An Inquiry into the Influence of the Constituents of a Bacterial Emulsion on the Opsonic Index.

By A. F. Hayden, M.B., B.S., F.R.C.S., I.M.S., and W. Parry Morgan, M.A., B.Sc., M.B.

(Communicated by Sir A. E. Wright, F.R.S. Received August 24,—Read November 2, 1911.)

(From the Inoculation Department, St. Mary's Hospital.)

In preparing an emulsion of bacteria for opsonic estimations it is necessary to break up the masses so far as possible into their constituent bacterial elements and then to separate these from any clumps by centrifugalisation. The rate at which the suspended particles of an emulsion settle depends not only on the centrifugal force applied, but also on the fineness of the particles, and therefore on the efficiency of the method of breaking up the masses. If this is not efficient the suspended matter will fall in the form of coarse particles, leaving a relatively clear supernatant fluid containing very little in suspension.

In the case of a tubercle emulsion we find that the best results are given by triturating a small quantity of dried bacilli with a pestle and mortar of which the grinding surfaces have the same curvature; using these, five minutes' grinding is ample. The mass of dried bacilli is first ground up in the dry state and then made into a paste with a little 1-per-cent. saline (the strength used in all our experiments). The crude emulsion is then made by taking up the paste with 1 to 1½ c.c. of saline. When this emulsion is thoroughly centrifugalised it separates out into a deposit and an opaque supernatant fluid which is practically free from bacilli but which contains a considerable amount of bacterial detritus. If this supernatant fluid be pipetted off and the deposit again mixed with fresh saline and thoroughly centrifugalised, the second supernatant fluid will contain much less detritus and will be correspondingly clearer. By repeating this process several times it is possible to get a supernatant fluid which is almost clear and free from detritus. The deposit will then consist wholly of washed bacteria.

It is thus seen that the usual bacterial emulsions which are employed for measuring opsonic power consist of three elements, the saline used as a menstruum, bacillary detritus, and intact bacilli.

We have set ourselves the problem of determining the effect of the bacillary detritus on phagocytosis and its influence in the estimation of the

opsonic index. With this end in view we have sought answers to the following questions.

What is the Effect of Removing Detritus from a Bacterial Emulsion and of Adding it to such an Emulsion ?

Experiment 1.—An emulsion giving with the usual technique a count of 0·5 per cell in 15 minutes was centrifugalised; the supernatant fluid containing much detritus was pipetted off and an equal volume of saline added and shaken up with the deposit. The resulting emulsion then gave a count of 1·9 per cell. *Thus by removing some of the detritus the phagocytosis was increased about four times.*

Experiment 2.—Two phagocytic mixtures were made, consisting of equal volumes of washed corpuscles; suspension of washed bacilli; serum; and, in the one case, saline, and in the other, suspension of bacillary detritus.

The following were the counts :—

(1) With saline.	(2) With suspension of detritus.
1·61	0·62

Similar experiments gave the following counts :—

	(1)	(2)
(*a*)	4·92	3·17
(*b*)	2·05	1·40
(*c*)	1·65	0·64

Thus—and this is the converse of the conclusion above arrived at—the *addition of detritus causes a well marked reduction in the phagocytosis.*

Is this Reduction Due to an Effect Exerted by the Bacillary Detritus on the Phagocytes, or on the Serum ?

Experiment 2B.—Three phagocytic mixtures were taken—equal volumes of corpuscles, suspension of washed bacilli, and—

(*a*) In the first mixture saline and serum.

(*b*) In the second, suspension of detritus and serum.

(*c*) In the third, two volumes of mixture of equal volumes of the suspension of detritus and serum, previously incubated for 20 minutes.

The following were the phagocytic counts :—

(*a*)	(*b*)	(*c*)
2·05	1·40	0·37

And a similar experiment gave

1·65	0·64	0·27

Experiment 3.—The following phagocytic mixtures were used:—

(*a*) Equal volumes of
Corpuscles.
Suspension of bacilli.
Two volumes of a mixture of equal parts of a suspension of detritus and serum which had been previously incubated for 20 minutes.

(*b*) Equal volumes of
Corpuscles.
Suspension of detritus.
Two volumes of an incubated mixture of the suspension of bacilli and serum.

(*c*) Equal volumes of
Corpuscles.
Suspension of detritus, and
Two volumes of an incubated mixture of saline and serum.

The following were the phagocytic counts:—

	(*a*)	(*b*)	(*c*)
Patient i.....	0·15	1·59	1·20
Normal i.....	0·22	1·81	1·09
Patient ii.....	0·19	1·37	
Normal ii.....	0·19	1·74	1·20
Patient iii.....	0·20	1·36	1·12

These experiments supplement the previous ones, in the respect that they show that the addition of detritus diminished phagocytosis by removing the opsonic power from the serum—in other words, that the detritus functions as a receptor. And we may conveniently speak of the receptors of the detritus as free receptors, in contrast to the fixed receptors of the intact bacilli.

Column (*a*) shows that the serum, when digested first with the free receptors, will combine with these to the practical exclusion of the fixed receptors which are afterwards added. Column (*b*) shows that the serum, when digested first with the fixed receptors, will now combine by preference with these, and Column (*c*) that, when free and fixed receptors are present, the serum at one and the same time will tend to combine more impartially with both.

How does the Absence or Presence of Detritus in the Bacterial Suspension Influence the Opsonic Index which is arrived at?

Experiment 4.—Indices were estimated by using phagocytic mixtures of—

Corpuscles,
Suspension of bacilli, and
Serum.

And in (i) Saline, and
(ii) Suspension of detritus.

The following were the results:—

	(1) Saline.		(2) Suspension of detritus.	
	Phagocytic count.	Index.	Phagocytic count.	Index.
Normal serum	1·61	1·00	0·62	1·00
Patient's serum	1·50	0·93	0·20	0·31
A similar experiment gave				
Normal serum	2·47	1·00	2·14	1·00
Patient's serum	1·99	0·81	0·63	0·29

The following method of experimentation was also resorted to:—

Experiment 5.—Indices were estimated with two emulsions—(A) consisting of a suspension of washed bacilli, (B) of a suspension containing detritus.

The following were the results:—

	(A)		(B)	
	Phagocytic count.	Index.	Phagocytic count.	Index.
Normal 1	1·30, 1·18 } 1·24	1·00	1·53, 1·70 } 1·61	1·00
„ 2				
Patient 1	1·40	1·12	1·44	0·84
„ 2	1·08	0·86	1·12	0·69
„ 3	1·10	0·88	0·75	0·46
„ 4	1·15	0·92	0·82	0·51
„ 5	1·35	1·08	1·98	1·23

A similar experiment with the serum of another patient and two emulsions, (*a*) containing some detritus, and (*b*) containing a larger quantity of detritus, gave the following results:—

	(A)		(B)	
	Phagocytic count.	Index.	Phagocytic count.	Index.
Patient	2·77	1·18	3·24	1·63
Normal i.....	2·40, 2·28 } 2·24	1·00	1·90, 1·84 } 1·87	1·00
„ ii.....				

In this last experiment, the appearance as regards the degree of opacity, and so the quantity of detritus, in the emulsion A, was such as to lead us to believe that, had a serum with a low index been tested with it, a low index would have been obtained. The emulsion B was far more opaque than A, and so a further experiment was performed. Three emulsions were prepared, using such quantities of dried tubercle bacillus as to obtain varying amounts of detritus: (A) containing a small quantity; (B) a larger quantity; (C) a very large quantity.

Indices were estimated with these three emulsions, using two normal sera and two patients' sera, one whose index was expected to be high, and another whose index was usually low. The following were the results:—

	(A)		(B)		(C)	
	Phagocytic count.	Index.	Phagocytic count.	Index.	Phagocytic count.	Index.
Normal i	1·44 } 1·37	1·00	2·00 } 1·83	1·00	1·84 } 1·71	1·00
„ ii	1·30 }		1·66 }		1·58 }	
Patient i	1·66	1·21	2·19	1·19	3·44	2·01
„ ii	0·92	0·67	1·12	0·61	1·00	0·58

And a repetition of this experiment, using two different emulsions—(A) containing a moderate quantity of detritus, and (B) containing a large quantity—and using the sera of three other patients, gave the following results:—

	(A)		(B)	
	Phagocytic count.	Index.	Phagocytic count.	Index.
Normal i	1·38 } 1·41	1·00	1·28 } 1·37	1·00
„ ii	1·44 }		1·47 }	
Patient i	1·55	1·10	1·93	1·41
„ ii	1·73	1·22	2·53	1·85
„ iii	0·48	0·33	0·48	0·34

Thus, when little or no detritus was contained in the emulsion, the indices were in all cases within normal limits. When detritus was present in moderate quantities, differences emerged between bloods of normal and subnormal opsonic power, but supernormal bloods were not differentiated from normal bloods. When emulsions which contained larger quantities of detritus were employed, both subnormal and supernormal bloods were clearly differentiated from normal bloods.

Why does a Suspension of Washed Bacilli such as used in the foregoing Experiments fail to give Satisfactory Indications in Opsonic Power? And why does the Addition of Detritus improve it in this respect?

Reflection shows that, when we are testing a series of sera with a view to eliciting differences in their bactericidal agglutinating or opsonic powers, we cannot expect that the full differences will be revealed unless the bacterial suspension which we employ contains more microbes than the strongest blood is competent to kill, agglutinate, or opsonise. And we can be certain that if the bacterial suspension contains only such number of microbes as the weakest blood is able to kill, agglutinate, or opsonise, no differences can be expected to emerge between the several bloods. We cannot, for instance, in the case when we are dealing with a batch of sera, of which the strongest is competent to kill 6,000,000 typhoid bacilli, while the weakest is able to kill only 600,000 bacilli, expect to elicit any differences of power with a suspension which contains no more than 600,000 bacilli. Nor could we, if we employed a suspension containing only 3,000,000, hope to differentiate between bloods that can kill 3,000,000 and bloods which can kill up to 6,000,000.

In the same way, when we are dealing with a batch of sera, of which the strongest would be competent to opsonise sufficient microbes to give an average ingest of 10 bacilli per cell, and the weakest only enough to give an average ingest of 2·5 microbes per cell, we cannot expect to bring out any differences between the bloods with a suspension which could provide at most 2·5 microbes per cell. Nor again, if we employ an emulsion which could not provide more than five bacilli per cell, could we hope to differentiate between bloods which could be competent to opsonise sufficient microbes to give a count of 5 per cell, and bloods which would be able to opsonise sufficient microbes to give us a count of 10 bacilli per cell.

It might, in view of this reasoning, seem as if the only satisfactory suspension would in the bactericidal test above mentioned be a suspension containing six or more millions of typhoid bacilli, and for the opsonic test a suspension which would give an average ingest of 10 microbes per cell. But this is not so.

All that is required is that each suspension should contain a quantity of receptors equal to that which will be contained in these suspensions of the required strengths, and Wright and Windsor* have shown in connection with the bactericidal power that if the bloods are partially depleted of their bactericidal power by the addition of receptors in the form of dead typhoid

* 'Journal of Hygiene,' Oct., 1902, vol. 2, No. 4, and 'Studies of Immunisation,' p. 45.

bacilli, quite weak suspensions of living typhoid bacilli can be used for the purposes of a differential test.

The same thing holds, as our experiments have shown, in connection with the opsonic power. If, as in Experiments 4 and 5, we partially deplete our sera of opsonic power by the addition of detritus to the phagocytic mixture, we can use quite weak suspensions of intact bacilli for the purposes of the differential opsonic test, and this will give us a sensitive indicator which will keep the bacterial ingest within the limits which will allow of its being accurately enumerated.

And we have here also a new and important point. If only a small quantum of detritus is present it will be possible to differentiate subnormal from normal indices, but not supernormal from normal; and not until we have sufficient detritus can we hope to differentiate both subnormal and supernormal from normal. We can, in short, only obtain a perfect indicator of differences in opsonic power by preparing our emulsions in such a way as to contain this sufficiency. This is clearly brought out in the latter experiments of Series V. And further, perhaps the most important result of these experiments is to explain the fact that with one and the same blood very different opsonic indices may be obtained by different workers, and even by the same worker at different times.