

## FURTHER OBSERVATIONS UPON CERTAIN SOURCES OF ERROR IN THE OPSONIC TECHNIQUE.<sup>1</sup>

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### INTRODUCTION.

IN a former communication (1909<sup>1</sup>) we stated that though the opsonic technique of Wright and Douglas is rather inaccurate, "we entirely disagree with those critics who appear to maintain that it is practically useless as a means of comparing degrees of phagocytosis."

We demonstrated that the accuracy was closely dependent upon two variable factors—(a) the number of leucocytes enumerated, for with tubercle and staphylococcus the error was diminished by some 20 per cent., when 120 instead of 60 leucocytes were counted; (b) the strength of the bacterial emulsion—for "by increasing the concentration of a tubercle or staphylococcus emulsion from two to four bacteria per cell the error diminishes about 25 per cent."

The observations summarised in this paper were undertaken to ascertain the influence of certain other factors upon the experimental error, and also upon the amount of phagocytosis, such as the effect of allowing the bacterial emulsion, or the leucocytic cream, to stand some time before putting up the phagocytic mixture, and also of preventing the phagocytic mixtures from separating into two layers of corpuscles and serum respectively during incubation.

One would naturally imagine that the settling of the leucocytes in the opsonic pipette prevents them from having equal opportunities of engulfing the food prepared for them, and thus a source of error would be introduced.

Rosenow (1906<sup>2</sup>) states that "previous investigators have commonly completely disregarded the influence upon phagocytosis that movement of the mixtures might have." He devised a machine for shaking the mixture, and found that about 120 vibrations per minute was sufficient to keep the corpuscles and leucocytes equally suspended, and that phagocytosis is thereby "perceptibly increased, and the results obtained would seem to be more reliable."

<sup>1</sup> Communicated to the Pathological Society of Great Britain and Ireland, January 6, 7, 1911. [Received for publication September 21, 1911.]

We have considered the following question :—

*What is the effect upon phagocytosis and upon experimental error of: (a) constantly rotating the phagocytic mixture in the opsonic pipette during incubation; (b) using leucocytes which have stood for two hours at room temperature; (c) using an emulsion which has been constantly stirred?*

#### TECHNIQUE.

A special apparatus was constructed to rotate the opsonic pipettes during incubation. It consisted of a series of interlocking cog-wheels, with a perforation in the centre of each, fitted upon the outside of a Hearson's opsonic incubator. The pipette was inserted through the hole in the cog-wheel, and fixed to it by a piece of rubber. A clockwork attachment caused the whole series of cog-wheels, with their contained pipette, to rotate together at the same speed; the speed of rotation could be varied at will.

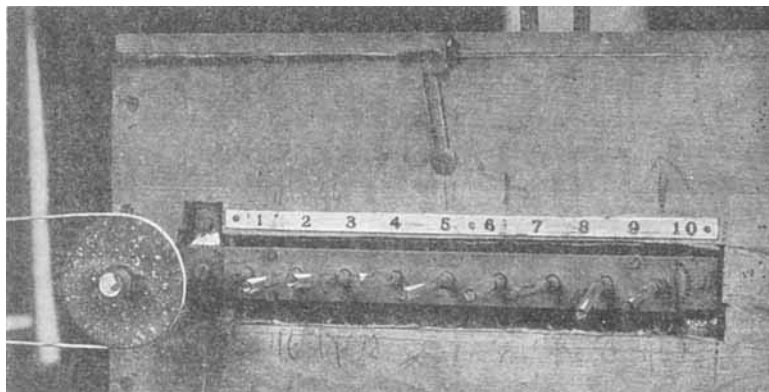


FIG. 1.—Apparatus used by Authors.

No settling in the phagocytic mixture occurred if the pipettes were rotated once every two minutes. The organism tested was *staphylococcus albus*, and the technique was exactly similar to that employed by us on previous occasions. The emulsion was strong enough to yield an average of about four cocci per cell. There was no agglutination of the staphylococci during any of the experiments. Four separate experiments were performed on different days. The total number of phagocytic mixtures put up was 61.

The bacteria in 150 leucocytes were enumerated from every mixture, 75 being counted by each observer, thus 9150 leucocytes were enumerated in all. The incubation period was fifteen or twenty minutes.

The amount of experimental error was estimated by comparing the results of duplicate or triplicate phagocytic mixtures taken in pairs, *e.g.* in Experiment 1 the counts from the three stationary phagocytic mixtures were: 1041, 1035, and 1027. If taken in pairs we have 1041 and 1035, 1041 and 1027, 1035 and 1027. The percentage deviations from the arithmetic means of the three pairs is 0.3, 0.7, and 0.4. The average for the three experiments is therefore 0.5 (see Table II. A).

It is unnecessary to describe all four experiments in detail; one, however, is given in full (see Table I.).

TABLE I.—*Error estimated by the Percentage Deviation from Arithmetic Mean (Experiment II.).*

State of Pipette.	Number of Phagocytic Mixtures.	Phagocytic Count.	Mean of Phagocytic Counts.	Percentage Deviation from Arithmetic Mean.
A Stationary . . . . .	{ 1 2 3	{ 1031 897 977	{ 968	{ 7.0, 2.7, 4.3 Average, 4.7
B Revolving once in 2 minutes	{ 1 2	{ 1178 1038	{ 1108	{ 6.3
C „ „ 1 minute	{ 1 2	{ 760 828	{ 794	{ 4.2
D „ „ $\frac{1}{2}$ minute	{ 1 2	{ 828 750	{ 787	{ 5.0
E „ „ $\frac{1}{4}$ minute	{ 1 2	{ 715 880	{ 797	{ 10.3
F „ „ 7-8 seconds	{ 1 2	{ 817 838	{ 827	{ 1.2
*G Stationary (emulsion stirred)	{ 1 2 3	{ 941 785 893	{ 873	{ 9.0, 2.6, 6.4 Average, 6.0
*H „ (emulsion not stirred)	{ 1 2 3	{ 803 784 933	{ 840	{ 1.1, 7.5, 8.6 Average, 5.7

\* NOTE.—The leucocytes have here stood at room temperature for two hours.

TABLE II.—*Summary of Error from Four Experiments by the Percentage Deviation from the Arithmetic Means of the various Counts taken in Pairs.*

State of Pipette.	Experiment I.	Experiments II. and III.	Experiment IV.	Average.
A Stationary . . . . .	0.5 (3)	2.7 (6)	4.2 (15)	3.3
B Revolving once in 2 minutes . .	6.4 (3)	3.2 (2)	4.7 (15)	4.0
C „ „ 1 minute . . . . .	3.1 (3)	2.5 (2)	...	2.9
D „ „ $\frac{1}{2}$ minute . . . . .	...	6.1 (2)	}	5.6
E „ „ $\frac{1}{4}$ minute . . . . .	...	6.8 (2)		
F „ „ 7-8 seconds . . . . .	...	3.8 (2)		
*G Stationary (emulsion stirred). .	...	4.3 (6)	}	5.0
*H „ (emulsion not stirred) . .	...	5.7 (6)		

\* NOTE.—The leucocytes have here stood at room temperature for two hours.

The numbers in brackets indicate the number of pairs upon which the calculations are based. The average error for the whole series is calculated in the same manner as in the preceding table.

The figure for the stationary pipette A is taken as 100; the others proportionately corrected. The numbers in brackets indicate the number of phagocytic mixtures upon which the percentages are based.

The percentages are averaged in the last column, allowance being made for the number of phagocytic mixtures, thus: the average of No. 2 is  $(102.2 \times 3 + 101.1 \times 4 + 98.4 \times 6) \div 13 = 100.1$ .

TABLE III.—*Summary of the Effect of Rotation upon Phagocytosis.*

State of Pipette.	Experiment I.	Experiments II. and III. Average.	Experiment IV.	Average.
A Stationary . . . . .	100 (3)	100 (6)	100 (6)	100
B Revolving once in 2 minutes . .	102.2 (3)	101.1 (4)	98.4 (6)	100.1
C „ „ 1 minute . . . . .	96.4 (3)	85.9 (4)	...	90.4
D „ „ $\frac{1}{2}$ minute . . . . .	...	80.2 (4)	}	83.7
E „ „ $\frac{1}{4}$ minute . . . . .	...	87.1 (4)		
F „ „ 7-8 seconds . . . . .	...	83.7 (4)		
*G Stationary (emulsion stirred) .	...	89.7 (6)	...	89.7
*H „ (emulsion not stirred) . .	...	87.9 (6)	...	87.9

\* NOTE.—The leucocytes have here stood at room temperature for two hours.

#### CONCLUSIONS.

1. The amount of phagocytosis and of experimental error is practically the same whether the opsonic pipette is stationary during incubation or rotated once every two minutes; but when it is rotated once every half-minute or less the amount of phagocytosis definitely diminishes, and the experimental error increases. This is contrary to the conclusion arrived at by Rosenow (1906<sup>2</sup>). His phagocytic mixtures, however, were shaken, not rotated.

2. There is a diminution, about 10 per cent., in the phagocytic capacity of leucocytes which have been allowed to stand in normal saline at the room temperature for two hours, and a marked increase in the amount of experimental error. The diminution in phagocytic capacity upon standing is similar to that observed by us on a previous occasion (1909<sup>3</sup>).

3. An emulsion of staphylococcus in normal salt solution, standing in a tube of 6 mm. diameter, does not settle appreciably in two hours, and the experimental error is not diminished by constant stirring of the emulsion.

#### REFERENCES.

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3. ERNEST GLYNN AND LISSANT COX *Journ. Path. and Bacteriol.*, 1909, vol. xiv. p. 121.