iodine (Lugol's solution)	1:650	1:3520	0.2	1:3370	0.2
Hexylresorcinol	1:1450	1:1620	0.9	1:1770	0.8
Metaphen	1:2070	1:1370	1.5	1:5665	0.4
Phenol	1:224	1:110	2.0	1:186	1.2
Mercurochrome	1:90	1:12½*	7.2+	1:140	0.6
Merthiolate	1:4220	1:25*	169+	1:2660	1.6

* Failed to kill. A more concentrated solution could not be prepared.
†All results are the average of 4 determinations.

This method aims to measure the relative efficiency of germicides which are to be used to kill bacteria in the presence of living tissue. While it is not claimed that actual conditions are duplicated, it is believed that the procedure approaches nearer an "ideal" *in vitro* method than any which has been reported. The organic matter is a mixture of serum and embryonic extract, a medium somewhat analogous to the tissue-fluid found *in vivo*. The correct proportion of organic matter cannot be known since it varies greatly with different infections and conditions. The 10-minute period of exposure is probably the maximal time of effective germicidal action on a cut surface.

Of the compounds examined by this method iodine showed the lowest toxicity-index and merthiolate the highest. Iodine (in aqueous solution) appears to be the best germicide of those tested. It combines low tissue-toxicity with high bactericidal potency against both gram+ *(Staph. aureus)* and gram— *(E. typhosa)* organisms. Of the newer organic preparations hexylresorcinol was

more effective against *Staph. aureus* (gram+) while metaphen gave a better score against *E. typhosa* (gram—).

The toxicity-index indicates the relative efficiency of germicides. It does not mean that a germicide with a low toxicity-index will be harmless to living tissue-cells at any concentration. Due consideration should be given to the killing dilutions of the germicides, in the presence of organic matter, on both tissue and bacteria. The germicides, when actually used, should be diluted to the proper strengths. It may be seen from the results that a 1:100 dilution of iodine would be more toxic to tissue than a 1:100 dilution of mercurochrome. However, if a 1:1,000 solution is used, the iodine would be harmless to tissue but still bactericidal, whereas mercurochrome, though not harmful to tissue, would exert no bactericidal effect. On the basis of the results, mercurochrome and merthiolate cannot be relied upon as germicides to kill *Staph. aureus* in the presence of organic matter as used in the above experiments. However, the two germicides rate considerably better against *E. typhosa,* mercurochrome giving a lower toxicity index than merthiolate.

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A New Method of Arterial Anastomosis for Acute Experiments Without Use of an Anticoagulant.

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In cross-circulation experiments and in acute transplantation experiments, it is often necessary to use coagulable blood because surgery is contemplated after the blood vessels have been connected. Various methods have been proposed to avoid thrombosis and assure adequate blood flow in such arterial anastomoses.[1] End-to-end anastomosis with silk sutures is satisfactory but is too time-consuming for acute experiments. Arterial anastomosis by means of Payr cannulae, the method recommended by Heymans and his coworkers,[2] proved unsatisfactory in our laboratory.

The method of arterial anastomosis to be described is very simple and can be performed rapidly. It has been successful every time it

[1] Markowitz, J., *Experimental Surgery,* Wm. Wood and Co., 1937.

[2] Heymans, C., Bouckaert, J. J., and Regniers, P., *Le Sinus Carotidien et la zone homologue cardio-aortique,* G. Doin et Cie, Paris, 1933.