VARIATIONS IN THE INHERENT PHAGOCYTIC POWER OF LEUCOCYTES.¹

By ERNEST E. GLYNN, M.A., M.D.(Cantab.), M.R.C.P.(Lond.),
*Lecturer in Clinical Pathology and Morbid Anatomy, University
of Liverpool, Bacteriologist to the Liverpool Royal Infirmary; and*
G. LISSANT COX, M.A., M.B., B.C.(Cantab.), M.R.C.S., L.R.C.P.,
Holt Fellow in Pathology, University of Liverpool.

THE teaching of Metchnikoff, that phagocytosis depends mainly upon the activity of the leucocyte—that immunity itself is essentially an expression of that activity—has received less support than ever in this country since the brilliant demonstration by Wright and his colleagues (1903³⁵), who showed that before a marked degree of phagocytosis can occur, the bacterium must be prepared, *i.e.* “opsonised,” by substances present in the plasma or serum.

Wright and Douglas (1904³⁸) compared the phagocytic power of leucocytes from a normal man with that of one previously immunised with staphylococcic vaccine and possessing in consequence a high opsonic index. They found that the high phagocytic count obtained in the latter depended entirely upon the opsonin. It was immaterial which strain of leucocytes was employed.

The researches of Bulloch and Atkin (1905³) apparently confirmed this conclusion. They found that “the leucocyte is practically an indifferent factor when the phagocytic power of different bloods is compared”; it is the opsonin that varies. For example, one individual is less resistant to a staphylococcic infection than another, partly because his blood is deficient in opsonin, certainly not because his leucocytes are less active.

Fleming (1908¹⁰), from Wright's Laboratory at St. Mary's Hospital, quoted in 1908 additional figures which support this view.

The opinions of the Wright school were summed up in 1907 by the founder himself when, referring to certain experiments which we shall quote in full (p. 123), he said: “It clearly emerges that the phagocytic count is uninfluenced by the variety of corpuscle employed. This conclusion, in addition to its theoretical interest, has also, be it remarked, an absolutely fundamental practical importance. It will make it clear that we may take the opsonic index of any blood as an index of the patient's power of phagocytic response”⁽³⁶⁾. It is obvious that if the leucocyte were also a variable factor, the estimation of the opsonic index might be of comparatively little value

¹ This paper formed part of an M.D. thesis, Cambridge, for one of us (E. E. G.).
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unless the innate phagocytic power of the individual's leucocytes apart from opsonin, were also measured.

It will be recollected that when determining the opsonic content of various sera, each sample is mixed with an equal volume of washed leucocytes and of bacterial emulsion respectively. The washed leucocytes therefore are from the *same* source, the sera from *different* sources.

The opsonic index is calculated by dividing the number of bacteria taken up per leucocyte in the presence of the various tested sera, by the number taken up per leucocyte in the presence of the serum of a normal individual, which latter is taken as control and regarded as unity. In seeking to demonstrate that the phagocytic power of leucocytes from different sources is the same, Wright, Bulloch, and Fleming slightly modified the usual opsonic technique, for they compared the phagocytosis of staphylococci which obtained with various strains of leucocytes in the presence of the *same* serum.

PREVIOUS OBSERVATIONS BY GLYNN AND YORKE.

On 4th January 1908 one of us (E. E. G.), in conjunction with Dr. W. Yorke, read a paper at the Pathological Society of Great Britain and Ireland, entitled "Variations in the Phagocytic Power of Leucocytes from different Individuals."

Accepting the technique of Wright and Douglas, we brought forward several observations to show—*First*, that there may be a marked and often very constant difference in the phagocytic capacity of various strains of polymorphonuclear leucocytes. *Secondly*, that these differences may be apparent not only in pathological blood, as some writers have recently found, but also in normal blood. *Thirdly*, the opsonic index does not necessarily coincide with the index obtained by mixing a person's *own* leucocytes with his *own* serum.

NEW OBSERVATIONS.

In order to confirm these results a new and extended series of observations have been undertaken, especially devised to eliminate certain sources of error which subsequent reflection and a recent paper on the "accuracy" of the opsonic method, published from Wright's laboratory (1908 ³⁴) suggested.

For these reasons the blood of three individuals, G, L, and A, was systematically examined at intervals of three days from 25th May to 12th June 1908, and again from 14th July to 10th August at similar intervals, except on three occasions. Two of these individuals were in normal health, namely, one of us, G, and the laboratory assistant, L; the third, A, also a laboratory attendant, suffered from chronic acne indurata of the face due to *Staphylococcus albus*.

L and A were selected partly on the grounds of convenience, partly because preliminary investigation showed that the intrinsic phagocytic

power of their leucocytes to *Staphylococcus albus* differed from that of G, partly because A being subject to a chronic staphylococcic infection, it was hoped that some interesting facts might come to light; and, lastly, because their washed corpuscles were not agglutinated by any of the sera used.

THE CYTOPHAGIC AND OPSONO-CYTOPHAGIC INDICES.

As already stated, there may be a marked and often very constant difference in the phagocytic power of leucocytes from various persons. This difference can be denoted by an index calculated on the same principles as the opsonic index. Like the opsonic index it is also a comparison of two results, one being taken as the standard. As will presently appear, such an index is a definite entity. It is unfortunately necessary, in order to make this paper intelligible and concise, to use such a word to express this index.

The first term which occurred was *Phagocytic Index*, but this has already been employed by Wright and others to indicate the number of bacteria engulfed by the leucocytes of one individual alone, without reference to the leucocytes of a second individual taken as unity, *i.e.* Wright's phagocytic index is not a ratio. *Leucocytic Index* is objectionable, for it suggests the number of leucocytes, not their phagocytic capacity.

Professor Myres, whom we consulted, suggested *Cytophagic*, which is simply an inversion of the term phagocytic; it also conveys the idea of the capacity for eating cells. The manner of calculating the cytophagic index will be given later.

Let us assume for a moment that the extent to which a given bacillus is phagocytosed depends upon *two* variable factors, not only the serum, *i.e.* opsonin, as Wright maintains, but also upon the inherent phagocytic capacity of the leucocyte. In order, therefore, to ascertain in any given blood the effect on both these factors, we may estimate the influence upon phagocytosis of (*a*) the serum alone, (*b*) the leucocytes alone, (*c*) the serum and leucocytes combined. The last method is best, for it gives the total phagocytic response of any blood, *i.e.* we estimate the effect upon phagocytosis of two variants, the inherent phagocytic (*i.e.* cytophagic) power of the leucocytes and the opsonic power of the serum.

We have expressed the result of comparing the combined action of leucocytes plus serum of two persons by the term *Opsono-cytophagic Index*. Such an index denotes, as far as *in vitro* experiments can denote it, the total phagocytic power of any blood. It corresponds to the "hæmophagocytic" index of Shattock and Dudgeon, who, however, have no term to express the inherent phagocytic capacity of the leucocytes alone.

RECAPITULATION.

If we compare the phagocytic powers of the blood of two persons, P (patient) and C (control), taking the latter as unity, we may express the proportion due to the leucocytes, or to the serum, or to both, by three separate indices. We may compare P's serum with C's put up with the *same* leucocytes, and measure the opsonising influence of the serum upon phagocytosis. This is the *Opsonic Index*.

We may compare P's leucocytes with C's put up with the *same* serum, and measure the influence of the leucocytes upon phagocytosis. This is the *Cytophagic Index*.

Lastly, we compare P's leucocytes put up with P's serum, and C's leucocytes put up with C's serum, and compare the total phagocytic power of their blood. This is the *Opsono-cytophagic Index*.

TECHNIQUE.

(A) *Introductory.*

It has already been mentioned that Wright and others compared the intrinsic phagocytic capacity of various strains of leucocytes by adopting the ordinary opsonic technique, and mixing together equal volumes of washed leucocytes, bacterial emulsion, and serum.

The same comparison might have been made by omitting the serum altogether, for if washed leucocytes are added to bacterial emulsion containing an appropriate amount of NaCl, phagocytosis will still occur. Wright designates this as "spontaneous" to distinguish it from "induced" phagocytosis, this latter being, of course, mainly due to the opsonin of the serum.

According to Wright (1907³⁷), "spontaneous" differs from "induced" phagocytosis in the following particulars:—

1. It is a slow process, and the "number of bacteria ingested attains ordinarily only very modest proportions."

2. The ingestion of bacteria is very irregular, a "few leucocytes taking part immoderately while others abstain."

3. "The ingestion of the bacteria can be completely suppressed by employing in the phagocytic mixture certain concentrations, in the case of the tubercle bacillus, a concentration of slightly over 1 per cent. of NaCl."

The first reason, therefore, for putting up the phagocytic mixture with serum in this research, is the irregular nature of spontaneous phagocytosis. The ordinary opsonic technique is inaccurate enough as it stands, without additional difficulties. We wished also to ascertain how far the opsonic index is an indication of the total phagocytic power of the blood, and whether there is any connection between the opsonic and cytophagic indices combined, and the opsono-cytophagic index.

A valuable paper has recently been published from Wright's laboratory by Fleming (1908¹¹), which has an important bearing upon this research. He states that:

1. A serum which agglutinates the washed red corpuscles in the phagocytic mixture "increases the amount of phagocytosis." It was necessary, therefore, to select persons whose washed erythrocytes were not agglutinated

by any of the sera used. The corpuscles and sera from G, L, and A fulfilled these conditions in every combination.

2. "With a diminution of the number of washed corpuscles in the opsonic mixture, there is an increase in the amount of phagocytosis." This statement implies that if two samples of washed corpuscles were prepared from the same individual so that one, A, contained twice as much salt solution as the other, B, and both were put up as usual with serum and bacterial emulsion, then the leucocytes from sample A would exhibit the greater phagocytic activity, *i.e.* taking B as unity, A's cytophagic index would be above normal.

In order to eliminate errors from this source, the following technique was adopted:—

After each of the three samples of corpuscles had been finally centrifuged, as much as possible of the supernatant salt solution was pipetted off. The tubes containing the corpuscles were then inclined at the same angle, namely, about 45°, and allowed to rest for five minutes. The corpuscles would now be completely separated from the salt solution, which latter was drawn off with a fine pipette. It was easier to remove the same quantity of salt solution from each sample when inclined in this way. The tubes were next placed vertically, and immediately before a quantum of corpuscles was removed by the opsonic pipette, each was vigorously rolled to and fro between the palms of the hands to shake up the corpuscles and fluid.

The actual number of washed red corpuscles per c.mm. in the three samples was ascertained by means of a Thoma-Zeiss apparatus, but it was impossible to count the leucocytes with accuracy, as some had clumped.

3. Ledingham and Bulloch (1906²⁰) have shown that washed corpuscles, taken during the leucocytosis following talianine injections, give a much smaller phagocytic count than normal corpuscles.

To avoid fallacies from this cause, it was necessary to make a routine enumeration of leucocytes in the blood of the three persons examined.

(B) *Details of Technique.*

Collecting and washing the blood.—The arm of each individual was rotated from the shoulder for a few seconds, and a rubber catheter wound round the finger from the knuckle to the distal end of the second phalanx. A prick was made on the dorsal surface of the last phalanx, and four-tenths of a c.c. of blood were collected by means of a graduated pipette, and immediately transferred to 20 c.c. of 1.5 per cent. solution of sodium citrate in 0.85 per cent. NaCl. As the blood was flowing, a sample was taken up in a Thoma-Zeiss pipette in order to estimate the number of leucocytes. As a considerable quantity of blood was often wasted by running off the finger, the technique was modified in the second series of experiments beginning in July, in the following way. Immediately after the application of the catheter a small piece of rubber tubing $\frac{1}{4}$ in. in diameter was fixed on to the dorsal surface of the third phalanx by means of a piece of tape, so as to form a well an eighth of an inch deep. The tissue of the finger at the bottom of this well was then stabbed, and the blood flowing into it was prevented from escaping, and consequently much easier to collect. This manoeuvre also ensured that the blood for the leucocyte counts, and for the washed corpuscles was taken from the same sample.

Sometimes it was impossible, especially in the first series of experiments, and particularly in the case of A, whose fingers were less fleshy, to obtain enough blood without removing the rubber ligature from the finger, and then reapplying it. As a rule, the whole operation of applying the rubber band to the finger, and collecting the blood, lasted from twenty to thirty seconds for each individual. The three samples of blood obtained in this way were invariably collected within a few minutes of each other. About the same time a sample of blood was obtained from each individual in a Wright's capsule;

this was allowed to clot and was centrifuged, to furnish serum for the experiment.

Washing the blood.—The three tubes of citrated corpuscles were centrifuged simultaneously, and after the supernatant fluid had been pipetted off, an equal amount of normal salt solution was added to each. The washing with normal salt solution was performed twice in the first series, once in the second.

The greatest care was exercised that each sample of blood should be drawn as far as possible at the same moment, and be subjected to exactly the same treatment both as regards the time of centrifuging, the amount of citrate and salt solution employed in washing, and in the quantity of supernatant fluid ultimately removed before the washed leucocytes were ready for use. Careful records were taken of the exact time of day when the blood was collected, the order of collecting the blood, whether the rubber band had to be reapplied to the finger or not, the time occupied with centrifuging, the number of times washing with salt solution was performed, and the time which elapsed between collecting the blood and beginning to put it up in the phagocytic mixture.

Preparation of the emulsion.—The emulsion was freshly prepared each day by mixing tubercle bacilli supplied by Allen & Hanbury, or a 24-hours' culture of *Staphylococcus albus*, in a mortar with sodium chloride, centrifuging for at least a quarter of an hour at low speed and filtering through two thicknesses of blotting-paper. The staphylococci were emulsified with 0.85 per cent. NaCl, and the tubercle bacilli with 1.5 per cent. till July, when, for reasons to be presently stated, 0.85 per cent. NaCl was employed for both.

The elimination of spontaneous phagocytosis.—The percentage of NaCl in the solutions from which the emulsion of tubercle and staphylococcus are prepared, must be considered before proceeding further.

Oponic workers are instructed (^{23. 28. 18}) to make a staphylococcus emulsion with 0.85 per cent., and a tubercle bacillus emulsion with 1.5 per cent. of NaCl. Now the latter strength will give a salt content in a phagocytic mixture of over 1 per cent., i.e. considerably stronger than that obtaining in the phagocytic mixtures with staphylococcus, and also stronger than that to which the leucocytes are accustomed.

It is believed that spontaneous phagocytosis, that is to say, the capacity of leucocytes to ingest bacteria in the absence of serum only, ceases when the salt content in the phagocytic mixture is 0.85 for staphylococcus, and slightly over 1 per cent. for tubercle bacillus. The assumption is made that the maximum influence of opsonin can only be measured by the complete elimination of spontaneous phagocytosis.

We have found that there is a considerable amount of spontaneous phagocytosis of the staphylococci and tubercle bacilli when employed in 0.85 per cent. saline, also that the degree of phagocytosis was approximately the same for both bacteria.

In a series of observations, therefore, beginning 14th July, we used 0.85 NaCl for both the tubercle and staphylococcus, in order that the conditions for phagocytosis of both organisms might be equally favourable. As a matter of fact, the cytophagic indices are approximately the same whether the tubercle bacilli were emulsified in 0.85 or 1.5 per cent. NaCl.

Because the tubercle bacilli tended to agglutinate slightly, after standing for some hours with normal salt solution, the emulsion was freshly prepared each day.

Incubation.—The phagocytic mixtures, consisting of equal parts of corpuscles serum and bacterial emulsion, were placed for fifteen or twenty minutes at 37° C. in a patent oponic incubator.

Owing to deficient circulation of the water in this incubator, the temperature of the various tubes was liable to differ by $\frac{1}{2}$ ° C. or more, at the same

time. This defect was completely remedied by continually agitating the water with a rotating paddle.

After incubation,¹ the contents of the test were remixed, and three films prepared from each one. The smears were made in the usual way by placing the drop at one end of the slide, and drawing it out with another narrower slide held at an angle, great care being taken to make the termination of the smear as rectangular as possible (*vide* diagram *infra*).

The staphylococcus films were stained by Leishman's method, and the tubercle with carbol fuchsin and alkaline methylene blue.

Counting.—The numbers of bacteria phagocytosed by sixty polymorphonuclear leucocytes were enumerated in two out of three films made from each phagocytic mixture. Thirty cells were taken from the two borders, beginning at the corners B_2 and passing backwards to B_1 . Thus the *same part* of every film was examined; the advantage of this precaution will be stated later. All the eosinophiles and all polymorphs with indistinct edges, owing to damaged cytoplasm, were eliminated from the counts. Clumps of more than three leucocytes were also omitted.

When the batch of films was finished, the figures were added up, and if there was a deviation from the mean of the counts of any two duplicate films of more than 5 per cent., another sixty leucocytes were taken from the third uncounted film and an average struck from the three sets of figures.

Half the total number of leucocytes counted (120) were selected from one

Diagram of slide with blood smear

film, and half from another, in order to diminish errors due to improper smearing, unequal staining, or inaccurate counting; and lastly, because it is obviously advantageous, when there is a marked difference between the figures obtained from the two films to count an additional sixty from the third film. If the figures, when added up were contrary to expectation, recounts were not made, nor were an extra sixty cells counted in the hope that "all might be well." No counts were discarded on the grounds that the films were improperly stained. The usual number of polymorphonuclear leucocytes counted was sixty, but occasionally eighty or fifty according to the strength of the emulsion. The number once fixed by an examination of the first film was invariably the same for all the batch. As duplicate films were used, it is clear that the total number of cells counted from each phagocytic mixture was usually 120, sometimes 180 or 100.

According to Fleming, it is a "*great mistake*" to count any "arbitrary number of leucocytes," for a number that will give a good average of a slide with one emulsion will not furnish a true estimate of a slide with another emulsion; "intelligence must be brought to bear." In most of his published cases 100 cells were counted, "but this rule was never strictly adhered to; more being counted in some, and less in others, according to the regularity of the count; in very few cases, however, were more than 200 cells counted." After considerable deliberation we decided that it was much better to count the same arbitrary number of leucocytes, usually sixty, in every slide

¹ For full details, see *Biochemical Journal*, vol. x. p. 302.

on the same day, omitting, of course, damaged cells, rather than varying the numbers according to the regularity of the count; the reasons for this decision will be seen later.

Elimination of auto-suggestion.—In order to eliminate any auto-suggestion in counting, all the stained films, usually twenty-four in all, were placed in numbered compartments and the figures upon them rubbed off. An attendant handed these unnumbered films indiscriminately to the observer, who thus remained ignorant of which film he was counting.

Calculation of indices.—As previously stated, one sample of washed corpuscles and one of serum were prepared from each of the three individuals, G, L, and A, and the phagocytic mixture was composed of equal volumes of serum, cream, and emulsion.

The way in which the combinations of serum and leucocytes and the estimation of indices were invariably made is given below in tabular form, with the figures obtained on 20th July. The methods of calculating errors in the technique are also given at the same time for the sake of convenience. The important question of "errors" will be discussed elsewhere.

Table illustrating in complete detail the routine methods of combining Sera and Washed Leucocytes (Cream) from G, L, and A; of calculating Indices, and estimating Errors.

Date, July 20, 1908. Organism, *Staphylococcus albus*.

PART I.

(Combinations of Sera and Washed Leucocytes (Cream) obtained from G, L, and A.)

Phagocytic Mixture.			Number of Cocci phagocytosed per Slide by fifty ¹ Leucocytes in the twelve separate Phagocytic Mixtures.				Total.
No.	Source of Cream.	Source of Serum.	Slide 1.	Slide 2.	Percentage Variation from the Arithmetic Mean.	Slide 3.	
1	A	A	386	307	8.7	360	610 (mean of the two estimations).
1A	A	A	266	267	0.0	...	
2	G	A	390	394	0.5	...	
3	L	A	316	316	0.0	...	
4	A	G	288	264	4.3	...	621 (mean of the two estimations).
5	G	G	305	286	3.0	...	
5A	G	G	380	312	7.1	305	
6	L	G	283	302	3.1	...	
7	A	L	286	278	1.4	...	565 (mean of the two estimations).
8	G	L	348	336	1.8	...	
9	L	L	265	279	2.6	...	
9A	L	L	279	315	6.0	284	
			3.2 ²				

¹ Fifty leucocytes, instead of the usual sixty, were counted because the emulsion was very strong.

² Average percentage deviation from the arithmetic mean of twelve pairs of slides.

KEY FOR CALCULATION OF INDICES.

A taken as patient. G taken as control.

1. OPSONIC INDEX.

$$\frac{\text{No. of cocci phagocytosed by A's or G's or L's leucocytes respectively + A's serum.}}{\text{No. of cocci phagocytosed by A's or G's or L's leucocytes respectively + G's serum.}}$$

2. CYTOPHAGIC INDEX.

$$\frac{\text{No. of cocci phagocytosed by A's leucocytes + A's or G's or L's serum respectively.}}{\text{No. of cocci phagocytosed by G's leucocytes + A's or G's or L's serum respectively.}}$$

3. OPSONO-CYTOPHAGIC INDEX.

$$\frac{\text{No. of cocci phagocytosed by A's leucocytes + A's serum.}}{\text{No. of cocci phagocytosed by G's leucocytes + G's serum.}}$$

PART II.

(1) Calculation of Indices of A, G taken as unity. (2) Estimation of Errors.

1. CYTOPHAGIC INDICES.

Note.—"Phag." = Phagocytosed; "Cream" = Washed Leucocytes.

No. of Estimation.		Indices.
<i>First</i>	$\left\{ \begin{array}{l} 610 \text{ cocci phag. by A's cream + A's serum} \\ 784 \text{ cocci phag. by G's cream + A's serum} \end{array} \right\}$	$= 0.778$
<i>Second</i>	$\left\{ \begin{array}{l} 552 \text{ cocci phag. by A's cream + G's serum} \\ 621 \text{ cocci phag. by G's cream + G's serum} \end{array} \right\}$	$= 0.889$
<i>Third</i>	$\left\{ \begin{array}{l} 564 \text{ cocci phag. by A's cream + L's serum} \\ 684 \text{ cocci phag. by G's cream + L's serum} \end{array} \right\}$	$= 0.825$
		Mean of the three indices = 0.831

2. ESTIMATION OF ERRORS.

(i.) Average difference between Triple Indices taken in Pairs.

Pairs of Indices.		Difference.
0.778	0.889	0.111
0.778	0.825	0.047
0.889	0.825	0.064
		Average difference 0.07

1. OPSONIC INDICES.

Note.—"Phag." = Phagocytosed; "Cream" = Washed Leucocytes.

No. of Estimation.		Indices.
<i>First</i>	$\left\{ \begin{array}{l} 610 \text{ cocci phag. by A's cream + A's serum} \\ 552 \text{ cocci phag. by A's cream + G's serum} \end{array} \right\}$	$= 1.105$
<i>Second</i>	$\left\{ \begin{array}{l} 784 \text{ cocci phag. by G's cream + A's serum} \\ 621 \text{ cocci phag. by G's cream + G's serum} \end{array} \right\}$	$= 1.262$
<i>Third</i>	$\left\{ \begin{array}{l} 632 \text{ cocci phag. by L's cream + A's serum} \\ 585 \text{ cocci phag. by L's cream + G's serum} \end{array} \right\}$	$= 1.080$
		Mean of the three indices = 1.149

2. ESTIMATION OF ERRORS.

(i.) *Average difference between Triple Indices taken in Pairs.*

Pairs of Indices.		Differences.
1·105	1·262	0·157
1·105	1·080	0·025
1·262	1·080	0·182
		—
		Average difference = 0·121

OPSONO-CYTOPHAGIC INDEX.

Note.—"Phag." = Phagocytosed; "Cream" = Washed Leucocytes.

$$\frac{610 \text{ cocci phag. by A's cream + A's serum}}{621 \text{ cocci phag. by G's cream + G's serum}} = 0·98.$$

This rather complicated scheme offers the following advantage with the least possible number of phagocytic mixtures, twelve in all.

1. Simultaneous estimation of the opsonic, cytophagic, and opsono-cytophagic indices.

2. Each final opsonic and cytophagic index is the mean of three completely separate estimations, whereby accuracy is increased. Accuracy is especially necessary in this research where the indices are rarely above 1·2 or below 0·8.

3. The errors in technique can be estimated by comparison of the triplicate indices.

4. It can be ascertained whether any particular combination of serum and leucocytes tends to produce a higher or lower index than any other combination.

The scheme has, however, certain disadvantages.

1. The opsono-cytophagic indices are put up in duplicate, not triplicate, and therefore are less likely to be accurate than the others.

2. Though the figures from which the triplicate indices are calculated come from separate phagocytic mixtures, yet the same figure is sometimes used more than once in the whole series of indices for one individual.

An examination of the fractions, pages 97–99, shows that the figures from the phagocytic mixtures Nos. 5 and 1 are used three times, Nos. 4 and 2 twice, and Nos. 3, 6, 7, and 8 once. See also Table IX., page 114.

THE ERROR IN OPSONIC TECHNIQUE.

Any one who has compared the figures obtained for the opsonic index of the same serum, as estimated quite independently by two

observers, will be aware that the difference between their results is often considerable even after an extensive experience of opsonic technique, and even when the precaution has been taken of enumerating a large number of leucocytes.

The latest statement from the Wright school is given by Fleming (1908¹²). He states that "two practised observers can count the *same slides* and obtain results in almost all cases within 10 per cent." Wright (1907³⁶) said, "The error of calculation in the case of normal bloods and in the hands of a good worker" is "rarely greater than plus or minus 5 per cent." But next year Fleming (1908¹²), who had carefully investigated the matter, concluded that "duplicate estimations of the tuberculo-opsonic index of tuberculous patients can be performed, the results differing from each other, by less than 20 per cent., except in rare instances."

Estimation of error in this paper.—The simplest and most practical way of estimating the degree of error in opsonic work is to compare the difference between indices calculated from duplicate phagocytic mixtures. This is a much more searching test than a comparison of the differences between two indices by recounting the same slides. In this paper three sets of indices have been calculated from triplicate phagocytic mixtures, i.e. the control blood and the tested bloods were each put up three times; in order, therefore, to compare our results with those of Fleming we have taken the mean of the difference between each of the three arranged in pairs. (See examples, pages 98 and 99.) This method of estimating the error of technique may be rather elementary for a statistician, but it has the merit of being easily understood.

Our average error so calculated is 0.17 for all duplicate staphylococcus indices, 0.15 for all duplicate tubercle indices.

From an examination of the figures given by Fleming (1908¹³) in Table VIII., in which thirty-eight duplicate indices are calculated by two observers putting up separate opsonic mixtures from tuberculous and normal persons, we find that the average difference between these thirty-eight pairs is 0.071.

A theoretical opsono-cytophagic index may be calculated by combining the figures actually obtained for the opsonic and cytophagic indices respectively. Such a theoretical opsono-cytophagic index may be compared with the opsono-cytophagic index actually worked out from the phagocytic mixtures. The difference between the theoretical and the actual index will represent the experimental error. For example, if the calculated opsonic index is 1.0, and the calculated cytophagic is 1.1, then the theoretical opsono-cytophagic would obviously be 1.1, and this can be compared with the opsono-cytophagic as worked out experimentally. Or again, if the opsonic and cytophagic indices were both 0.8, the combined opsono-cytophagic should be 0.6, because each of the separate indices is 0.2 *below* unity.

ESTIMATION OF ERROR BY THE DIFFERENCE BETWEEN ACTUAL AND THEORETICAL¹
OPSONO-CYTOPHAGIC INDEX. July 20, 1908.

Opsonic Index.	Cytophagic Index.	Actual Opsono-cytophagic Index.	Theoretical Opsono-cytophagic Index.	Error.
1.15	0.83	0.98	0.98	0

At first sight a comparison between the opsono-cytophagic index calculated in a theoretical manner from the opsonic and the cytophagic indices, and the opsono-cytophagic index calculated from actual figures obtained by putting up each man's leucocytes with his own serum, should be a very accurate method of ascertaining the error inherent in technique. But it is not so satisfactory as at first sight appears, for, as previously mentioned, two out of the eight sets of figures on which the indices of one individual are based, are used twice, and two thrice, either as the denominator or the numerator of fractions. This interlocking of figures tends to make the difference between the theoretical and actual opsono-cytophagic index *less* than it really is. However, broadly speaking, there is not the least doubt that a comparison between the actual and theoretical opsono-cytophagic indices does give a rough estimate of the accuracy of technique.

Unfavourable opinions have been expressed by various writers (2. 19. 22. 25. 31. 33) upon the accuracy of the opsonic technique, notably by Fitzgerald, Whiteman, and Strangeways (1907⁹). We have considered this question at length elsewhere (Biochemical Journal) (17), and have demonstrated that in our technique, at any rate, the error was least when the stronger bacterial emulsions were employed, namely, those yielding an average of four to six bacteria per cell. The following table, taken from the paper in question, gives the error in duplicate estimations of tuberculo-opsonic indices made by Fitzgerald, Whiteman, and Strangeways (1907⁹), Fleming, and ourselves. Full details as to the method of obtaining these figures of Fitzgerald, Whiteman, and Strangeways are given elsewhere (1908¹⁷).

We have omitted the counts of two phagocytic mixtures from our results because, as the triple indices show, they are obviously wrong. In all probability the error is mainly due to the employment of too weak an emulsion, namely, one yielding 1.3 and 2.2 bacteria per cell. On 1st August, the phagocytic mixture containing L's leucocytes with A's serum gave an exceedingly high count, namely, 42 per cent. higher than the next highest, and 40

¹ METHOD OF CALCULATION.—A's opsonic index is 0.15 above unity. A's cytophagic index is 0.17 below unity. Therefore the combined opsono-cytophagic index should theoretically be $0.17 - 0.15 = 0.02$ below unity. But 0.02 below unity is 0.98. The actual index, however, was 0.98, therefore the error in this case is 0.

per cent. above the mean of the whole series. This count which has been deleted affected two sets of indices, producing an abnormally high opsonic index for A and cytophagic index for L of 1·657 and 1·657 respectively.

Paper by	Observer.	Average Difference.	Number of Indices preceding Figures based on	Approximate Number of Cells counted.	Approximate Number of Bacteria per Cell.	Organism.
Fleming	Two	0·07	76	100	2*	Tubercle
E. G. .	E. G.	0·17	240	120	3·5	Staphylococcus
E. G. .	E. G.	0·15	120	120	3·2	Tubercle
Strangeways	F. G. & W.	0·36	36	50	1·2	"
"	W.	0·36	18	50	1·2	"
"	F. G.	0·51	18	50	1·2	"

The following are the triple indices for that date :—

A's Opsonic.	L's Opsonic.	A's Cytophagic.	L's Cytophagic.
1·657	0·883	1·169	1·657
1·078	0·952	1·004	0·926
0·926	1·117	0·981	1·086

The great diminution produced in the calculated error is sufficient justification for the omission, as shown by the following table :—

Alteration in the indices and the diminution in the calculated error resulting from the omission of one abnormally high count on 1st August.

L.							
	Opsonic.		Cytophagic.		Opsono-cytophagic.	Theoretical Opsono-cytophagic.	Error.
	Mean Index.	Error.	Mean Index.	Error.	Index.	Index.	
Count included	0·98	0·16	1·22	0·49	1·03	1·20	0·17
Count omitted	0·98	0·16	1·00	0·16	1·03	0·98	0·05
A.							
Count included	1·22	0·49	1·03	0·16	1·08	1·25	0·17
Count omitted	1·00	0·15	1·03	0·16	1·08	1·03	0·05
Note.—The emulsion yielded about 1·3 staphylococci per cell.							

* Note.—The exact strength of emulsion employed by Fleming's workers cannot be ascertained ⁽¹³⁾.

On 4th August the phagocytic mixture containing A's leucocytes with G's serum gave an extraordinarily low count, which was 30 per cent. below the next lowest, and 45 per cent. below the mean of the whole series. This count which has been deleted affected three sets of indices and produced two very high opsonic indices for A and L, namely, 1·457 and 1·671, and one low cytophagic index of 0·436 for A.

The following are the triple indices for that date:—

A's Opsonic Indices.	L's Opsonic Indices.	A's Cytophagic Indices.	L's Cytophagic Indices.
1·457	1·676	0·436	0·750
1·037	1·258	0·612	0·832
0·937	0·842	0·582	0·557
This count has been deleted.			

Alteration in three indices and the diminution in the calculated error resulting from the omission of one abnormally low count in L and A respectively on 4th August.

L.							
	Opsonic.		Cytophagic.		Opsono-cytophagic.	Theoretical Opsono-cytophagic.	Error.
	Mean Index.	Error.	Mean Index.	Error.	Index.	Index.	
Count included	1·26	0·55	0·71	0·18	0·70	0·97	0·27
Count omitted	1·05	0·41	0·71	0·18	0·70	0·76	0·06
A.							
Count included	1·14	0·52	0·54	0·11	0·64	0·68	0·04
Count omitted	0·99	0·10	0·60	0·03	0·64	0·61	0·03
Note.—The emulsion yielded about 2·2 staphylococci per cell.							

These two counts are the only ones which have been omitted out of those obtained from nearly 350 consecutive phagocytic mixtures. One of us (E.G.) recounted about a dozen of the 700 slides, either because he was interrupted, or because his attention was wandering, but the recounts were made before he had the slightest idea what the indices were likely to be.

No counts were discarded on the grounds that the smears were spoilt.

The following tables give in tabular form the indices and errors (calculated as previously described on p. 98) for L and A, during May, June, July, and August.

These figures are graphically displayed in Charts I. to VI. (p. 129).

Table of Indices and Errors.

For L with *Staphylococcus albus*.

Note.—The error is the average difference between the triple estimations, taken in pairs, of the opsonic or cytophagic indices respectively.

Date.	Opsonic Index.	Error.	Cytophagic Index.	Error.	Actual Opsono-cytophagic Index.	Theoretical Opsono-cytophagic Index.
May 25 .	1.03	0.18	0.90	0.09	1.02	0.93
„ 28 .	0.90	0.23	0.75	0.16	0.67	0.65
„ 31 .	1.02	0.09	1.02	0.08	0.99	1.04
June 3 .	1.00	0.23	0.90	0.18	0.91	0.90
„ 6 .	0.93	0.11	0.92	0.15	0.90	0.85
„ 9 .	1.08	0.21	0.83	0.17	0.82	0.91
„ 12 .	0.88	0.04	0.91	0.17	0.74	0.79
July 14 .	0.97	0.19	0.81	0.03	0.72	0.78
„ 17 .	0.99	0.23	0.91	0.20	0.93	0.90
„ 20 .	1.03	0.09	0.86	0.09	0.91	0.89
„ 23 .	0.81	0.13	0.89	0.35	0.86	0.70
„ 26 .	0.91	0.15	0.95	0.00	0.80	0.86
„ 30 .	1.03	0.20	0.92	0.18	0.93	0.95
Aug. 1 .	0.98	0.16	1.00	0.16	1.03	0.98
„ 4 .	1.05	0.41	0.71	0.18	0.70	0.76
„ 8 .	0.98	0.16	0.99	0.08	0.88	0.95
„ 10 .	1.05	0.14	0.82	0.04	0.88	0.87
Total .	16.64	2.95	15.09	2.31	14.69	14.71
Mean Ave.	0.98	0.17	0.89	0.13	0.86	0.86

Table of Indices and Errors.

For A with *Staphylococcus albus*.

Note.—The error is the average difference between the triple estimations, taken in pairs, of the opsonic or cytophagic indices respectively.

Date.	Opsonic Index.	Error.	Cytophagic Index.	Error.	Actual Opsono-cytophagic Index.	Theoretical Opsono-cytophagic Index.
May 25 .	1.12	0.19	0.69	0.16	0.80	0.81
„ 28 .	1.15	0.34	1.06	0.13	1.03	1.21
„ 31 .	1.06	0.12	0.94	0.11	0.93	1.00
June 3 .	1.04	0.16	1.07	0.17	1.10	1.11
„ 6 .	0.93	0.24	0.71	0.20	0.56	0.64
„ 9 .	0.99	0.15	0.81	0.13	0.74	0.80
„ 12 .	0.96	0.18	1.11	0.07	1.09	1.07
July 14 .	1.01	0.08	0.88	0.23	0.78	0.89
„ 17 .	1.22	0.27	0.77	0.12	0.81	0.99
„ 20 .	1.15	0.12	0.83	0.07	0.98	0.98
„ 23 .	1.01	0.31	0.82	0.07	0.97	0.83
„ 26 .	1.00	0.21	1.09	0.21	1.07	1.09
„ 30 .	1.15	0.23	0.87	0.17	0.98	1.02
Aug. 1 .	1.00	0.15	1.03	0.16	1.08	1.03
„ 4 .	0.99	0.10	0.60	0.03	0.64	0.61
„ 8 .	0.96	0.11	0.87	0.14	0.76	0.81
„ 10 .	1.04	0.04	0.74	0.08	0.83	0.78
Total .	17.78	3.00	14.89	2.25	15.15	15.77
Mean Ave.	1.04	0.18	0.87	0.13	0.89	0.93

Table of Indices and Errors.

For L with Tubercle Bacilli.

Note.—The error is the average difference between the triple estimations, taken in pairs, of the opsonic or cytophagic indices respectively.

Date.	Opsonic Index.	Error.	Cytophagic Index.	Error.	Actual Opsono-cytophagic Index.	Theoretical Opsono-cytophagic Index.
May 25 .	0.89	0.10	0.86	0.03	0.79	0.75
„ 28 .	1.09	0.11	0.61	0.06	0.64	0.70
„ 31 .	1.07	0.24	0.87	0.25	1.02	0.94
July 17 .	0.93	0.11	0.95	0.11	0.89	0.88
„ 20 .	0.97	0.11	1.01	0.11	1.01	0.98
„ 23 .	1.04	0.04	0.99	0.11	0.98	1.03
„ 26 .	0.98	0.25	1.16	0.19	1.30	1.14
„ 30 .	0.99	0.30	0.90	0.28	0.87	0.89
Aug. 1 .	0.97	0.21	1.09	0.34	1.12	1.06
„ 4 .	1.03	0.02	0.93	0.16	0.88	0.96
Mean Average	1.00	0.15	0.94	0.16	0.95	0.93

Table of Indices and Errors.

For A with Tubercle Bacilli.

Note.—The error is the average difference between the triple estimations, taken in pairs, of the opsonic or cytophagic indices respectively.

Date.	Opsonic Index.	Error.	Cytophagic Index.	Error.	Actual Opsono-cytophagic Index.	Theoretical Opsono-cytophagic Index.
May 25 .	1.02	0.17	0.87	0.11	0.83	0.89
„ 28 .	1.35	0.11	1.03	0.11	1.29	1.38
„ 31 .	1.20	0.23	0.82	0.10	1.05	1.02
July 17 .	1.09	0.07	0.90	0.04	0.94	0.99
„ 20 .	1.02	0.08	0.98	0.07	0.99	1.00
„ 23 .	1.23	0.13	0.90	0.08	1.09	1.13
„ 26 .	1.01	0.27	1.09	0.33	1.16	1.10
„ 30 .	1.01	0.18	0.88	0.14	1.03	0.87
Aug. 1 .	1.16	0.21	0.96	0.13	1.13	1.12
„ 4 .	0.98	0.16	0.74	0.05	0.69	0.72
Mean Average	1.11	0.16	0.92	0.12	1.02	1.02

SUPPLEMENTARY OBSERVATIONS.

Before passing on to the interpretation of results, we must briefly describe some of the earlier observations by one of us (E. G.) from June 1907 to April 1908.

A. *Observations upon the cytophagic indices of Dr. X, a normal individual, and F, a man æt. 25 years, with chronic tubercular hip, were made with Staphylococcus albus in October, November, and December 1907.*

The technique differs from that already described on p. 94 et seq. in the following particulars:—

(a) The phagocytic mixtures were put up with the “pooled” serum of two or more individuals.

(b) The leucocytes were washed three times in normal salt solution.

(c) The method of counting was the same, but usually 100 leucocytes were enumerated on each slide.

(d) Duplicate phagocytic mixtures were employed, so that the indices set down are the mean of two indices.

Unfortunately the opsonic indices were not estimated.

TABLE I.—*Cytophagic Indices of Dr. X, healthy, and two Hospital Patients to Staphylococcus albus, G's Leucocytes taken as Control.*

	Dr. X.	F. Tubercle.	P. Cirrhosis.
Oct. 21	1·23	...	0·98
„ 26	1·20	0·72	...
Nov. 1	...	...	0·99
„ 12	1·12	0·75	...
Dec. 9	1·32	...	...
„ 10	1·33	...	...
„ 11	1·26	0·68	...
„ 14	1·27	0·54	...
„ 22	1·09	...	...
„ 31	1·06	0·79	...
Average	1·21	0·70	0·985

B. *Observations upon L, the laboratory assistant, previously mentioned and R, another laboratory attendant, were made in April 1908, the technique was exactly the same as that described on p. 94. But as it was found that R's washed corpuscles were agglutinated by L's serum in the phagocytic mixture the work was abandoned.*

The count from this phagocytic mixture has been omitted in the calculation of the indices given below.

Indices of R, healthy Laboratory Attendant.

Tubercle Bacillus.				Staphylococcus.		
Date.	Opsonic.	Cytophagic.	Opsono-cytophagic.	Opsonic.	Cyto-phagic.	Opsono-cytophagic.
April 7 . .	0·98	1·08	1·05	1·09	1·12	1·20
„ 9 . .	0·96	1·19	1·08	1·06	1·11	1·18
Mean . .	0·97	1·13	1·06	1·07	1·11	1·19

Indices of L, healthy Laboratory Assistant.

Tubercle Bacillus.				Staphylococcus.		
Date.	Opsonic.	Cytophagic.	Opsono-cytophagic.	Opsonic.	Cytophagic.	Opsono-cytophagic.
April 7 .	1.07	0.81	0.88	1.17	0.93	0.98
„ 9 .	1.06	0.97	0.97	1.02	0.88	0.95
Mean .	1.06	0.89	0.92	1.09	0.90	0.96

The next table gives the cytophagic indices to *Staphylococcus albus* of several hospital patients selected at random in June and October 1907, at least a hundred cells were counted, G's leucocytes taken as control.

TABLE II.

June 1907	Diabetes	0.83
	Bright's disease	1.01
	Septicemia	0.71
	Bright's disease	0.77
October 1907	Chronic bronchitis	1.03
	Axillary abscess	0.92
	Appendicitis	0.70

INTERPRETATION OF RESULTS.

The following conclusions may be drawn from the systematic examination of the blood of L, G, and A, during May, July, and August 1908.

1. *The opsonic index is not an index of the "power of phagocytic response, and the leucocytes are "not" practically an indifferent factor in phagocytosis."*

An average of the seventeen consecutive observations with staphylococcus shows that although L's opsonic index is 0.98, *i.e.* practically the same as the control G, yet the phagocytic power of his own leucocytes with his own serum is only 0.86, because his leucocytes are deficient in inherent phagocytic power, the cytophagic index being 0.89. (See Charts 1 and 2.) Exactly the same obtains for L, with tubercle.

The estimations with A for tubercle bacillus and staphylococcus are specially interesting, because his opsonic index is above that of the control G, namely, 1.11 to tubercle and 1.04 to staphylococcus, yet the phagocytic power of his own leucocytes with his own serum is only 1.01 and 0.89 respectively. (See Charts 3 and 4.)

The want of correspondence between the opsonic and the opsono-cytophagic indices is also specially well seen on Table III. p. 108, which gives an average of all the indices upon the days when the opsonic index was above and below 1 respectively.

TABLE III.—*Summary of the Averages of all the Opsonic, Cytophagic, and Opsono-cytophagic Indices, when the Opsonic Indices are above and below 1 respectively, demonstrating that the Opsonic Index does not measure the total Phagocytic Power of the Blood.*

		Name.	Organism.	Occasions.	Average Opsonic Index.	Average Cytophagic Index.	Average Opsono-cytophagic Index.
1	Opsonic index, above 1	L	S	7	1.04	0.86	0.89
2	„ below 1	L	S	9	0.93	0.90	0.84
3	„ above 1	A	S	10	1.09	0.86	0.91
4	„ below 1	A	S	5	0.96	0.81	0.77
5	„ above 1	L	T	2	1.03	0.96	0.99
6	„ below 1	L	T	5	0.97	1.02	0.99
7	„ above 1	A	T	6	1.09	0.95	1.08
8	„ below 1	A	T	1	0.98	0.74	0.89

It is obvious, therefore, that we cannot take the opsonic index of the blood of these two individuals, L and A, tested with a given strain of *Staphylococcus albus* and tubercle bacillus as an index of their power of phagocytic response, and that it is not right as far as they are concerned to regard their leucocytes “as practically an indifferent factor in phagocytosis.” These conclusions are confirmed by the two observations in April upon R and L. (See pp. 106, 107.)

Tables I., p. 106, and II. p. 107, also show that the inherent phagocytic power of washed corpuscles is not the same in all individuals. Unfortunately the opsonic indices were not estimated in this series.

2. *The inherent phagocytic power of leucocytes from the same individual may vary from time to time.*

An examination of A's cytophagic indices with *staphylococcus* shows considerable variation, which cannot be accounted for by errors of technique for the following reasons:—

(1) The average error calculated for the cytophagic indices of L and A is exactly the same, namely, 0.13. (See Tables, pp. 104.) Yet the variations in the cytophagic curves of the latter are much greater than those of the former.

(2) The striking similarity in A's curves for May and July.

(3) The great accuracy of the triple indices obtained on three occasions when A's mean cytophagic index was *high*, and on three occasions when it was *low*. These indices are given in Table IV., p. 109, as well as some for L arranged in the same manner.

There is also definite evidence of similar variations in the cytophagic indices of L and A for tubercle.

TABLE V.—*Demonstrating Variations in the Cytophagic Indices of A and L respectively.*
Organism, Tubercle Bacillus.

The triple observations are given to show that the variations in the charted indices are not the result of errors of technique.

A's HIGH CYTOPHAGIC INDICES.			
Observation.		Dates.	
		May 28.	July 20.
1		0·948	1·033
2		1·114	0·973
3		1·023	0·933
Mean (<i>i.e.</i> index charted)		1·028	0·980
A's LOW CYTOPHAGIC INDICES.			
Observation.		Dates.	
		August 4.	May 31.
1		0·780	0·753
2		0·728	0·808
3		0·704	0·910
Mean (<i>i.e.</i> index charted)		0·737	0·824
L's HIGH CYTOPHAGIC INDICES.			
Observation.		Dates.	
		July 20.	July 26.
1		1·035	1·053
2		0·921	1·340
3		1·089	1·082
Mean (<i>i.e.</i> index charted)		1·015	1·158
L's LOW CYTOPHAGIC INDICES.			
Observation.		Dates.	
		May 28.	May 25.
1		0·557	0·826
2		0·655	0·880
3		0·630	0·870
Mean (<i>i.e.</i> index charted)		0·614	0·860

The cytophagic indices of Dr. X, made in 1907, also show the occurrence of slight but regular differences in the cytophagic indices.

3. *The variations in the cytophagic indices are probably not specific in normal persons.*

(1) As an index is a ratio between two variable quantities, so variations in the cytophagic index may result from changes either in the control or in the tested leucocytes; if both vary in the same direction to the same extent, the index will remain the same, but if both vary in opposite directions the index will vary considerably. The only means of ascertaining whether the variations in the cytophagic indices of L and A are probably due to variations in their own leucocytes or in those of the control G is to compare the indices actually obtained on the same day with staphylococcus or with tubercle bacillus, or, better still, with both organisms simultaneously.

The variations in L's and A's staphylo-cytophagic and tuberculo-cytophagic indices from 1st August to 10th August are most probably produced by variations in the phagocytic power of the control leucocytes from G.

(2) The tuberculo-cytophagic and the staphylo-cytophagic indices of A tend to vary *together*; the same obtains for L. Having regard to the inaccuracy of the technique and the fact that the observations are made with different bacteria, this last correspondence is too striking to be merely a coincidence, and in curves of this nature the positive is of more value than the negative. (See Chart 5, p. 131.)

(3) A comparison of the average cytophagic indices with tubercle bacillus and with staphylococcus during the same period supports the contention that simultaneous variations in the indices of the same individual towards both organisms vary together.

TABLE VI.—*Table showing the Similarity of Cytophagic Indices for Tubercle Bacillus and Staphylococcus in the same Individual.*

Name.	Average Index with Tubercle Bacillus.	Average Index with Staphylococcus.	Number of Days upon which Observation was made.
L	0·89	0·90	2, viz. April 7 and 9.
R	1·13	1·11	2, „ „
L	0·94	0·89	10 { May 25, 28, 31.
A	0·92	0·88	10 { July 17, 20, 23, 26, 30. August 1, 4.

There is therefore no definite evidence that the leucocytes of one individual phagocytose tubercle bacilli better than staphylococci, or *vice*

versa. Such a specific phagocytosis or want of phagocytosis, is hardly probable seeing that three of the tested individuals, namely, G, L, and R, were in their normal health, while the fourth, A, had only a slight local infection which has scarcely altered his opsonic index.

4. *The cytophagic indices of L and A show greater variations from the normal than the opsonic indices.*

Apart from a perusal of the charts this question may be decided in two ways:—

(a) An average may be taken of all the opsonic and cytophagic indices in order to ascertain which differs more from unity. This method is only a rough one because the indices of the same individual are sometimes above and sometimes below unity; still it demonstrates that the cytophagic indices vary more than the opsonic.

TABLE VII.

Name.	Tubercle.		Staphylococcus.	
	Mean Opsonic Index.	Mean Cytophagic Index.	Mean Opsonic Index.	Mean Cytophagic Index.
A . . .	1.11	0.92	1.04	0.87
L . . .	1.00	0.94	0.98	0.89

(b) We may estimate the variations which occur every three days in the indices of the *same* individual; this may be done by ascertaining the difference between each consecutive pair of opsonic or cytophagic indices. For example, taking the opsonic indices of L for tubercle bacillus, we find the difference in May is 0.22, thus—

Index.		
May 25	0.89	} Difference = 0.20
„ 28	1.09	
„ 31	1.07	Difference = 0.02
		Total . 0.22

The next table shows that the cytophagic indices of the *same* individual tend to vary every three days more than the opsonic in every instance except that of A for tubercle bacillus, when the figures are practically the same.

TABLE VIII.

			A.		L.	
Organism.	Date.	No. of Pairs.	Differences in Opsonic Indices.	Differences in Cytophagic Indices.	Differences in Opsonic Indices.	Differences in Cytophagic Indices.
Tubercle bacillus }	May	2	0.48	0.37	0.22	0.51
	July	6	0.83	0.86	0.26	0.86
Total difference . .			1.31	1.23	0.48	1.37
Staphyl. ,,	May	6	0.34	1.35	0.69	0.73
	July	9	0.82	1.68	0.76	1.09
Total difference . .			1.16	3.03	1.45	1.82

It must be remembered that in estimating the opsonic indices, different sera are put up with the *same* leucocytes, but in the cytophagic indices the same sera are put up with *different* leucocytes. Though it will be demonstrated (see p. 119) that slight alterations in the amount of fluid in the washed corpuscles cannot possibly explain why L's and A's cytophagic indices are persistently low, yet the fact that the dilution of the corpuscles cannot be quite the same, may in a large number of results tend to make the variations in the cytophagic indices appear greater from day to day than they actually are. If, however, this were so one would expect that the calculated error for all the cytophagic indices would be *greater* than for all the opsonic, but the reverse obtains, the figures being 0.168 (opsonic), 0.136 (cytophagic).

The evidence, therefore, supports the view that the cytophagic indices may fluctuate at least as much, probably more than the opsonic.

After all, it is not very surprising that the variations in the cytophagic indices should be as great or even greater than in the opsonic, because opsonin may be regarded as merely a secretion, whereas the leucocytes are living organisms, and phagocytosis is probably the most characteristic manifestation of their vital activity.

5. *Observations on the opsonic indices.*¹

(1) L's opsonic indices for tubercle bacilli and staphylococcus are remarkably regular, especially as only one serum is used as control, not a pool of several. They are always within Bulloch's normal limits of 1.2 and 0.8, and on twenty-four out of twenty-seven occasions within the narrower normal limits of 1.1 and 0.9 recently set by Fleming, (1908¹⁴).

¹ We have not considered, in this paper, the question raised by Simon (1906, 1907³²), Marshall (1908²¹), and Walker (1907³⁴) whether the ordinary bacterial emulsion is strong enough to absorb all the opsonin present in the serum tested.

(2) A's opsonic indices for tubercle bacilli and staphylococcus are relatively higher than that of the control, and show more variations for both organisms.

The staphylo-opsonic index is on the average 1.04, and above 1.1 on four occasions (p. 104). A relatively high index sometimes obtains in chronic infections (¹).

There is no apparent cause for the high and irregular tuberculo-opsonic index which averages 1.11 (p. 105).

It will be noticed that when the opsonic indices are high the cytophagic indices tend to be low, and *vice versa*. This effect may be due partly to the method of calculating the indices already described (p. 98), and summarised again below.

TABLE IX.—*Table summarising Method of calculating Indices.*

Number.	Source of Leucocytes in Phagocytic Mixture.	Source of Serum in Phagocytic Mixture.
1 2 3	A G L	A
4 5 6	A G L	G
7 8 9	A G A	L

Fractions used in calculating A's *cytophagic* indices— $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{3}$.
Fractions used in calculating A's *opsonic* indices— $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{3}$.
Fraction used in calculating A's opsono-cytophagic index— $\frac{1}{2}$.

It will be noticed that phagocytic mixture No. 1 appears as the numerator, and No. 5 as the denominator, in the opsonic and the cytophagic indices, so that the effect exercised by these two figures is the same; on the other hand, 4 and 2 appear as numerator and denominator in turn.

Now in the case of A the opsonic indices are usually high, and the cytophagic indices usually low. If the count of No. 2 is higher than that of No. 4, the cytophagic index will be somewhat lowered, but at the same time the opsonic index will be somewhat raised. In other words, the tendency will be to separate the opsonic and cytophagic indices from each other.

Again, if the count of No. 2 is lower than that of No. 4, it will tend to approximate the opsonic and cytophagic indices. This tendency will be masked by the figures in the fraction $\frac{1}{2}$ and modified by the last fraction, which is different for both, namely, $\frac{7}{8}$ for the cytophagic indices, and $\frac{3}{8}$ for the opsonic indices.

The system of employing four out of eight counts to estimate two of the three opsonic indices, and also two of the three cytophagic

indices for the same person, is all that can be fairly objected to in our technique, and the objection is not a serious one. The reason for adopting such a system has been stated on page 99. It is partly or wholly responsible for the tendency of the opsonic and cytophagic indices to vary in opposite directions, and it tends to make the experimental error in demonstrating the close relationship which exists between the opsonic and cytophagic indices, and the opsono-cytophagic index appear less than it actually is.

But it must be distinctly understood that each of the three cytophagic indices is calculated from *six separate* phagocytic mixtures, and each of the three opsonic indices is also calculated from *six separate* phagocytic mixtures.

6. *Fluctuations in the opsono-cytophagic index.*

The objection may be raised that it is impossible for the total power of phagocytic response, as shown by the opsono-cytophagic index of A and L, to vary occasionally from 25 to 50 per cent. in three days. A, however, was infected with staphylococcus, and the greatest variations occur in his staphylococcal opsono-cytophagic index; namely, on 6th June and 4th August. But the variations probably appear greater than they actually are: first, because some may be partly due to error in technique; secondly, because these indices are only ratios between two quantities which may fluctuate simultaneously in opposite directions, and thereby produce considerable change in the index.

If the phagocytic response obtained by mixing an individual's washed leucocytes with serum *in vitro* gives any indication of the *in vivo* phagocytic power of his blood, it is not very remarkable that fluctuations should occur, for they may be an expression of variation in the "resisting" power—as it is called for want of a better term. It is recognised that the "resistance" of a normal man may vary from time to time. We assume that it falls before an attack of pneumonia or an outbreak of boils. In this connection it is worth noting that when on 4th August in the control G a severe cold in the head was beginning, the inherent phagocytic power of his leucocytes rose considerably above that of A and L, but fell four days later. It was at this period that a slight leucocytosis occurred. The opsonic index was stationary.

7. *Other variations in the blood of normal persons as marked as the variations in phagocytic power already described.*

It has been proved that L's polymorpho-nuclear leucocytes phagocytose on the average about 10 per cent. fewer bacteria, and Dr. X's leucocytes 20 per cent. more bacteria than those of the control G.

Some may urge that it is absurd to believe such marked differences can exist between the inherent phagocytic power of leucocytes of three healthy men when tested *in vitro*.

But there are plenty of instances of similar idiosyncrasy in physiology; thus a comparison of the normal pulse or respiration rate in adults might easily give indices of 1.2 or 0.8. The various

constituents of the blood in normal persons may yield indices which differ considerably, for example :—

(a) *The number of erythrocytes.*—The average number of red blood corpuscles is 5,000,000 per c.mm., “but 6,000,000 is by no means rare among healthy young men, and higher figures are occasionally seen (6).” If the latter figure is taken as the control it gives an erythrocyte index of $\frac{5,000,000}{6,000,000} = 0.83$.

(b) *The number of leucocytes.*—The number of white cells in the blood varies between 5000 and 10,500 per c.mm., and averages 7500 (7). Taking the latter figures as control we obtain the indices $\frac{5000}{7500} = 0.6$, and $\frac{10,500}{7,500} = 1.4$.

These indices deviate from unity as much as or more than the average deviation of the cytophagic indices of L, A, R, or Dr. X.

OBJECTIONS.

OBJECTION I.—The first and most vital objection which can be raised is that, *owing to the inaccuracy of the opsonic technique, no conclusions whatever can be drawn from it, especially as the majority of the indices set down in this research are admitted to be within Wright's normal limits of 0.8 and 1.2.* In this connection we wish to point out the following facts :—

1. That the phagocytic mixtures were put up and counted entirely by one observer (E. G.), who has had three years' experience of opsonic work.
2. Our error has been minimised by calculating each opsonic and cytophagic index three times from separate phagocytic mixtures in the observations since May 1908.¹
3. In order to further establish the proposition that the inherent phagocytic power of leucocytes varies, we have repeated these triplicate estimations sixteen times for staphylococcus and nine times for tubercle.

The error in Wright's technique is greater than was at first believed, but those critics who maintain that it is hopelessly inaccurate should note :

1. The similarity between the triple indices in Tables IV. and V., pages 109, 110, especially those on 8th and 10th August, when each index is calculated nine times instead of the usual three.

2. The fact that the inherent phagocytic power of L's leucocytes to staphylococcus was below that of the control in fifteen out of seventeen occasions, and for tubercle in seven out of ten; in the case of A, for tubercle in eight out of ten occasions. It is almost incredible that these figures signify nothing but an imperfect technique.

It may, however, be argued that any support derived from the uniformity of the indices in question is removed by the fluctuations in A's staphylococcus indices. But the similarity of the triple estimations when the mean index is high and low respectively indicate that the fluctuation cannot be wholly due to error. (See Table IV. p. 109.)

3. The marked tendency of the staphylo- and tuberculo-cytophagic indices for A and L to vary together is shown in Chart 5. This is all the more striking because the tubercle bacillus films were counted several weeks after the

¹ All the other cytophagic indices were calculated twice from separate phagocytic mixtures.

staphylococcus, and we had no suspicion of any correspondence in the curves until they were plotted out.

Had we never employed an emulsion yielding less than four bacteria per cell our results would have been more accurate (¹⁷).

OBJECTION II.—Variations in the cytophagic indices are due to leucocytosis.

Ledingham and Bulloch injected sodium cinnamate, nuclein, and other substances into rabbits and guinea-pigs, and found that during the period of hyper-leucocytosis the phagocytic power of the polymorpho-nuclear leucocytes is considerably reduced.

Leucocytosis, therefore, in the blood of "L" and "A" might explain their low cytophagic indices. This possibility is negated by an examination of Table X., giving systematic leucocyte counts of the three individuals as previously described from the same samples of blood as that furnishing the washed corpuscles. It should be remembered that the blood was drawn in nearly every case about two hours after a meal.

As a matter of fact "G's" leucocyte count is on the average rather *higher* than that of the other two. The reverse would be the case if L and A's low indices were the result of leucocytosis. It is interesting to note that the relative number of leucocytes and lymphocytes in the blood of "A," who is the subject of chronic acne indurata, varies considerably, but that the percentage of leucocytes in the case of the control "G" remains much more constant.

"G's" white corpuscle counts show an interesting periodic rise and fall. At the acme of the first period 20th July, he was in his usual health, but at the acme of the second, 8th August, was recovering from an ordinary cold in the head. At the latter period only was the rise due to a well-marked leucocytosis as distinct from a lymphocytosis.

TABLE X.—Table of White Blood Corpuscle Counts.

Date.				G.	L.	A.
May 31	.	.	.	8,437	6,250	10,312
June 3	.	.	.	4,687	5,312	5,312
" 6	.	.	.	7,812	11,874	8,124
" 9	.	.	.	8,125	6,250	10,624
" 12	.	.	.	10,000	13,750	15,624
G.						
Date.	Hours after Meal.	Leucocytes per cm.	Lymphocytes per cm.	Total White Cell Count.	Percentage of Leucocytes.	Percentage of Lymphocytes.
July 14	3	5625	2500	8,125	69·2	30·8
" 17	2½	6875	2500	9,375	63·6	36·4
" 20	2½	9687	3125	12,812	67·7	32·3
" 23	1½	8437	3437	11,874	58·1	41·9
" 26	2	5937	4083	10,000	59·4	40·6
" 30	1½	4375	2812	7,187	60·9	39·1
Aug. 1	1½	5321	3125	8,446	63·0	37·0
" 4	3	6563	3437	10,000	65·6	34·4
" 8	2	9375	2500	11,875	78·9	21·1
" 10	4	5321	3125	8,446	61·8	38·2

TABLE X.—*continued.*

L.						
Date.	Hours after Meal.	Leucocytes per cm.	Lymphocytes per cm.	Total White Cell Count.	Percentage of Leucocytes.	Percentage of Lymphocytes.
July 14	3	3592	1715	5,312	52.2	47.8
" 17	3	7812	1562	9,374	80.0	20.0
" 20	2½	6875	4062	10,937	40.9	59.1
" 23	1½	4688	2188	6,876	53.3	46.7
" 26	2½	5312	2187	7,500	58.9	41.1
" 30	1½	5321	3125	8,446	63.0	37.0
Aug. 1	2	4687	3187	6,874	68.2	31.8
" 4	2½	4687	3125	7,812	56.5	43.5
" 8	2	4688	2812	7,500	62.5	37.5
" 10	3½	4687	3125	7,812	56.5	43.5
A.						
July 12	2½	6875	2500	9,375	63.6	36.4
" 17	3	3437	1562	4,999	54.6	45.4
" 20	2½	5312	2343	7,656	55.9	44.1
" 23	1½	3916	2031	5,947	48.2	51.8
" 26	2	3437	2343	5,770	31.9	68.1
" 30	1½	6562	2500	9,062	72.4	27.6
Aug. 1	1	2656	3594	6,250	42.5	57.5
" 4	2½	5547	1797	7,344	77.0	23.0
" 8	2	6250	2344	8,594	71.6	28.4
" 10	3½	4062	2656	6,718	60.5	39.5

OBJECTION III.—The variations in the cytophagic indices are due to *unequal dilutions of the washed corpuscles.*

Fleming (1908¹⁵) compared the amount of phagocytosis obtained with a given sample of washed corpuscles with that of the same sample diluted several times with normal salt solution. He states "that in twofold dilution the results varied from 10 per cent. to 20 per cent. *higher* than when the undiluted corpuscles were used, with one exception, out of six observations where probably an error crept in." It is not clear whether he means by "twofold dilution" the addition of one equal volume or of two equal volumes of salt solution to the washed corpuscles.

These observations suggest that L's low cytophagic index might possibly be due to G's washed corpuscles being more diluted with salt solution than L's.

The precaution adopted to ensure that the percentage of salt solution was the same in the three samples of washed corpuscles from G, L, and A respectively have already been described with the technique (p. 95). The actual corpuscular dilution of each sample was always ascertained by counting the number of red blood corpuscles per c.mm., and Table XI. indicates that the dilution was always approximately the same on each day, especially in the second series of observations when the counter (G. L. C.) became more expert.

TABLE XI.—Table giving Number of Red Blood Cells per Cubic Millimetre in the Mixed Washed Corpuscles.

Date.	G.	L.	A.
May 31	5,822,222	6,866,666	7,866,666
June 3	4,183,333	6,400,000	6,616,666
„ 6	7,916,666	8,633,333	7,016,666
„ 9	6,826,500	9,490,500	8,144,625
„ 12	6,937,500	9,712,000	5,855,250
July 17	8,497,500	8,497,500	8,965,000
„ 20	7,782,500	7,755,000	9,033,750
„ 23	8,126,250	8,497,500	7,218,750
„ 26	8,167,500	9,391,250	8,910,000
„ 30	8,470,000	9,033,750	8,868,750
Aug. 1	8,387,500	9,157,500	8,785,250
„ 4	9,006,250	8,332,500	7,988,750
„ 8	6,215,000	3,863,750	5,417,500
„ 10	6,545,000	2,708,750	3,286,250

On one occasion only, namely, 10th August, could the washed corpuscles of L and A be described as diluted with an equal volume of salt solution when compared with those of the control G.

If an unequal dilution of the three samples of washed corpuscles was really responsible for low cytophagic indices, then on 4th, 8th, and 10th August the index of both L and A should be *above*, not below, that of G, for a “diminution in the number of washed corpuscles” in the phagocytic mixture “increases the amount of phagocytosis” (1908¹⁵).

These facts prove that there is no connection whatever between the cytophagic indices of the three individuals and the amount of dilution of their washed blood corpuscles.

OBJECTION IV.—The variations in cytophagic indices are due to agglutination of the red blood corpuscles in the phagocytic mixture.

Agglutination of the red corpuscles in the phagocytic mixtures, which according to Fleming increases phagocytosis (1908¹⁵), cannot explain our results. For no agglutination occurred in any of the various combinations of sera and washed corpuscles employed, except in the experiments with R's corpuscles in April, described p. 106.

OBJECTION V.—The variations in the cytophagic indices are due to some *fortuitous combination of serum and leucocytes employed*.

Fleming gives experiments to show that the opsonic index is the same whether the washed corpuscles employed are from a healthy or infected person.

Potter (1907²⁶), on the contrary, maintains “that the opsonic indices with sera vary with the source of the leucocytes, and sometimes even when the sources are two or more apparently normal individuals.”

Shattock and Dudgeon (1907²⁹) found that immune or active cells do more work in immune than in normal serum, “which points to some interaction between the patient's cells and the patient's serum.”

If these statements are correct, it may be objected that A's and L's cytophagic indices are low because the sera employed are unfavourable to the

phagocytic power of their leucocytes, but favourable to the phagocytic power of the control leucocytes from G.

Table XII., however, proves that as far as these three *normal* individuals are concerned, L's and A's cytophagic and opsonic indices for tubercle bacillus and staphylococcus are practically the same, no matter which strain of serum or leucocytes is employed.

TABLE XII.

CYTOPHAGIC INDICES.					
Leucocytes.	Bacterium.	Number of Observations.	Cytophagic Indices with A's Serum.	Cytophagic Indices with G's Serum.	Cytophagic Indices with L's Serum.
A's . . .	S	21	0·857	0·846	0·864
L's . . .	S	21	0·906	0·860	0·912
A's . . .	T	10	0·918	0·946	0·880
L's . . .	T	10	0·917	0·966	0·933
OPSONIC INDICES.					
Serum.	Bacterium.	Number of Observations.	Opsonic Indices with A's Leucocytes.	Opsonic Indices with G's Leucocytes.	Opsonic Indices with L's Leucocytes.
A's . . .	S	21	1·046	1·039	1·090
L's . . .	S	21	1·000	0·980	1·024
A's . . .	T	10	1·089	1·146	1·088
L's . . .	T	10	0·976	1·100	0·998

OBJECTION VI.—The variations in the cytophagic indices are due to *one strain of leucocytes being more susceptible to injury from washing, centrifuging, or from standing at the room temperature.*

A's low cytophagic indices might conceivably be explained by assuming that his leucocytes are more injured than G's by the rough usages of *in vitro* experimentation.

Fleming states that the freshness of the washed corpuscles affects their phagocytic power, but Wright and Douglas (1903⁴⁰) found no indication of variation within the space of a few hours. Even after the lapse of three days the phagocytic power has not declined to less than one-half or one-third of that of the blood freshly drawn.

With the view of elucidating these points the washed corpuscles of G, L, and A were put up with staphylococcus in the usual manner, but the whole series of observations was repeated three times with forty-five minutes' interval between each. The emulsion was constantly stirred to prevent loss of strength from sedimentation of the bacteria. A second capsule of serum was employed in the third series in case the opsonin in the first capsule should have been appreciably diminished by contamination with cocci introduced by the points of the pipettes.

TABLE XIII.—*Total Number of Cocci Phagocytosed by G's, L's, and A's Leucocytes put up in the usual way and estimated three times on the same day at forty-five minutes' interval.*

Series I.			Series II.			Series III.		
	Cocci Phagocytosed.	Hours Blood Standing.	Cocci Phagocytosed.	Hours Blood Standing.	Percentage Change.	Cocci Phagocytosed.	Hours Blood Standing.	Percentage Change.
Aug. 8 .	3282	1½	3365	2½	+2·5	2946	3¼	-9·4
„ 10 .	5710	1	5296	1½	-7·2	5166	2½	-9·5

It is clear from this table that the combined phagocytic capacity of the three strains of washed corpuscles which have previously stood about one and a half hours, diminished nearly 10 per cent., after standing for a further period of one and a half hours.

The counts in the second series, on 8th August, show a slight increase, possibly because the whole batch of slides was stained rather more intensely with Leishmann's stain, so that cocci, usually faintly stained, became visible.

The next table proves conclusively that the slight decline in phagocytic capacity affects all three strains in leucocytes equally, for there is practically no alteration in the cytophagic indices of any one strain in the three series.

TABLE XIV.—*Mean of Triple Estimations of A's and L's Cytophagic Indices estimated three times on the same day at forty-five minutes' interval.*

	Series I.	Series II.	Series III.
L, August 8	0·96	0·98	1·02
L, „ 10	0·79	0·85	0·80
A, „ 8	0·87	0·83	0·91
A, „ 10	0·75	0·75	0·70

The possibility that L's or A's low cytophagic indices are due to the phagocytic power of their leucocytes declining more rapidly than that of the control G, can also be excluded by the daily record, kept in the second series of experiments, of the time elapsing between drawing the blood and putting up the phagocytic mixture. (See Table XV. p. 122.)

Taking, for example, A's cytophagic indices for staphylococcus, the table shows that on 26th July and 1st August, when three hours had elapsed before the blood was put up, the cytophagic indices were 1·09 and 1·03, yet upon 17th and 23rd July and 8th August, when the same period also elapsed, the indices were 0·77, 0·82, and 0·87 respectively.

Lastly, in order to prevent any one strain of washed corpuscles having an unfair advantage with regard to the time of standing on any particular day, the order of putting up the phagocytic mixtures for the three cytophagic indices of each individual was always varied thus—first series, corpuscles from A, then from G, then from L; second series, corpuscles from G, then

from L, then from A; third series, corpuscles from L, then from A, then from G.

In Table XV., however, for the sake of simplicity the phagocytic mixtures are given as if they were put up in the same order for each series.

TABLE XV.—*Illustrating various Points in connection with the Preparation of the Washed Blood Corpuscles, and the putting up of the Phagocytic Mixtures.*

Date.	I. Time of drawing Blood.	II. Hours after Meal.	III. Time each Sample of Corpuscles in Centrifuge.	IV. No. of Times washed in a NaCl.	V. Time elapsing between drawing and putting up Blood.	VI. Incubation Times.	VII. Strength of Emulsion.
			Minutes.		Hours.	Minutes.	
July 14 .	11 a.m.	{ G, 3 L, 3 A, 2½	... 39 ...	... 2 ...	S. 2¼	... 15 ...	 S. 5·1
July 17 .	11.15 ..	{ G, 2½ L, 3 A, 3	... 55 ...	... 2 ...	T. 2¼ ... S. 3	... 15 ...	T. 4·7 ... S. 3·4
July 20 .	11 ..	{ G, 2¼ L, 2½ A, 2½	... 42 ...	... 2 ...	T. 1½ ... S. 2½	... 15 ...	T. 4·3 ... S. 6·2
July 23 .	10.15 ..	{ G, 1½ L, 1½ A, 1½	... 65 ...	... 2 ...	T. 2 ... S. 3	... 20 ...	T. 3·4 ... S. 1·3
July 26 .	12 noon.	{ G, 2 L, 2½ A, 2	... 40 ...	... 2 ...	T. 2 ... S. 3	... 20 ...	T. 2·7 ... S. 2·2
July 30 .	10 a.m.	{ G, 1½ L, 1½ A, 1½	... 39 ...	... 1 ...	S. 2½ ... T. 2¼	... 20 ...	S. 2·2 ... T. 3·3
August 1	10.10 ..	{ G, 1¾ L, 2 A, 1½	... 50 ...	... 1 ...	S. 3 ... T. 4	... 20 ...	S. 2·3 ... T. 1·7
August 4	11.30 ..	{ G, 3 L, 2½ A, 2¼	... 40 ...	... 1 ...	S. 2¼ ... T. 3¼	... 20 ...	S. 2·2 ... T. 2·8
August 8	2.45 p.m.	{ G, 2 L, 2 A, 2	... 40 ...	... 1 ...	... S. 3 ...	... 20 ...	... S. 6·5 ...
August 10	12.30 ..	{ G, 4 L, 3½ A, 3½	... 40 ...	... 1 ...	... S. 2 ...	... 20 ...	... S. 3·0 ...

OBJECTION VII.—The variations in the cytophagic indices are due to some *other variable factor* in technique.

1. *The duration of centrifuging* clearly has no influence on the indices, for we find on 17th and 23rd July and 1st August, A's cytophagic indices for staphylococcus were 0·77, 0·82, and 1·03, while the time of centrifuging

was fifty to sixty-five minutes, while on 26th July and 4th and 10th August the indices were 1·09, 0·60, and 0·74, and the time of centrifuging was 40 minutes. It is necessary to repeat that on each day the three samples of blood were all prepared as Fleming advises "at the same time and under the same conditions," that they were drawn within a few minutes of each other, they were washed in the same quantity of citrate or normal salt solution, and they were centrifuged together.

2. *The number of washings* in normal salt solution has no effect. In the experiments with Dr. X quoted in another place, the corpuscles were washed three times in normal salt solution.

3. *The strength of emulsion, the time elapsing between a meal and drawing the blood, and duration of incubation period* also exercise no influence upon the phagocytic indices. (See Table XV. p. 122.)

Doubtless many of the objections just raised are hardly worthy of serious consideration, but we have mentioned them in order to exclude every possible fallacy in the interpretation of our results.

These considerations also dispose of all the objections which might be urged against the work with the blood of Dr. X and R, except one. No systematic blood count was made, but there is not the slightest reason to believe that either was subject to leucocytosis at any time. It is quite possible, however, that a leucocytosis was present in some of the hospital patients on pages 106, 107.

CRITICISM OF THE EVIDENCE UPON WHICH THE WRIGHT HYPOTHESIS IS BASED.

We have thought it advisable to quote fully the actual experiments made by Wright and Douglas (1904) and others. Upon these depends to a very great extent, the statement that "the phagocytic count is uninfluenced by the variety of corpuscles employed" (1906 ³⁷).

A. OBSERVATIONS OF WRIGHT AND DOUGLAS (1904 ³⁹).

EXPERIMENT A.

Immunised patient's washed corpuscles	3 vols.
Immunised patient's serum	3 "
Suspension of staphylococcus culture	1 vol.
Phagocytic index (<i>20</i> ¹ patient's washed blood corpuscles), 25·7.	

EXPERIMENT B.

Washed corpuscles from normal man	3 vols.
Serum from normal man	3 "
Suspension of staphylococcus culture	1 vol.
Phagocytic index (<i>15</i> ¹ patient's washed blood corpuscles), 13.	

EXPERIMENT C.

Immunised patient's washed corpuscles	3 vols.
Serum from normal man	3 "
Suspension of staphylococcus culture	1 vol.
Phagocytic index (<i>15</i> ¹ patient's washed blood corpuscles), 13.	

¹ The italics are ours.

Washed corpuscles from normal man	.	.	.	.	.	3 vols.
Serum from immunised patient	.	.	.	.	.	3 "
Suspension of staphylococcus culture	.	.	.	.	.	1 vol.

Phagocytic index (15¹ patient's washed blood corpuscles), 28.2.

Rabbit's serum mixed with emulsion of staphylococci and human leucocytes (from seven persons) in the proportion of 3 : 1 : 3. Phagocytic count obtained by counting the number of cocci in *thirty-five*¹ polynuclear leucocytes, and then calculating the number per leucocyte.

				Cocci per Leucocyte.
1.	Rabbit's serum + cocci + corpuscles	(of W. B., a normal male)	.	=9·8
2.	" + " + "	(of F. T., ")	.	=9·3
3.	" + " + "	(of O. G., ")	.	=9·7
4.	" + " + "	(of R. D., ")	.	=9·6
5.	" + " + "	(of C. H., ")	.	=9
6.	" + " + "	(of H. M., an anæmic female)	.	=9·9
7.	" + " + "	(of S. M., male, facial acne)	.	=9

The test material in this experiment was an emulsion of tubercle bacilli, and the number of tubercle bacilli in 100 leucocytes was counted, and the average struck as usual.

						Tubercle bacilli per Leucocyte.
1.	Serum of normal individual	+	tubercle bacilli emulsion	+	leucocytes of normal individual	5·7
2.	"	"	+	"	" + leucocytes of lupus patient	5·4
3.	"	"	+	"	" + "	5·2
4.	"	"	+	"	" + "	5·3
5.	Serum of lupus patient	+	"	"	" + leucocytes of normal individual	2·5
6.	"	"	+	"	" + "	2·4
7.	"	"	+	"	" + "	3·2

	Number of Staphylococci per Cell.
A, healthy nurse	4.47
B, child with acute rheumatism	4.38
C, healthy student	4.52
D, laboratory workers	4.08
E, „	4.00
F, patient with tuberculous enteritis	3.99
G, patient with tuberculous and staphylococcal infection of hip	4.02

The painful experience of the last few years has taught all opsonists that the number of corpuscles enumerated by Wright and Douglas (fifteen to twenty) and by Bulloch and Atkin (thirty-five in experiment A) is too small for observations of such great importance.

¹ The italics are ours.

The employment by Bulloch of a strong emulsion, *i.e.* one yielding about nine cocci per leucocyte, may compensate for the small number of leucocytes counted. Wright's emulsion was abnormally strong, and it must be extremely difficult to count accurately twenty-five or more cocci in a leucocyte.

Assuming for the sake of argument that the figures *are* correct, they do not support the conclusions which the observers draw from them, for this reason. Cytophagic indices very similar to our own may be obtained in each set of experiments by taking the highest phagocytic count as the control and the lowest phagocytic count as the test.

A. WORK OF WRIGHT AND DOUGLAS.

$$\text{Cytophagic Index} \left\{ \frac{\text{No. of cocci phagocytosed by patient's corpuscles, patient's serum, 25.7}}{\text{No. of cocci phagocytosed by normal corpuscles, patient's serum, 28.2}} \right\} = 0.91.$$

B. WORK OF BULLQCH AND ATKIN.

EXPERIMENT A.

$$\text{Cytophagic Index} \left\{ \frac{\text{No. of cocci phagocytosed by C. H.'s corpuscles, rabbits' serum, 9}}{\text{No. of cocci phagocytosed by H. M.'s corpuscles, rabbits' serum, 9.8}} \right\} = 0.91$$

EXPERIMENT B.

$$\text{Cytophagic Index} \left\{ \frac{\text{No. of cocci phagocytosed by normal corpuscles, lupus serum, 2.5}}{\text{No. of cocci phagocytosed by normal corpuscles, lupus serum, 3.2}} \right\} = 0.75$$

C. WORK OF FLEMING.

EXPERIMENT C.

$$\text{Cytophagic Index} \left\{ \frac{\text{No. of cocci phagocytosed by laboratory worker's corpuscles serum, 3.9}}{\text{No. of cocci phagocytosed by nurse's corpuscles, serum, 4.47}} \right\} = 0.89$$

It is true that the deviations of these cytophagic indices from unity are comparatively slight, though they have been purposely made as large as possible. But frequent repetition of the experiments would have demonstrated that some of the differences were constant, unless the observers have been particularly unlucky in the selection of their cases.

It is clear from what has been said that the experimental evidence in favour of the fundamental generalisation that the phagocytic power of corpuscles from different sources is "the same" (Fleming), or "practically the same" (Bulloch), is very slender both as regards the number of observations, the technique employed, and the deductions drawn.

OTHER OBSERVATIONS SUPPORTING THE AUTHORS' CONCLUSIONS.

That the leucocyte is not an entirely indifferent factor in phagocytosis was noted by Ledingham in 1906, who found, as Dean (1907⁸) points out, that in certain cases of myelogenous leukæmia the serum may appear to have quite a normal opsonic content, as tested with normal leucocytes, but a markedly subnormal one when tested with the patient's own leucocytes.

Ledingham and Bulloch (1906²⁰) injected sodium cinnamate, nuclein, etc. into rabbits and guinea-pigs, and found that during "the period of hyper-leucocytosis the phagocytic power of the neutrophil leucocytes is considerably reduced."

Bushnell (1907⁵), using staphylococci, compared the phagocytic power of the polymorphs of a leucocythæmic patient with those of a normal person and

found that it was considerably less. In the same year Park and Biggs (1907²⁴) state that the leucocytes from different persons vary not only in "their activity, but also in their selective action." Potter (1907²⁵) concludes that "leucocytes from patients the subjects of bacterial infection do not always agree in phagocytic activity with those from supposedly normal individuals." During the recovery from certain infections, *e.g.* pneumonia, their phagocytic power is above, and in convalescence below, that of normal leucocytes.

Fleming (1908¹⁶) himself admits finding the phagocytic power of leucocytes diminished in acute pneumonia. This he states may be due to the great increase in the number of leucocytes.

Shattock and Dudgeon (1907³⁰), in a valuable series of experiments, compared the phagocytic power of the immune washed leucocytes from infected persons to melanin or *Bacillus coli* with normal or immune serum, and concluded that "the cells vary in value, like the serum, and the only method of arriving at a correct estimate of the patient's hæmophagocytic resistance is to allow the immune cells to work in the immune sera."

GENERAL SUMMARY AND CONCLUSIONS.

1. The Wright school asserts that the inherent phagocytic power of the leucocytes is "the same," or "practically the same," in health and in disease; consequently the opsonic index is an index of the "power of phagocytic response."

2. Adopting Wright's methods, and excluding all the recently described fallacies, a large number of comparisons have been made under precisely similar conditions of the phagocytic power of three strains of leucocytes to tubercle bacillus and staphylococcus, and the bacteria contained in over 50,000 cells enumerated by one of us after three years' experience of opsonic work, in such a way as to exclude the possibility of auto-suggestion influencing the counts.

3. The average error in our duplicate indices is 0.17 for staphylococcus; it is 0.15 for tubercle bacillus as compared with 0.07 for tubercle bacillus, the latest figures from the Wright school, and 0.36 and 0.51 by Fitzgerald and Strangeways. But we have more than compensated for the greater inaccuracy of our technique, compared with that of Wright's assistants, by making duplicate or triplicate estimations of every index. Further, our work is probably more accurate than that of the earlier observers (Wright and Douglas, etc.), on account of the larger number of cells counted.

4. The results of this investigation combined with those upon Dr. X, R, and L, etc. show—

- (a) That the inherent phagocytic power of the leucocytes of different individuals towards bacteria may tend to be constantly below a given standard, not only in disease but to a lesser extent in health.

- (b) The phagocytic power of different leucocytes is subject to variations in the same individual which are at least as marked as those in the amount of opsonin (in undiluted serum), but there is no evidence that the variations are specific in the uninfected.

- (c) The extent of the phagocytosis obtained when washed leucocytes and serum from the same individual are incubated together can be correctly

estimated by separately measuring the proportions due to the serum and washed leucocytes respectively.

5. These variations in inherent phagocytic power were not dependent upon the agglutination of the erythrocytes, and in the case of L, A, and G, and probably the other individuals, upon unequal dilution of the creams, or greater susceptibility of one strain of leucocytes to a particular serum, or to the artificial conditions of centrifuging, washing, or standing at the room temperature, nor were they dependent upon leucocytosis.

6. Owing to the variations in inherent phagocytic capacity of leucocytes the opsonic index is *not* necessarily an index of the power of phagocytic response, when that power is measured *in vitro* and by Wright's technique.

7. Variations of functional activities of different individuals' homologous organs occur in health as well as in disease. Variations also occur in the blood itself, in its opsonic or agglutinative power, and the number of its cells, not only in disease but also in health. It may be inferred, therefore, that the inherent phagocytic power of different strains of leucocytes should also vary, as phagocytosis is a function of the leucocyte.

8. The evidence brought forward by the Wright school to establish their fundamental proposition regarding the leucocyte is unconvincing because the observations are few; but assuming that their results are correct, their deductions are incorrect.

9. Some of our conclusions are supported by the recent observations of other workers, especially by Shattock and Dudgeon, but we have demonstrated that the leucocytes vary in value in persons who cannot be described as "patients" or as possessing "immune cells."

10. Our observations support the hypothesis of a partially independent rôle for the leucocytes in phagocytosis, so ably brought forward by Metchnikoff and so vigorously assailed by the members of the Humoral school.

11. We have been obliged to criticise some of the conclusions of the Wright school, but we fully appreciate the value of their work and the enormous stimulus it has given to research by placing the theory of vaccines on a rational basis and greatly extending its scope in the treatment of disease.

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CHART 1.—*L's Opsonic and Opsono-cytophagic Indices to Staphylococcus albus.*—*L's serum contains the normal amount of opsonin, the index averaging 0.98. The phagocytic power of his leucocytes when influenced by his own serum is subnormal, the index averaging 0.86 (see p. 104).*

CHART 2.—*L's Opsonic and Cytophagic Indices to Staphylococcus albus.*—*The inherent phagocytic power of L's leucocytes is subnormal, the index averaging 0.89. This explains why the phagocytic power of his leucocytes when influenced by the opsonin in his own serum is also subnormal, the index averaging 0.86 (see Chart 1, also pp. 104, 109).*

Note.—The fall in the cytophagic index on 4th August is apparently due to a rise in the phagocytic power of the control leucocytes (see Chart 6).

CHART 3.—A's Opsonic and Opsono-cytophagic Indices to *Staphylococcus albus*.—Although A's serum contains the normal amount of opsonin, the index averaging 1.04; yet the phagocytic power of his leucocytes, when influenced by his own serum, is *subnormal*, the index averaging 0.89 (see p. 109).

CHART 4.—A's Opsonic and Cytophagic Indices to *Staphylococcus albus*.—The inherent phagocytic power of A's leucocytes is *subnormal*, the index averaging 0.87. This explains why the phagocytic power of his leucocytes when influenced by the opsonin in his own serum is also *subnormal*, the index averaging 0.89 (see Chart 3, also pp. 104, 104).

Note.—The fall in the cytophagic index on 4th August is apparently due to a rise in the inherent phagocytic power of the control leucocytes (see Chart 6).

CHART 5.—*L's and A's Cytophagic Indices to Tubercle bacillus and Staphylococcus albus respectively.*—This chart shows the tendency of the staphylo- and tuberculo- cytophagic indices to vary together in the same individual, and indicates that in normal individuals the cytophagic index is *not* specific (see pp. 109, 110).

CHART 6.—*A's and L's Cytophagic Indices to Tubercle bacillus and Staphylococcus albus.*—This chart shows that the variations in the cytophagic indices after 26th July are apparently mainly due to variations in the control leucocytes from G.