Cream and deposit after centrifugalizing examined microscopically:

Cream:	Streptococci	} present
	Staphylococci	
	Epithelial and pus cells	
Deposit:	Streptococci	} present
	Staphylococci	
	Epithelial and pus cells	

Cultures on blood serum of creams and deposits showed the presence of a large Gram-positive, spore-bearing bacillus, probably *B. subtilis*, only (not inoculated or investigated further).

Specific examination after pasteurization—

<i>B. coli communis</i>	absent from	...	1 c.cm.
Klebs-Loeffler bacillus	"	...	1 "
<i>B. enteritidis</i> (Gaertner)	"	...	1 "

No tubercle or diphtheria bacilli were detected in cream or deposit.

CAN OPSONIC ESTIMATIONS BE RELIED ON IN PRACTICE?

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It frequently happens in hospital practice and in private that a reliable method of diagnosing difficult cases of infection is urgently needed. In no disease is this more true than in tuberculosis. For general use, where ordinary methods fail us, the cutaneous methods of von Pirquet, Moro, and Wolff Eisner, including Calmette's modification of these by conjunctival instillation, are not of pre-eminent value. In private practice the dangers and fallacies of

Chart showing Different Opsonic Indices of Identical Serums, as Estimated by Different Observers.

Calmette's test effectually bar its universal acceptance. Of Professor Courmont's modification of the serum diagnosis of tuberculosis there are not as yet sufficient data to judge. In this country, and in America, it is widely believed that in the opsonic index we have at last a method which is trustworthy not only for diagnostic purposes, but also for prognosis, and in determining treat-

ment. After extensive trial of its merits, mainly in tuberculosis, over a period of three years, I am not able to endorse the view that the index is a safe guide to diagnosis and treatment, even when estimated by acknowledged masters of the opsonic art.

In order to test its value as presented to us to-day, I have from my own private practice taken a series of cases, some of which had been sent for diagnosis, and in whom a trustworthy opinion, apart from physical signs, was therefore of real importance. No sort of selection has been made, and results that favour the opsonic method of estimation have been impartially recorded side by side with the unfavourable ones. In all cases the nature of the test has been explained to the various observers, who have without exception encouraged the inquiry. One observer, indeed, expresses the hope that the tests will be published in order to stimulate further efforts in improving the technical details. Such devotion to the highest traditions of scientific research cannot but command our admiration. Each observer mentioned in the accompanying chart is the same throughout, and is designated always by the same letter. It is, perhaps, unnecessary to say that only experts with years of practice and only those directly connected with well-known laboratories have been asked to examine the various serums supplied. In each of these twelve cases two or more samples of serum drawn at the same time and under precisely similar conditions, have been sent to two or more workers, sometimes in the same laboratory, sometimes in different laboratories. In addition to this, as will be seen, occasionally two samples of the same serum have been sent to one observer, who was not informed that they were samples of identical serums.

In each case the following precautions were taken:

1. Whether taken from vein, finger, or ear, the serum was separated from the clot at the end of six hours after puncture of skin or vein.
2. No serum was estimated unless quite free from contamination from red cells.
3. In sealing the tubes all possibility of heating the serum was obviated by using tubes with long drawn ends.
4. All tubes were sealed within five minutes of collection.
5. All indices were estimated within twenty-four hours of collection.
6. All samples of each batch of serum were taken from blood provided by one bleeding, in most cases by venipuncture.

TESTS 1 AND 2.

Disease suspected: renal tuberculosis.

A dose of 0.005 mg. T.R. tuberculin was administered by the mouth and the indices to tubercle bacillus were estimated by two observers in different laboratories, both before and after inoculation. The interval between the two sets of observations was fifteen hours. The indices returned were:

<i>Before Inoculation.</i>			
Observer A.	O.I. to T.B. ...	1.29	Phagocyte count not kept
Observer T.	"	0.97	"

<i>After Inoculation.</i>			
Observer A.	O.I. to T.B. ...	1.15	Phagocyte count not kept
Observer T.	"	1.30	"

In this case observers A. and T. differed in their estimation of the index after inoculation by 0.15, a small difference. Before inoculation they differed by 0.32, a not inconsiderable difference. Further whilst A. estimated that inoculation was followed by a fall of index, Observer T. registered a rise. Two indices were within normal limits and two outside it.

TESTS 3 AND 4.

Disease suspected: tuberculosis, position of focus unknown. In this case there was no direct evidence of any organ being directly the seat of tuberculous infection, but in view of anomalous pyrexia it was necessary to exclude, if possible, such a contingency. Two series of observations were therefore undertaken with a week's interval. In the first test two samples of the same serum were sent to one observer, and a third sample was sent to a second observer in a different laboratory.

The indices returned in the first test were:

Observer A.	O.I. to T.B. ...	1.34	Phagocyte count not kept
Observer T.	"	0.67	"
Observer T.	"	0.55	"

In the second test three tubes of the same sample of serum were sent to three observers in three laboratories a week after the first test.

The indices returned were:

Observer A.	O.I. to T.B. ...	1.06	Phagocyte count not kept
Observer B.	"	0.98	"
Observer T.	"	0.82	"

In the first test the maximum variation was 0.79, the minimum 0.12, and the middle variation 0.67. Observer T. differed from himself by 0.12. In no case was the index within normal limits.

In the second test the maximum variation was 0.24, the next largest 0.16, the minimum 0.8. In no case was the index outside the normal limits.

TEST 5.

Disease: pulmonary tuberculosis, with well-marked physical signs, fever, and tubercle bacillus in the sputum.

Four tubes of the same sample of serum were sent to three observers in three different laboratories. To Observer T. two tubes of the same serum were sent without his knowledge. The indices returned were:

Observer B.	O.I. to T.B.	0.88	Phagocyte count of 100 cells,	211 : 240
Observer T.	"	1.17	Phagocyte count not kept	
Observer T.	"	1.34	"	"
Observer A.	"	2.34	"	"

The maximum variation here was 1.46, the next largest 1.17, and the minimum 0.17, the figure by which Observer T. differed from himself. In this case two indices were within normal limits and two above.

TEST 6.

Disease suspected: miliary tuberculosis. In this case the patient was extremely ill with high fever, but the physical signs in the lungs were anomalous.

Four tubes of the same serum were sent to three observers in three different laboratories, two tubes of identically the same serum being given to Observer O. without his knowledge. The indices returned were:

Observer A.	O.I. to T.B.	2.20	Phagocyte count not kept	
Observer O.	"	0.96	Phagocyte count of 100 cells,	126 : 130
Observer O.	"	0.82	"	107 : 130
Observer B.	"	0.82	"	193 : 240

In this case the index returned by B. was practically identical with one of the indices returned by O., who differed from himself by 0.14. The maximum variation between A. and B. was 1.40. In a case almost certainly tuberculous clinically of four observations only one was outside normal limits.

TEST 7.—Serum from Normal Individual.

In this case six samples of the same serum were sent to three observers in three different laboratories, each observer receiving unknown to him two samples of the same serum.

The indices returned were:

Observer X.	O.I. to T.B.	0.72	Phagocyte count of 100 cells,	202 :
Observer O.	"	0.87	"	222 : 268
Observer B.	"	0.92	"	231 : 250
Observer B.	"	0.93	"	232 : 250
Observer X.	"	0.89	"	249 :
Observer O.	"	1.07	"	268 : 268

In this case B.'s observations were practically identical, and were very close to single observations of O. and X. The latter, however, differed from himself by 0.17, and O. differed from himself by 0.20. The maximum variation also was considerable—namely, 0.35. The fact, however, that all observers returned an index within normal limits is striking.

TEST 8.

Disease suspected: renal tuberculosis. In this case there was present, in addition to the signs incriminating the left kidney, a large inflamed tuberculous cervical gland undergoing rapid softening. Massage of the kidney was undertaken, and the opsonic indices were estimated before and after massage. In each case six samples of the same serum were sent to six observers in five different laboratories, each observer receiving, unknown to him, two samples of the same serum. Observer B. alone was given tubes before and after massage.

The indices returned were:

Before Massage.				
Observer A.	O.I. to T.B.	0.78	Phagocyte count of 100 cells,	—
Observer A.	"	0.83	"	"
Observer B.	"	0.93	"	193 : 208
Observer O.	"	1.00	"	228 : 226
Observer O.	"	1.03	"	233 : 226
Observer B.	"	1.17	"	245 : 208
After Massage (Twenty Hours).				
Observer T.	O.I. to T.B.	0.81	Phagocyte count of 100 cells,	227 : 281
Observer T.	"	0.82	"	229 : 281
Observer B.	"	0.89	"	220 : 250
Observer B.	"	0.93	"	232 : 250
Observer X.	"	0.89	"	246 : 277
Observer X.	"	1.59	"	443 : 277

In this case some of the results are remarkably consistent. Observer A. differs from himself by only 0.05, Observer O. by only 0.03, Observer T. by only 0.01, and Observer B. returns an identical index before and after massage of 0.93. On the other hand, the value of this unanimity is upset by the maximum variation before massage, 0.39, and a maximum variation after massage of 0.78. Again, whilst B. returns an identical index before and after massage, his second reading gives a drop after massage of 0.24, the difference between his first two observations. Further, Observer X. is seen to differ

from himself by 0.70. In conclusion, it is worth noticing that in a case obviously tuberculous only two indices were returned as being outside the normal limits, one being 0.02 below and the other 0.39 above.

The interval between the two sets of observations was twelve hours.

TEST 9.

Disease suspected: pulmonary tuberculosis.

In this case three samples of the same serum were sent to three observers in two laboratories.

The indices returned were:

Observer A.	O.I. to T.B.	1.42	Phagocyte count of 100 cells,	95 : 67
Observer T.	"	1.02	"	180 : 176
Observer O.	"	0.95	"	178 : 190

Here A. differed from O. by 0.47. The next largest variation was 0.40, and the smallest 0.7. Two indices were well within normal limits, and one much above.

TESTS 10, 11, 12, 13.

Disease suspected—pulmonary tuberculosis. Moderate fever.

In this case, on three successive days three samples of identical serums were sent on four different occasions to three different observers.

The indices returned were as follows:

11.30 a.m. first day:				
Observer A.	O.I. to T.B.	1.48	Phagocyte count of 100 cells,	136 : 92
Observer T.	"	1.28	"	230 : 180
Observer E.	"	1.18	"	159 : 134
6 p.m. first day:				
Observer A.	"	1.6	"	147 : 92
Observer T.	"	1.28	"	230 : 189
Observer E.	"	0.84	"	113 : 134
6 p.m. second day:				
Observer A.	"	2.16	"	145 : 67
Observer T.	"	1.25	"	221 : 176
Observer O.	"	0.95	"	178 : 185
6 p.m. third day:				
Observer A.	"	2.94	"	197 : 67
Observer T.	"	1.28	"	—
Observer O.	"	0.92	"	—

It is of interest to note that the temperature at the times when the various sets of observations were made were as follows:

First Set.	Second Set.	Third Set.	Fourth Set.
99.6°	100.6°	100.2°	99.0°

The observations of Observer T. were remarkably consistent, being on all four occasions practically identical. The maximum variations of T. from A. on each occasion were, however, 0.20, 0.32, 0.90, 1.66. The maximum variation in all the observations was 2.02.

That opsonins do exist as factors to be reckoned with appears to have been firmly established by Sir Almroth Wright and his many followers. That they represent more than a fractional part in the highly complex machinery of immunity production we may still be permitted to doubt. Granted, however, for the moment, that opsonins are the important factor in the phenomenon of immunization that many believe them to be, the question at the head of this paper is constantly arising. Technical considerations of standardization of emulsions, cultural difficulties, agglutination questions, and so forth concern only those experts whose responsibility it is to render such technicalities as free from sources of error as possible. They do not affect the practising physician, whose only concern is with the pertinent questions of reliability and cost. As regards cost, as every one knows, it is high and necessarily so, and a most serious objection to wide use of the method it is. Regarding the question of reliability, it is quite impossible to ignore the fact that countless observations have been reported in which estimations have appeared to be of the highest value, either in diagnosis, or in directing some particular line of treatment. This is by no means, however, a universal experience, even when every effort has been made to secure the services of the high priests of the art. There appears, indeed, to be a growing conviction that, except possibly in the hands of an extremely small band of experts, the method is not of the general utility with which it has been credited.

The results I have quoted seem to support this conviction. If there is a satisfactory answer to these results, no doubt it will be forthcoming.

I take this opportunity of acknowledging my indebtedness to Dr. A. Venning for continuous help in the collection and separation of the serums referred to.