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HUMAN PNEUMOCOCCAL OPSONIN AND THE ANTI- OPSONIC SUBSTANCE IN VIRULENT PNEUMOCOCCI.*†

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INTRODUCTION.

IN a previous article¹ I have shown that the pneumococcal action of pneumonic and other blood is the combined result of opsonification, phagocytosis, and intraphagocytic destruction. The observations were made upon avirulent pneumococci *in vitro*, and hence under conditions widely different from those in a pneumococcus infection in which it concerns virulent pneumococci which resist opsonification by human serum. No less than 75 strains of pneumococci from the blood in pneumonia have been shown to be insusceptible to phagocytosis when first isolated, no matter whether the serum used was obtained from normal persons or from patients in various stages of pneumonia. This resistance to phagocytosis is associated with virulence for rabbits and guinea-pigs. In order to define better the actual

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¹ *Jour. Infect. Dis.*, 1906, 3, p. 683.

rôle of phagocytosis in pneumococcus infection it therefore seemed important to determine the exact relation between opsonins and virulent pneumococci.

FILTERABILITY OF OPSONINS.

IN order to study the exact relation between pneumococci, virulent and avirulent, and the opsonin in human serum it was found necessary to develop a reliable technique for experiments on the absorption of opsonin by various bacteria. To avoid error in absorption experiments the bacteria must be separated completely from the serum. For this purpose the centrifuge has been used,² but prolonged centrifugalization is time-consuming, and unless great speed is obtainable it is often also inefficient. It was therefore determined to test the filterability of opsonin, and it was found that opsonin is not appreciably diminished by passing serum through porcelain filters. At first a so-called Maasen filter with very small pores was used, so that with a pressure of 25 pounds several hours were required to obtain a small quantity of serum. Subsequently coarser filters have been used requiring from 10 to 30 minutes to deliver 0.5 to 2 c.c. or more of serum.

SPECIFICITY OF HUMAN OPSONIN.

Experiments were now undertaken to determine whether or not human serum contains opsonin that is specific for pneumococci. The 24-hour surface growth of several large plain agar slants (approximately 250 sq. cm.) was washed off with 2.5 to 3 c.c. normal human serum. This mixture was then incubated from 3 to 12 hours or at room temperature for 24 hours, and then at 37° C. for two additional hours. The serum after dilution with an equal quantity of physiological salt solution was now filtered. The opsonic content of this serum was then compared with the untreated serum which was kept under the same conditions of temperature, etc. The mixtures for determining the relative degree of phagocytosis consisted of equal parts of washed normal blood, serum and salt solution, and bacterial suspension. The results obtained are presented in tabular form. (Table I.)

² Bullock and Western, *Proc. Roy. Soc.*, 1906, S. B., 77, p. 531; Potter, Dittman and Bradley, *Jour. Am. Med. Assoc.*, 1906, 47, p. 1722.

TABLE I.
SPECIFICNESS OF OPSONINS IN HUMAN SERUM.

BACTERIUM USED FOR ABSORPTION OF OPSONIN	PHAGOCYTOSIS AFTER 30 MINUTES UNDER INFLUENCE OF TREATED SERUM				
	Pneumococci "M"	Pneumococci "233"	Strepto- coccus "J"	Staphylo- coccus "C"	Tubercle Bacillus
Pneumococcus "M"	0	0	4	4.5	0.9
" " "233"	0	0	3.0	2.0	0.4
" " "240"	4.2	3.5	5.6	7.0	2.0
" " "241"	4.0	1.9	6.4	5.8	1.3
" " "237"	2.5	2.1	4.8	6.0	1.4
Streptococcus C	3.0	1.4	0	3.7	1.7
Staphylococcus T	2.5	1.0	3.0	0	0.9
PHAGOCYTOSIS UNDER INFLUENCE OF NORMAL SERUM					
	6.0	4.0	7.0	8.9	2.7

After contact with the serum for 24 hours at room temperature and for 4 hours at 37° C. the bacteria were removed by filtration.

It is shown that non-virulent pneumococci deopsonize human serum completely, so far as strains of avirulent pneumococci are concerned; at the same time they absorb some, but by no means all, of the opsonin for streptococci, staphylococci, and tubercle bacilli. Streptococci and staphylococci, on the other hand, absorb completely the opsonin for each respectively, but not all the other opsonins.

THE RELATION BETWEEN PNEUMOCOCCAL VIRULENCE AND SUSCEPTIBILITY TO PHAGOCYTOSIS AND THE POWER OF PNEUMOCOCCI TO ABSORB OPSONIN.

In the ordinary opsonic experiment the serum is allowed to act upon the bacteria for a comparatively short time—less than one hour, as a rule. As it was possible that this length of time of exposure to opsonic serum is insufficient to opsonize virulent pneumococci, a number of experiments were made in which small numbers of virulent cocci were acted upon by large quantities of sera at 37° C. for 24–48 hours, but nevertheless the cocci were not taken up by fresh leucocytes.

Human serum was treated with large quantities of pneumococci, virulent and avirulent, in order to determine whether the virulent absorb opsonin to the same degree as the avirulent. The technique employed was that just outlined, and need not be recounted here further than to say that blood agar (blood 1 part, plain agar 8–10 parts) was used to obtain large quantities of virulent pneumococci. In order to obviate the use of large amounts of blood in preparing this medium, agar was slanted in large tubes, allowed to cool, and 5 c.c. of the freshly prepared blood agar poured over the large slant and allowed to "set" before inoculation. The blood used in this way was found to have no opsonic effect upon the organisms grown on the medium.

Table 2 shows a distinct difference in the opsonic content of

TABLE 2.
ABSORPTION OF OPSONIN BY NON-VIRULENT AND VIRULENT PNEUMOCOCCI.
PHAGOCYTOSIS* (30 MINUTES)

Strain of Pneumococcus Used in Absorption of Opsonin from Serum	Normal Serum	Treated Serum
Non-virulent E	25.0	0
" N	10.0	0
" N	6.0	0
" M	6.0	0
" M	4.0	0
" 233	4.0	0
" 236	6.0	0
" 236 VI	12.0	0
" R _{51a}	6.4	0
" 235	5.2	0
Virulent 236 VI	10.0	0.6
" 235 I	4.0	1.0
" 236 III	3.0	0
" 240	10.0	6.0
" 240	6.0	9.0
" 241	10.0	3.0
" 241	6.0	2.2
" 241	4.0	1.9
" 237 II	6.3	5.5
" 237 IV	40.0	31.0
" 237 XI	10.0	5.5
" 237 XVIII	6.0	5.2

* The suspension of non-virulent pneumococci given in this and other tables to test the opsonic power of serum were either 24- or 48-hour cultures in plain broth. This has been found to be the most satisfactory way of procuring an even suspension of pneumococci of the proper density for routine work. Experiments proved that the presence of the broth has no appreciable influence upon opsonic action. The figures in the tables under the heading "Phagocytosis" represent the average number of bacteria per leucocyte, not less than 50 being counted at the end of 20 or 30 minutes.

serum treated with strains of non-virulent and virulent pneumococci. The non-virulent strains deopsonize the serum completely, while the virulent strains may or may not have this effect, depending upon the degree of virulency. It is interesting to note that the opsonin may be completely removed not only for the strains used in the absorption, but for other avirulent strains as well. Thus the organism R_{51a} isolated four years ago deopsonized serum completely for Strain 224 which had been isolated only four weeks previously. One may therefore conclude that the specific pneumococco-opsonin is common for practically all strains of phagocytatable pneumococci.

The influence of the reaction of the sera used was now studied. Table 3 shows that there is a great difference in the degree of phago-

TABLE 3.
RELATION BETWEEN THE OPSONIC POWER AND THE REACTION OF SERUM.

Sera	Reaction in Terms of n/40 Oxalic Acid	Phagocytosis (30 Minutes)
Fresh normal untreated serum	1.1 alkaline	38
Fresh normal untreated serum neutralized to litmus by addition of oxalic acid	Neutral	19
Alkalinity restored by adding NaOH	1.1 alkaline	28
Fresh normal serum + CO ₂	0.5 "	28
Fresh normal serum treated with non-virulent pneumococci	0.6 "	0
Fresh normal serum treated with virulent pneumococci	0.65 "	25

NaCl solution was added to the sera to bring the dilution to the proper point.

cytosis according as the reaction differs. Neutralization with $n/40$ oxalic acid reduces it one-half. This effect is not due to destruction of opsonin because the subsequent addition of sufficient NaOH to restore the original alkalinity increases materially the phagocytosis. A reduction in the alkalinity by passing CO_2 through serum is associated with some reduction. Whether this reduction is due to destruction of opsonin, to inhibited opsonic action, or to lessened activity of the leucocytes cannot be said without further experiments. The difference in the deopsonic power of avirulent and of virulent pneumococci is not due to differences in the reaction of serum treated with non-virulent and with virulent pneumococci, because the reaction though somewhat less alkaline than normally is approximately the same in both cases. In nearly all sera treated with virulent pneumococci there is a slight reduction in the opsonic effect, which perhaps may be due to diminished alkalinity of the serum.

Much work has been given to the study of the effects of cultivation on artificial media and of passage through rabbits upon the relation of pneumococci to opsonin and opsonificability. The results obtained are illustrated in Tables 4, 5, and 6. From Table 4 we see that as pneumococcus "240" which was isolated from the blood of a case of pneumonia is cultivated upon artificial media it becomes more and more susceptible to opsonin, and, *pari passu*, its power to

TABLE 4.
INCREASE IN SUSCEPTIBILITY TO PHAGOCYTOSIS AND IN POWER TO ABSORB OPSONIN OF PNEUMOCOCCUS 240 ON CULTIVATION UPON ARTIFICIAL MEDIA.

Days upon Artificial Media	Susceptibility to Phagocytosis of Washed Pneumococcus 240 after Experiment	Susceptibility to Phagocytosis of Culture Used to Deopsonize Serum before Absorption Experiment	PHAGOCYTOSIS (20 MINUTES)	
			Normal Serum	Treated Serum
1	0	0	12	10.5
9	2.3	+	10	6.0
14	6.3	++	6.0	3.0
23	10.0	+++	18	4.0

TABLE 5.
SUSCEPTIBILITY TO PHAGOCYTOSIS AND POWER TO ABSORB OPSONIN OF PNEUMOCOCCUS 237 AS AFFECTED BY PASSAGE THROUGH RABBITS.

Pneumococcal Strain Used to Absorb Opsonin	Days upon Artificial Media	Susceptibility to Phagocytosis	PHAGOCYTOSIS (20 MINUTES)	
			Untreated Serum	Treated Serum
237	2	0	—	—
IV	8	+	4.5	0.6
237 VIII	2	0	6.0	4.6
IX	2	0	28.0	24.0
237 XI	10	+	10.0	1.5
XIII	2	0	6.0	4.0
237 XVIII	2	0	15.0	11.4

The Roman numerals after 237 indicate the number of rabbits through which the pneumococcus has been passed at the time of each experiment.

TABLE 6.
INCREASING RESISTANCE TO PHAGOCYTOSIS AND LOSS OF POWER TO ABSORB OPSONIN AS VIRULENCE IS INCREASED.

Pneumococcus	Days Cultivated upon Artificial Media	Phagocytosis (20 Minutes)	Remarks
236	36	38.0	
236 II	31	25.0	
236 IV	23	9.5	Complete absorption; organisms opsonified
236 VI	11	0.5	Opsonin reduced only slightly while the serum failed to opsonize pneumococcus
236 VIII	7	0	
236 VIII	7	0	
236 X	4	0	
236 XII	2	0	No appreciable reduction of opsonin; 24 hours' contact with serum failed to opsonize pneumococcus

The Roman numerals after 236 indicate the number of rabbits through which the strain has been passed at the time of the experiment.

absorb opsonin grows. Of course the results obtained on different days are not strictly comparable, although the various conditions always were kept the same as nearly as possible.

In Table 5 is shown that the susceptibility to opsonin and the power to remove opsonin from opsonic serum, of one and the same pneumococcus ("237") may be increased and diminished at will by passage through rabbits and artificial cultivation.

Table 6 illustrates a really crucial experiment of similar scope with pneumococcus "236" which, originally avirulent, was brought to a high state of virulence by successive passages through rabbits. Cultures were made on blood agar from the heart's blood of each rabbit while alive; six passages of this kind were required before the strain acquired sufficient virulence to cause prompt death of animals. The results given were obtained at the same time as the tests which were all made at the end of 36 days from the beginning of the experiment. The results again show that contact, even prolonged, of highly virulent pneumococci with serum does not result in absorption of opsonin and consequent opsonification of the bacteria. Pneumococcus 236 VI which is, so to speak, at the dividing-line between the virulent and avirulent strains is not opsonized by 48 hours' contact with serum. Marked absorption of opsonin occurs only when the organisms used are susceptible to phagocytosis, to some degree at least. The question arises whether the opsonin in such cases is absorbed only by those individual bacteria which have lost their virulence. It seems likely that this is so, because on plating out virulent pneumococci which have been cultivated for some time, colonies are obtainable of markedly different degrees of virulence.

PNEUMOCOCCAL "VIRULIN," THE SUBSTANCE UPON WHICH VIRU-
LENCE AND RESISTANCE OF PNEUMOCOCCI TO
PHAGOCYTOSIS APPEAR TO DEPEND.

In order to investigate more closely the mechanism whereby virulent pneumococci defend themselves against opsonin and phagocytosis I undertook, in accord with Dr. Hektoen's suggestion, a study of the action of pneumococcal extracts upon opsonic serum. Highly virulent and avirulent pneumococci were suspended in m/8 NaCl solution and placed at 37° C. for 48 hours. The clear fluid drawn off after thorough centrifugalization was then tested as to its action upon opsonin (Table 7). The result is quite striking. The extract from

TABLE 7.

EFFECT OF EXTRACTS OF VIRULENT AND AVIRULENT PNEUMOCOCCI UPON OPSONIC SERUM.

Equal parts of extract or NaCl and serum were incubated at 37° C. for one hour when equal volumes of washed blood and pneumococcal suspension were added.

Mixtures	Phagocytosis (20 Minutes)
Virulent extract	0.13
Avirulent extract	4.6
NaCl solution	6.0

the virulent pneumococci inhibits phagocytosis almost completely, whereas the avirulent extract does so in much less degree.

Similar results have been obtained with many extracts representing 10 strains of virulent pneumococci obtained from the blood of pneumonia patients and from post-pneumonia empyema. The extracts of five strains of non-virulent pneumococci, cultivated upon artificial media for from four weeks to four and one-half years, have given only a moderate antiopsonic effect.

The extracts are now prepared from pneumococci grown upon large blood-agar slants or in glucose-free broth to which 1 per cent dextrose is added. This broth is prepared from beef and subjected to fractional sterilization. The reaction is 1 per cent acid to phenolphthalein. Virulent pneumococci grow more abundantly and more rapidly in this medium than in the ordinary broth made from beef extract and sterilized in the autoclave. The addition of 1 volume of sterile blood to 8 or 10 volumes of broth not only makes the cocci grow better but they also yield a stronger extract.

In order to get active extracts it is necessary to suspend rather large quantities of pneumococci in relatively small amounts of salt solution. The pneumococci in about 60 c.c. of broth after 48 hours'

growth are suspended in 4 to 5 c.c. of normal salt solution and kept at 37° C. for 48 hours, heated to 60° C. for one hour, the pneumococci centrifugated down and the supernatant clear fluid, drawn off.

The conclusion seems warranted that the extract contains some substance or substances which bind or neutralize the opsonin in the serum, because active extracts do not inhibit phagocytosis by washed leucocytes of previously sensitized pneumococci. Furthermore the antiopsonic effect of virulent pneumococcal extracts is largely specific for pneumococci; as Table 8 shows the extract does not prevent the

TABLE 8.
THE SPECIFIC ANTIOPSONIC ACTION OF EXTRACTS OF VIRULENT PNEUMOCOCCUS.

Mixtures of Pneumococcal extract and Serum or NaCl Solution at 37° C. for 1 hour before adding Washed Blood and Bacterial Suspension	PHAGOCYTOSIS (20 MINUTES)		
	Pneumo- coccus	Strepto- coccus	Staphylo- coccus
Serum 0.1 + pneumococcal extract 0.1	0.1	7.0	10.3
Serum 0.05 + pneumococcal extract 0.15	0	3.3	9.8
Serum 0.025 + pneumococcal extract 0.175	0	1.5	5.0
Serum 0.1 + NaCl 0.1	6.0	10.6	12.0
Serum 0.05 + NaCl 0.15	5.0	6.0	10.7
Serum 0.025 + NaCl 0.175	3.2	3.0	6.0

phagocytosis of streptococci and staphylococci. After being treated in the manner described virulent pneumococci appear to become phagocytatable. It must be remembered, however, that there are difficulties in the way of a clear demonstration on this point, because thoroughly extracted or autolyzed organisms are so disintegrated and stain so poorly that they are hard to see. However, if extracted organisms do become phagocytatable they should absorb opsonin from serum, and, if large enough quantities are added, the pneumococco-opsonin should be removed entirely; and it has been found that when equal quantities of highly virulent pneumococci, extracted and unextracted, are suspended for 24 hours in equal amounts of serum, the extracted remove all the opsonin while the unextracted diminish only slightly the opsonic power. Unfortunately it is impossible to carry out experiments with respect to the animal virulence of the extracted pneumococci, because extraction as carried out is associated with death of the cells.

When avirulent pneumococci are suspended for 24 hours in virulent pneumococcal extract and then washed rapidly in salt solution they become relatively insusceptible to phagocytosis (Table 9) at the same time as the extract employed loses its power to neutralize opsonin (Table 10) and also becomes less toxic. This interesting

TABLE 9.

EFFECT OF VIRULENT PNEUMOCOCCUS EXTRACT UPON AVIRULENT PNEUMOCOCCI.

Equal numbers of avirulent pneumococci suspended 24 hours in the same amounts of virulent extract and NaCl sol. The cocci washed and phagocytability determined.

Extract pneumococci	+ normal serum	+ washed blood	aa	2.0
NaCl	"	"	"	25.0
Extract	"	+ serum + NaCl	aa + washed blood	aa 6.0
NaCl	"	+ " + NaCl	aa + " "	aa 30.0

TABLE 10.

EFFECT OF AVIRULENT PNEUMOCOCCI ON PNEUMOCOCCAL EXTRACTS.

Avirulent pneumococci suspended in virulent extract 24 hours at 37° C. and then removed by filtration. The antipsonic effect of the extract so treated compared with untreated extract:

Mixtures				Phagocytosis (20 Minutes)
Treated extract	0.15	+ serum	0.05	2.4
Untreated "	0.15	"	0.05	0
Treated "	1	"	0.15	4
Untreated "	1	"	0.1	0.2
NaCl solution	1	"	0.1	5.0

result awakened the idea that possibly the induced resistance to phagocytosis brings with it restoration of virulence. To test this possibility by experiment there was injected into the peritoneal cavity of each of three guinea-pigs of nearly the same weight the 24-hour surface growth of two blood-agar slants (approximately 20 sq. cm.) after treatment of the pneumococci (avirulent 233) in each case as follows: (1) Guinea-pig No. 1 received the pneumococci in 3 c.c. of NaCl solution in which they had been suspended for 24 hours. (2) Guinea-pig No. 2 received the pneumococci in 3 c.c. of virulent pneumococcal extract in which they had been suspended for 24 hours. (3) Guinea-pig No. 3 received the pneumococci after they had been suspended for 24 hours in virulent pneumococcal extract and then washed rapidly. The results of the detailed study of the subsequent phenomena are shown in Table 11.

The results of the examination of the peritoneal fluid and blood cultures before and after death leave no doubt that death in guinea-pigs Nos. 2 and 3 was the result of pneumococcal growth, and this growth appears to have been made possible through the acquirement of virulence by the previous treatment of the cocci in the extract. The rapidity with which the leucocytes disposed of the untreated pneumococci is especially noteworthy. No free pneumococci were found at the end of six hours, notwithstanding that such a large quantity was inoculated. In the case of the treated pneumococci the results were diametrically different. The endothelial cells which were very numerous at the end of 24 hours in the peritoneal fluid showed marked phagocytosis of polymorphonuclear leucocytes in guinea-

TABLE II.

THE CONFERENCE OF VIRULENCE UPON AVIRULENT PNEUMOCOCCI BY TREATMENT IN EXTRACTS OF VIRULENT PNEUMOCOCCI.

Intraperitoneal inoculation of same quantity of avirulent pneumococcus after treatment for 24 hours in 3 c.c. of NaCl solution and in 3 c.c. of virulent extract.

	Guinea-pig 1 (320 grams) Pneumococci in Salt Solution	Guinea-pig 2 (325 grams) Pneumococci in Untreated Extract	Guinea-pig 3 (340 grams) Pneumococci in Untreated Extract Washed and Suspended in 3 c.c. NaCl Solution
4½ hours	Many leucocytes; phagocytosis of pneumococci marked; few free pneumococci, no endothelial cells.	Few leucocytes, many pneumococci; some phagocytosis. Seems in great pain.	Few leucocytes, some phagocytosis; no endothelial cells.
6 hours	Many leucocytes, no free pneumococci; slight phagocytosis.	More leucocytes; many pneumococci; considerable phagocytosis; seems ill.	Leucocytes abundant; many pneumococci; some phagocytosis.
24 hours	Leucocytes fairly abundant; many endothelial cells digesting leucocytes; as many as four per cell. Blood culture, negative. Seems perfectly well. Weight 310 gms.	Many leucocytes; some endothelial cells, phagocytosis of pneumococci but not leucocytes; free pneumococci abundant. Blood culture positive. Crouched, very ill. Weight 275 gms.	Very many leucocytes, phagocytic endothelial cells, phagocytosis for pneumococci but not for leucocytes. Blood culture positive.
48 hours	Leucocytes and endothelial cells few. Entirely well. Blood culture negative.	Leucocytes many; show no phagocytosis; phagocytosis of pneumococci and leucocytes by endothelial cells; free pneumococci present. Weight 240 gms. Very ill.	Less phagocytosis of pneumococci by endothelial cells; free pneumococci present. Weight 275 gms. Very ill.
72 hours	Entirely well. Weight 320 gms.	Death. Heart's blood—pure culture of pneumococci. Serofibrinous peritonitis.	Still very ill.
96 hours			Death. Findings as in Pig 2.

pig No. 1 and of pneumococci in guinea-pigs Nos. 2 and 3. It seems that the endothelial cells which appear later take up pneumococci of a higher grade of virulence than the leucocytes, for at this time the latter show no phagocytosis, even though pneumococci are present in abundance. Results similar to these have been obtained in rabbits as well as guinea-pigs with four other strains of avirulent pneumococci which had been cultivated for 3, 7, 8, and 15 months respectively. The animals receiving the pneumococci in virulent extract always showed the greater reaction, and death occurred earlier in them than in the animals which received pneumococci that had been washed after treatment in extract. That this in a measure is the result of toxic effect of the extract itself is probable, because by itself the extract is not without toxic action. It is likely also that by washing in salt solution pneumococci treated with extract a certain amount of the active substance is again extracted. Subsequent generations of the pneumococci isolated from the blood of the dead animals have virulence which increases as usual on animal passage.

The minute study of the antiopsonic body in pneumococcal extracts is now in progress. It may be stated that it resists boiling for two

minutes and that it does not appear soluble in alcohol or ether. To what extent if any it may be associated with the capsular substance of the pneumococci has not been determined. On morphological grounds there seems little reason to associate virulence of pneumococci with the capsule because it presents the same general appearance in virulent as in avirulent strains.

The chief points may be summarized as follows: It has been found possible to extract from virulent pneumococci which themselves originally do not take up pneumococco-opsonin a substance which neutralizes the opsonin in human serum; this substance unites with avirulent pneumococci and by so doing it confers upon them a degree of resistance to phagocytosis as well as of animal virulence. In other words it seems possible to extract from virulent pneumococci the substance upon which virulence would seem to depend, and at present the name "virulin," suggested by Dr. Hektoen, seems quite appropriate. While the action of "virulin" may be subject to several hypothetical explanations, at present it is probably best to look upon it simply as a substance or mixture of substances which when united with the pneumococcal cell prevent this from taking up opsonin, and which when free has special affinity for opsonin. That it does not merely concern free opsinophile cell receptors seems likely, for one reason because virulent pneumococci when extracted, i. e., freed from virulence, are found to absorb pneumococco-opsonin freely.

CONCLUSIONS.

Human serum retains its opsonic properties with respect to various bacteria after being filtered through porcelain.

The results of absorption experiments indicate that normal human serum contains several opsonins with specific affinities for pneumococci, streptococci, staphylococci, and tubercle bacilli.

Avirulent pneumococci absorb opsonin and become susceptible to phagocytosis; virulent pneumococci do not absorb opsonin and are insusceptible to phagocytosis; and these properties may be diminished or increased at will by passage through rabbits or cultivation on artificial media as the case may be.

Extraction or autolysis of virulent pneumococci in NaCl solution brings into the solution a substance or group of substances which

inhibits the action of pneumococco-opsonin; avirulent pneumococci take up this substance and now become not only resistant to phagocytosis but exhibit also to some degree the property of animal virulence; after extraction of the substance virulent pneumococci acquire the power to absorb pneumococco-opsonin.