

THE EFFECT OF CERTAIN SURGICAL ANTISEPTICS  
AND THERAPEUTIC AGENTS ON PHAGOCYTOSIS.

I. CARBOLIC ACID, MERCURIC CHLORIDE,  
BORIC ACID, QUININE HYDROCHLORIDE.<sup>1</sup>

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The present wide interest in opsonotherapy and belief in the importance of phagocytosis in immunity, make it desirable to determine the effect of the commoner medicinal agents on phagocytic power. The effect of the three most important surgical antiseptics, namely, mercuric chloride, carbolic acid and boric acid, and of the most widely used internal remedy in acute infections, quinine hydrochloride, have thus far been determined, and are here presented.

*Material and Technique.*—Except in the one experiment herein-after specially mentioned, the material used in this study was defibrinated human blood and streptococci suspended in physiological saline (0.85 per cent. sodium chloride). The streptococci were in all cases obtained from twenty-four to forty-eight hour cultures on blood serum (goat), sterilized at 100° C.

To test the effect of a chemical substance on phagocytosis, increasing amounts of a solution of that substance (whenever possible equimolecular with 0.85 per cent. sodium chloride) were added to a number of test-tubes, and the volumes in the tubes made constant (1 c.c.) with physiological saline. There was then added to each tube a constant amount (1 c.c.) of freshly drawn, defibrinated blood and, at stated intervals, a constant volume (1 c.c.) of streptococcus suspension.

Each tube was immediately immersed in a thermostatic water bath at 37.5° C., and fifteen, thirty and sixty minutes later, smears

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were made from it. These smears were stained by Wright's method and the number of bacteria in sixty polymorphonuclear leucocytes in each smear counted.

*Experimental Error.*—In beginning quantitative work in any field of biological chemistry, it is necessary, first of all, to determine the accuracy of the proposed experimental method—the limits of the experimental error. Without such determination, deductions from experimental data are comparatively valueless. An experiment was therefore undertaken to determine the range of error in work by the above technique.

To do this, ten duplicate tubes were prepared, each containing a mixture of equal parts (1 c.c.) of physiological saline, defibrinated human blood and streptococcus suspension. Smears were made from these tubes and counted, exactly as in the proposed investigation.<sup>2</sup> The data from this experiment are given in Table I.

TABLE I.

*Duplicate Bacterial Counts.*

	1	2	3	4	5	6	7	8	9	10	Average.	
15 Minute Counts	Wright's Index	5.63	5.58	6.18	5.23	5.87	5.83	5.72	5.27	5.53	5.21	5.61
	Error	+ .02	— .03	+ .57	— .38	+ .26	+ .22	+ .11	— .34	— .08	— .40	± .24
	Percentage Error	+ 0.4	— 0.5	+ 10.0	— 6.8	+ 4.6	+ 3.9	+ 2.0	— 6.1	— 1.4	— 7.1	± 4.3
30 Minute Counts	Wright's Index	[9.63]	8.40	7.65	7.48	7.38	7.35	7.88	8.15	7.83	7.50	7.73
	Error	[+1.90]	+ .67	— .08	— .25	— .35	— .38	+ .15	+ .42	+ .10	— .23	± .29
	Percentage Error	[+24.6]	+ 8.7	— 1.0	— 3.2	— 4.5	— 4.9	+ 1.9	+ 5.4	+ 1.3	— 3.0	± 3.8
60 Minute Counts	Wright's Index	12.60	11.80	12.15	12.05	11.93	11.35	11.48	10.87	11.03	11.08	11.64
	Error	+ .96	+ .24	+ .51	+ .41	+ .29	— .29	— .16	— .77	— .61	— .56	± .48
	Percentage Error	+ 8.2	+ 2.1	+ 4.4	+ 3.5	+ 2.5	— 2.5	— 1.4	— 6.6	— 5.2	— 4.8	± 4.1

From this table it is seen that the average error in the fifteen minute counts is 4.3 per cent., with a maximum error of 10 per cent. In the thirty minute counts, with the exception of a single smear, the average error is 3.8 per cent., and the maximum 8.7 per

<sup>2</sup>This experiment, of course, was not attempted till the technique had been mastered by preliminary work.

cent., the one exceptional smear giving an error of 24.6 per cent. In the sixty minute counts, the average error is 4.1 per cent., the maximum 8.2 per cent.

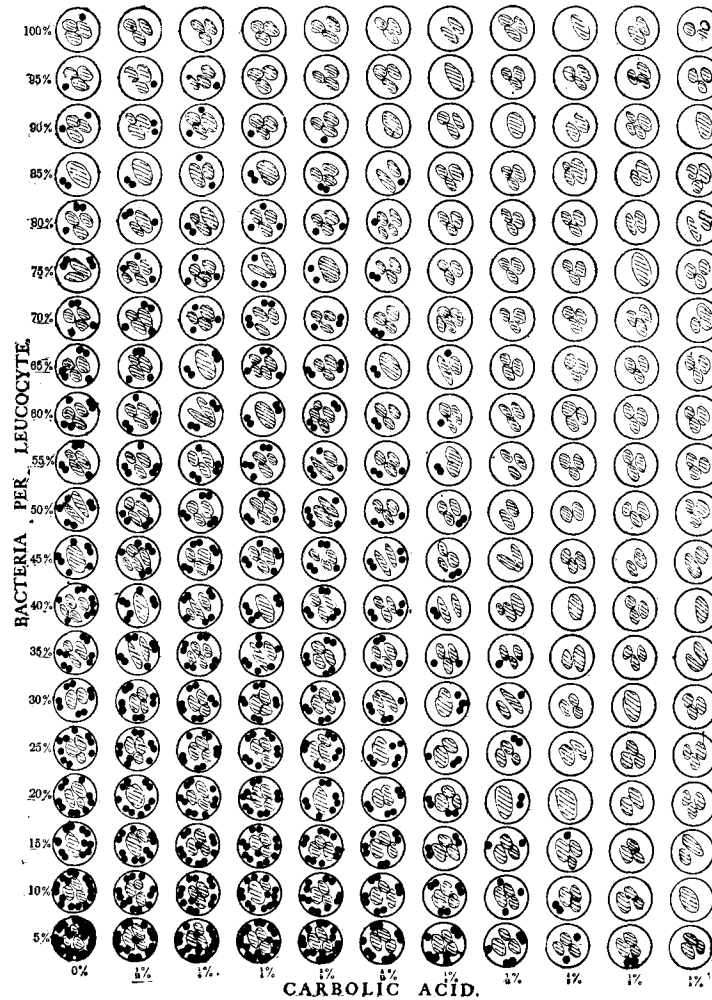


FIG. 1. Influence of carbolic acid on phagocytosis. Human leucocytes; average of 15, 30 and 60 minute counts.

In interpreting data obtained by the above technique, therefore, allowance must be made for a usual maximum error of about 10 per cent., but an occasional error (one count in thirty) of as much

as 25 per cent. must be expected. To eliminate this large experimental error, the average of a large number of data must be used.

*Graphic Representation.*—In recording data in most fields of quantitative chemistry, it is desirable to select a graphic method of representation. Such a method for the phenomenon of phagocytosis, must give not only the average number of the bacteria per leucocyte, and the number of leucocytes ingesting bacteria, but the numerical distribution of the bacteria among the different leucocytes, as well.

The graphic method selected is shown in the column "o/o" of Fig. 1. This column consists of twenty diagrammatic leucocytes,

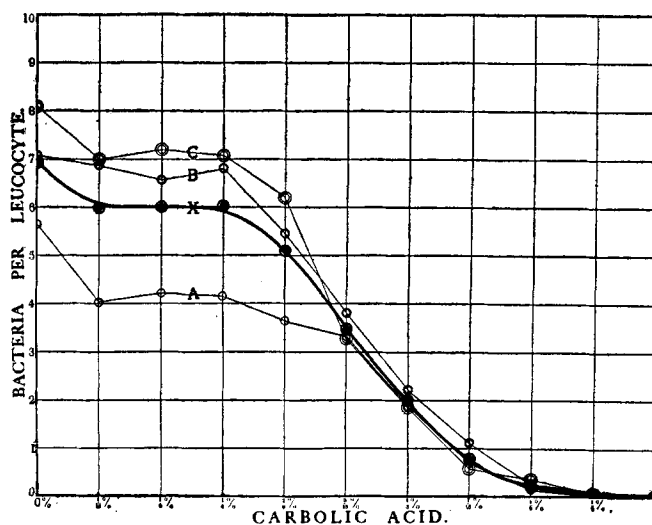


FIG. 2. Influence of carbolic acid on Wright's index. Data from Figure 1, A = 15-minute counts; B = 30-minute counts; C = 60-minute counts; X = average.

each leucocyte representing 5 per cent. of the polymorphonuclear neutrophiles present in a given smear. In each leucocyte is marked the average number of bacteria found in its corresponding 5 per cent.

*Carbolic Acid.*—The average of the three counts obtained by the above technique, with carbolic acid, gives the data recorded in Fig. 1. These data can be translated in terms of the currently used

Wright's index, by calculating the average number of bacteria taken up per leucocyte. Such calculations are shown graphically by the broken lines *A*, *B* and *C* of Fig. 2. The average of these observa-

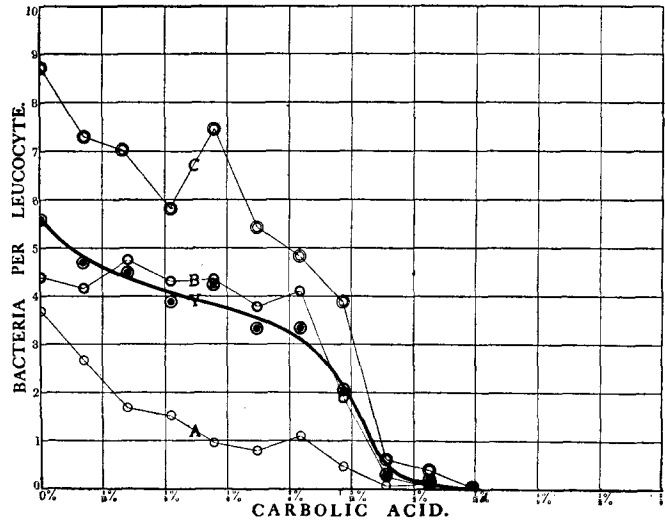


FIG. 3. Influence of carbolic acid on Wright's index. Rabbit leucocytes. *A*, *B* and *C* = 15-minute, 30-minute and 60-minute counts; *X* = average.

tions, plotted as smooth curves to eliminate experimental errors, as is customary in physical experimentation, is given in the heavy

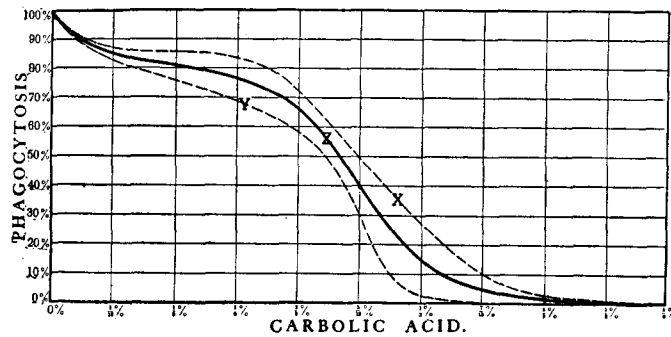


FIG. 4. Influence of carbolic acid on Wright's index; Curves *X* and *Y*, of Figs. 2 and 3, plotted to the same scale. *Z* = average.

curve *X*. A similar curve, obtained with rabbit blood in place of human blood, is given in Fig. 3. The curves of these two experi-

ments, reduced to percentage curves, by taking the phagocytosis in the control tubes of each experiment as 100 per cent., are shown in Fig. 4.

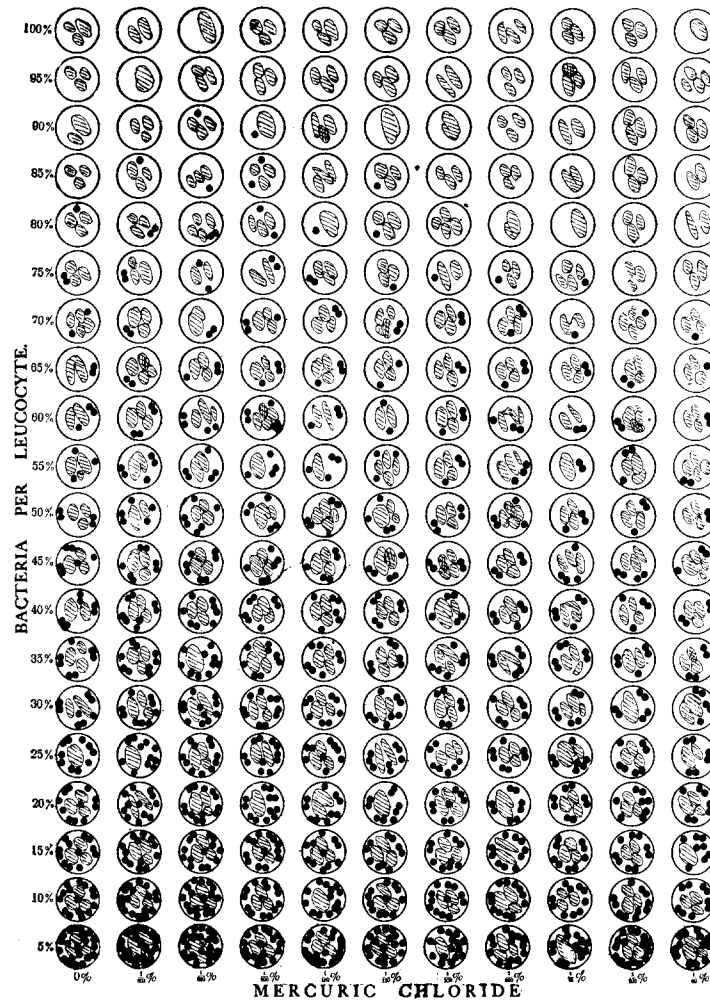


FIG. 5. Influence of mercuric chloride on phagocytosis. Human leucocytes; average of 15- and 30-minute counts.

From this figure, it is seen that the addition of carbolic acid to the experimental tubes causes, from the first, a decrease in phagocytic power, phagocytosis falling off about one third by the time the

carbolic acid reaches a concentration<sup>3</sup> of 2/9 per cent. A further increase in carbolic acid causes an almost precipitous fall in phagocytic power, phagocytosis being reduced seven eighths by the time

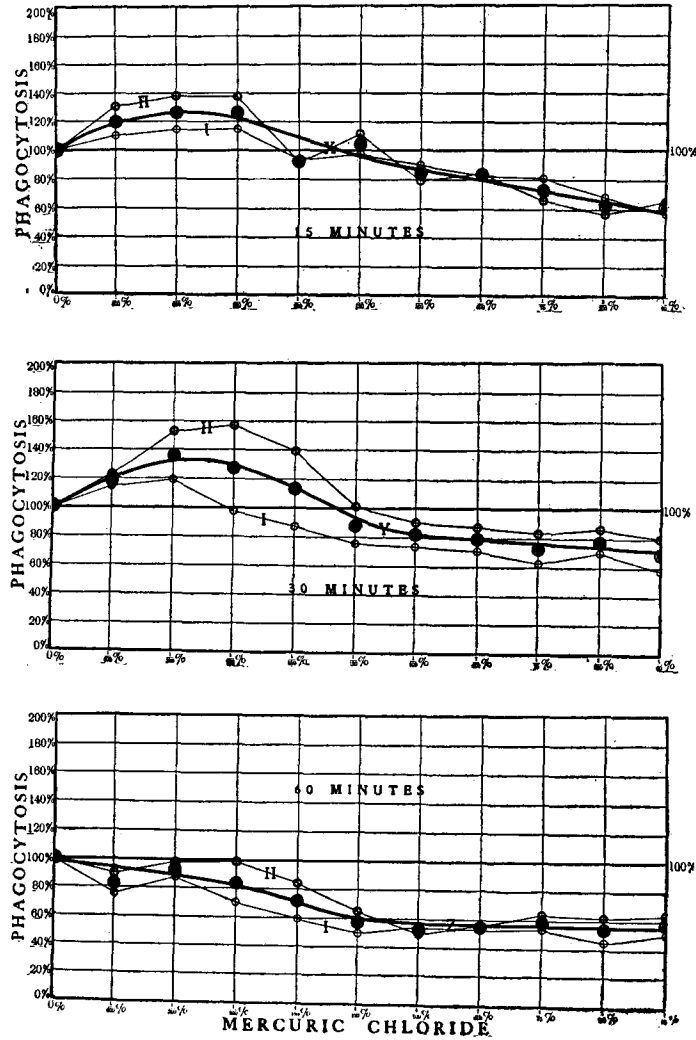


FIG. 6. Influence of mercuric chloride on Wright's index. 15, 30- and 60-minute data plotted separately. *I* = data from first experiment; *II* = data from second experiment; *X*, *Y* and *Z* = averages.

<sup>3</sup> Concentration measured in terms of final dilution.

the concentration reaches one third per cent., and entirely ceasing at one half per cent.

*Mercuric Chloride.*—The average of the fifteen and thirty minute counts, in two experiments with mercuric chloride are given in Fig. 5. Since the sixty minute counts and the earlier counts in these experiments show different effects of mercuric chloride, the influence of this antiseptic on phagocytosis can not be represented by a single curve, as was done with carbolic acid. Three percentage curves were therefore plotted, showing the changes in Wright's index, at the end of fifteen, thirty and sixty minutes. These curves are shown in Fig. 6. In each curve 100 per cent. stands for the

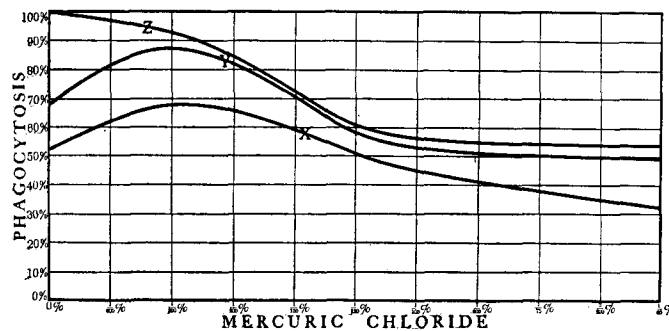


FIG. 7. Influence of mercuric chloride on Wright's index. Curves of Fig. 6 plotted to the same scale.

phagocytosis in the control tubes, containing no antiseptic.

These three curves may be reduced to the same scale by taking the normal sixty minute phagocytosis as 100 per cent. Thus reduced, they are shown in Fig. 7. From this figure, it is seen that mercuric chloride causes, in small amounts, a preliminary stimulation in phagocytosis, followed by a depression. The maximum preliminary stimulation is obtained with a bichloride concentration of 1/300 per cent. As the concentration increases beyond this amount, the preliminary stimulation decreases, and completely disappears at 1/120 per cent.

A further increase in mercuric chloride causes a pronounced fall in phagocytic power, early phagocytosis being reduced 50 per cent. by the time the concentration reaches 1/60 per cent. With con-



centration of 1/200 per cent. and over, phagocytosis practically ceases at the end of thirty minutes.

*Boric Acid.*—The average of the fifteen and thirty minute counts

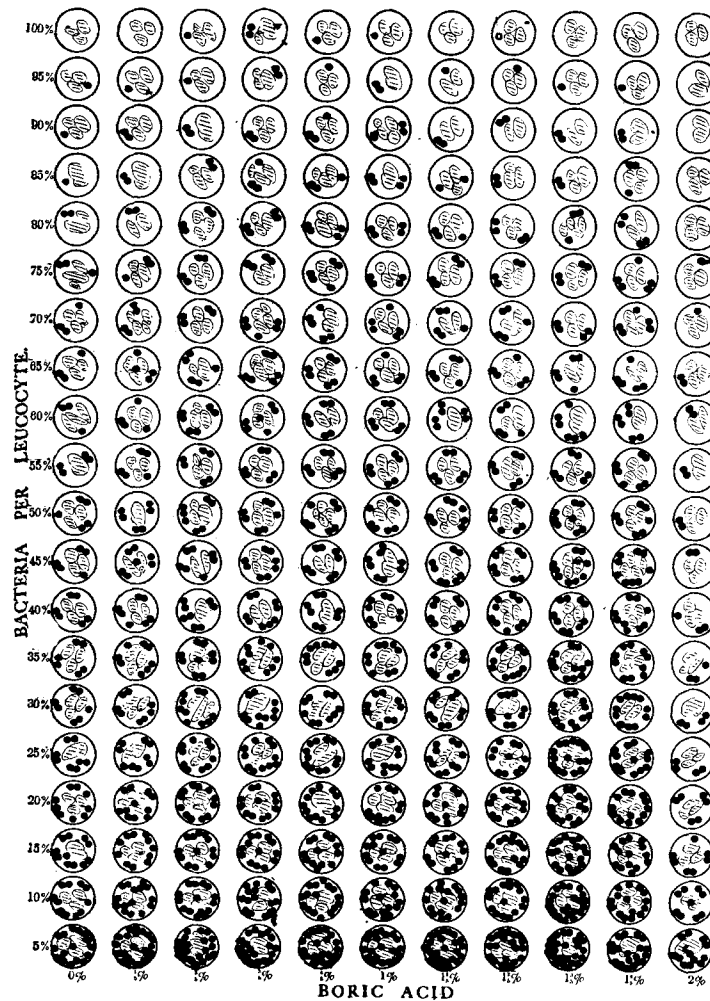


FIG. 8. Influence of boric acid on phagocytes. Human leucocytes, average of 15- and 30-minute counts.

in two experiments with boric acid are given in Fig. 8. With boric acid, as with mercuric chloride, the sixty minute counts

and the earlier counts show different effects on phagocytosis. Three percentage curves were therefore plotted (Fig. 9) showing the observed effect at the end of fifteen, thirty and sixty minutes. These curves are plotted to the same scale as given in Fig. 10.

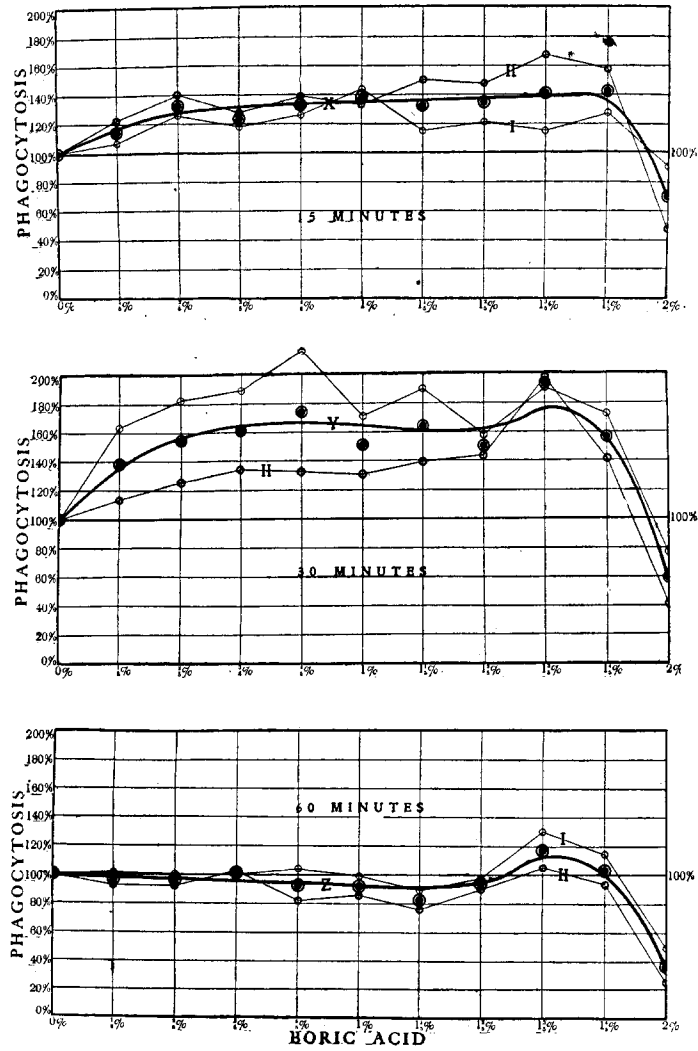


FIG. 9. Influence of boric acid on Wright's index. 15-, 30- and 60-minute data, plotted separately. *I* = data from first experiment, *II* = data from second experiment; *X*, *Y* and *Z* = averages.

From Fig. 10 it is seen that boric acid, in small amounts, causes a preliminary stimulation in phagocytosis, followed by a depression. This phenomenon becomes more marked as the percentage of boric acid increases, until a concentration of  $1\frac{1}{2}$  per cent. is reached. After this a further increase in boric acid causes a rapid fall in phagocytic power, phagocytosis apparently completely ceasing soon after the concentration reaches 2 per cent.

*Quinine Hydrochloride.*—The average of the three counts in an experiment with quinine hydrochloride are given in Fig. 11, and

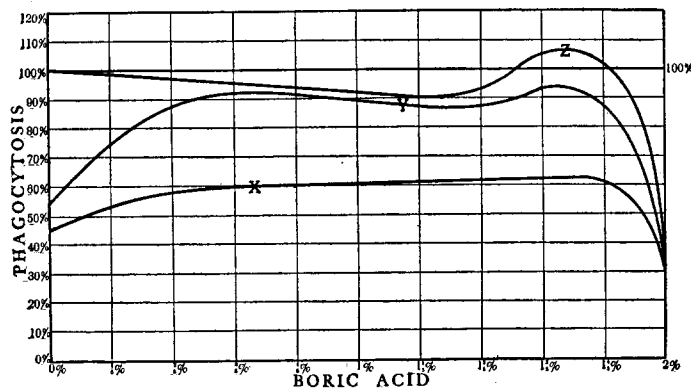


FIG. 10. Influence of boric acid on Wright's index. Curves from Fig. 9, plotted to the same scale.

the corresponding variations in Wright's index in Fig. 12. A duplicate experiment is given in Fig. 13, and the average of the two experiments in Fig. 14.

From this figure it is seen that the addition of quinine hydrochloride gives, from the first, an increase in phagocytosis, the increase reaching a maximum (20 per cent.) as soon as the concentration reaches  $1/200$  per cent. A further increase in quinine hydrochloride causes a decrease in phagocytosis, phagocytosis being reduced to normal by the time the concentration reaches  $1/120$  per cent., and apparently completely ceasing soon after the concentration reaches  $1/40$  per cent.

Whether the observed increase is in the nature of a permanent stimulation, or is only a temporary stimulation to be succeeded by a depression, has not been determined.

*Summary.*—Judging from counts made at the end of fifteen, thirty and sixty minutes the following influences on phagocytosis have been demonstrated:

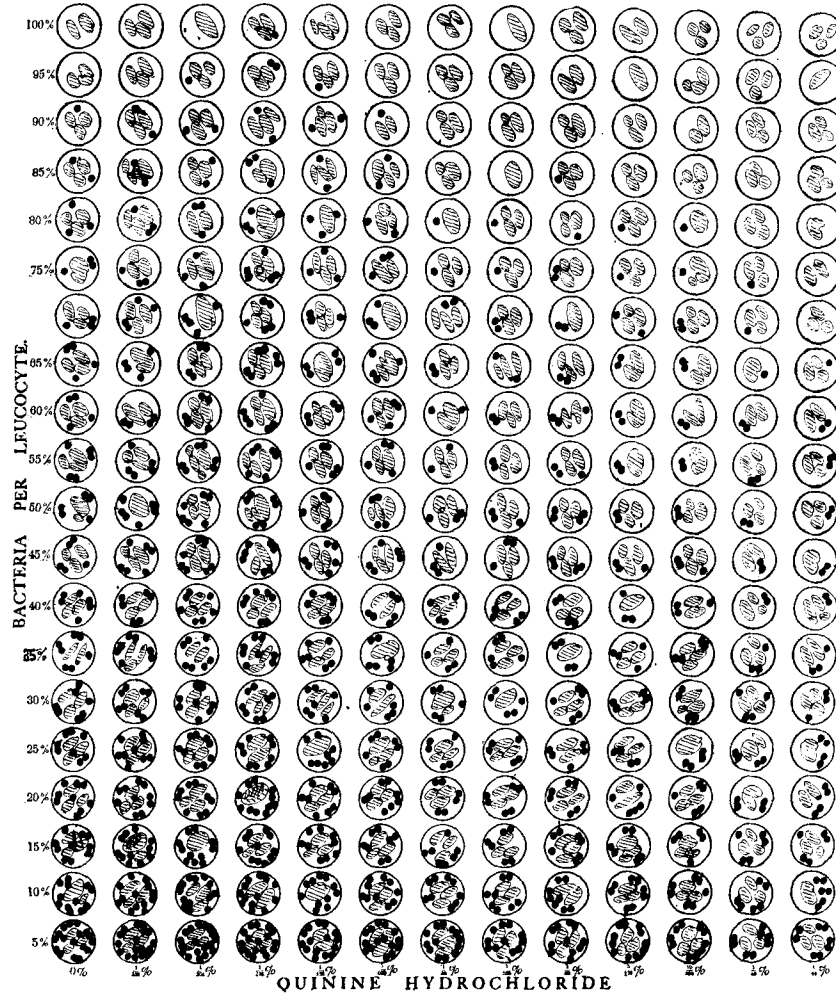


FIG. 11. Influence of quinine hydrochloride on phagocytosis. Human leucocytes; average of 15-, 30- and 60-minute counts; Experiment II.

1. Carbolic acid, added in increasing amounts to experimental tubes, causes from the first a decrease in phagocytic power, phagocytosis falling off one third by the time the concentration reaches

2/9 per cent., seven eighths by the time it reaches 1/3 per cent., and completely ceasing at 1/2 per cent.

2. Mercuric chloride, in concentrations less than 1/120 per cent.,

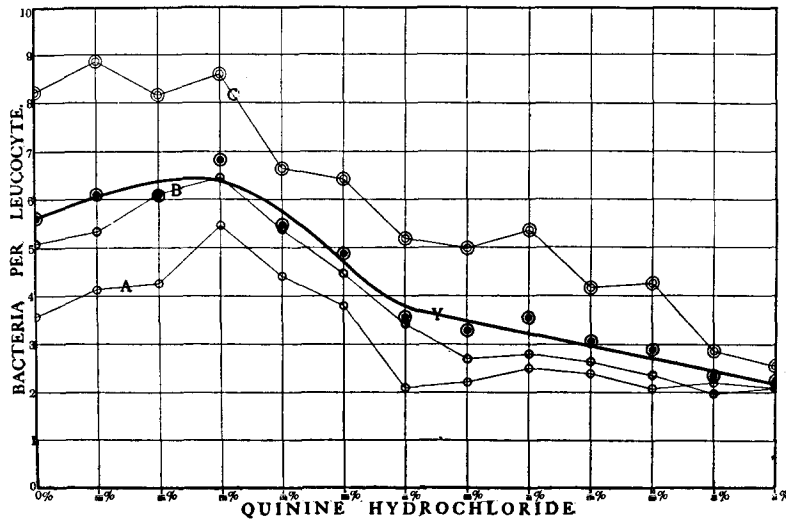


FIG. 12. Influence of quinine hydrochloride on Wright's index. Experiment II. A, B and C = 15-, 30- and 60-minute counts; Y = average.

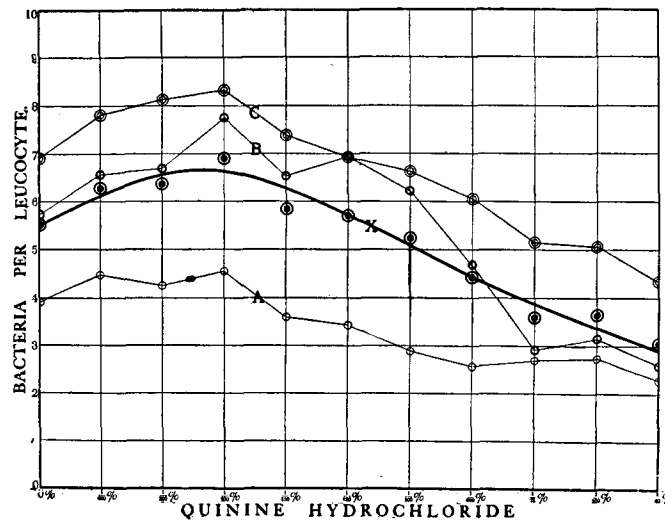


FIG. 13. Influence of quinine hydrochloride on Wright's index. Experiment I. A, B and C = 15-, 30- and 60-minute counts; X = average.

causes a transient stimulation in phagocytosis, followed by a depression. In larger amounts it causes a permanent depression from the first, phagocytosis apparently completely ceasing soon after the concentration reaches  $1/60$  per cent.

3. Boric acid, in concentrations less than  $1\frac{1}{2}$  per cent., causes a transient stimulation in phagocytosis, followed by a depression. As the concentration increases above  $1\frac{1}{2}$  per cent. there is a rapid fall in phagocytic power, phagocytosis apparently completely ceasing soon after the concentration reaches 2 per cent.

4. Quinine hydrochloride, added in increasing amounts, causes a

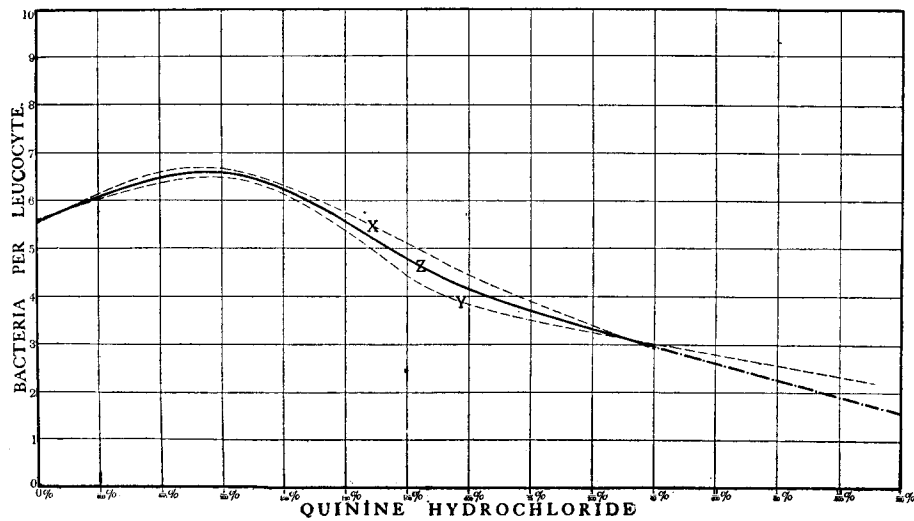


FIG. 14. Influence of quinine hydrochloride on Wright's index. Curves X and Y, from Figs. 12 and 13, plotted to the same scale; Z = average.

stimulation in phagocytosis, phagocytosis reaching a maximum as soon as the concentration reaches  $1/200$  per cent. A further increase in quinine hydrochloride causes a decrease in this stimulation, phagocytosis being reduced to normal as soon as the concentration reaches  $1/120$  per cent. In larger amounts quinine hydrochloride causes a depression in phagocytosis, phagocytosis apparently ceasing soon after the concentration reaches  $1/40$  per cent. Whether the observed stimulation is a permanent stimulation, or a transient stimulation to be succeeded by a depression has not been determined.