A STUDY IN DIPHTHERIA WITH SPECIAL REFERENCE TO OPSONINS.

by

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1934.

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GENERAL INTRODUCTION

Within recent years, medicine has undergone changes of a truly revolutionary nature, changes, indeed, which have served to establish our knowledge of human, and incidentally, other forms of biology on a more scientific foundation. These changes have been so fundamental, that perhaps now we may, with increasing justification, refer to medicine, both theoretical and practical, as a science and not purely as an art.

To the student, the more radical of these changes would appear to be contemporaneous with, and directly due to, the advent and development of that most youthful of sciences, - bacteriology. Indeed, considering the multidude and variety of diseases which may affect humanity and which are now known to be due to living micro-organisms, we may well wonder how incomplete and fragmentary our understanding of medicine must have been before bacteriology had a place in our equipment for the investigation and study of diseases. This recently established science has not only revealed to us the living causes of many diseases, but it has also furnished us with weapons, such as we had never previously known, both for the prevention and cure of disease. Consequently, the contribution of bacteriology to medicine as a biological science is equalled only in magnitude by its contribution to our knowledge of therapeutics and practical medicine.

The history of these developments in medicine is closely associated with the names of Leeuwenhock, the discoverer of bacteria, Jenner, who introduced vaccination, Pasteur, Koch, Klebs/

Klebs and Loeffler, Roux and Yersin and Von Behring. The work of the five last-named is especially identified with the elucidation of the cause and cure of diphtheria, with which this study is particularly concerned. To Klebs and Loeffler is due the discovery of the bacillus which now bears their name; Roux and Yersin demonstrated its specificity and its toxin and Von Behring put in our hands that most effective therapeutic agent, diphtheria antitoxin. The researches and discoveries of these few men have radically changed the whole aspect of medicine and were, in a sense, remarkably complete in that they supplied the answer to many problems which remained unsolved up to the closing years of last century. Thus within the short space of time between 1884, when Klebs and Loeffler established the identity of the causal agent of diphtheria and 1890, when Von Behring demonstrated the efficacy of antitoxin, the last chapters concerning diphtheria appeared to have been written and that very deadly disease was robbed of its terrors. To the biologist, however, these researches have presented many new and intricate problems which, in spite of many additions to our knowledge of bacteriological technique, biological and biochemical methods, even to-day remain unsolved. No subsequent work on diphtheria has been so productive of practical results as was that carried out during the years 1884 - 1890 and it is possible that the acquisition of an effective cure for the disease has resulted in the loss of that very stimulus which inspired that earlier and highly successful research. Notable contributions, it is true, have been made to our knowledge concerning diphtheria since 1890 and in particular, mention might be made of Schick's intradermal test for susceptibility, the introduction of effective methods of producing artificial active immunity, and Ramon's work on the nature of the interaction of toxin and antitoxin/

antitoxin which has contributed much to our knowledge of the structure of these bodies. Many problems however still remain to be solved even in connection with diphtheria, problems, the solution of which will serve to increase our understanding of the nature of immunity and help us to correlate the innumerable observations and facts we have accumulated in connection with specific infectious diseases generally. These problems which are so intimately associated with the nature and functions of living matter must command our interest and it is certain that the elucidation of the problems connected with the reactions of infective agent and host must be our immediate concern. The need for active research into the nature of the biological processes which are set up in the body in the production of immunity is surely obvious, for the elucidation of these problems alone will give us that understanding of fundamental principles which will enable us to combat and control infective diseases generally in a rational and effective manner. Then also our achievements will not be confined to individual infections only but will be capable of more general interpretation and have a wider field of application. Indeed, it may be said, that research into these matters can never be regarded as complete until the more elementary, and, at the same time, more intricate questions concerning them have been answered.

Further, it is a matter for some surprise that so many of the achievements in the field of research, not only in the case of diphtheria but of infectious diseases generally, have emerged, not from the isolation hospital with all its available clinical material, but from the laboratory. It would appear that perhaps closer co-operation between the clinician and the bacteriologist might be productive of further progress in the elucidation of the problems that still remain to be solved.

This present work embodies the results of clinical observations and laboratory investigations and was undertaken with a view to studying the development of the immunity in diphtheria. This particular infection was chosen for the subject of study because it has proved a fruitful source of productive research in the past; our knowledge of this disease is perhaps more complete than that of any other infection; and clinical material is always readily available for study.

NATURE OF IMMUNITY IN DIPHTHERIA

It has for long been a matter of established fact that diphtheria produces its effects in the body through the medium of a diffusible exotoxin and also that the chief agent in, or manifestation of, the immunity mechanism is the antitoxin as has been shown by biological tests, Schick tests and also by therapeutic tests. It has, however, been suggested that antitoxin in the blood cannot be the only factor in protection. Thus Park and Zingher (1)(1916) observed that mild cases of diphtheria with a positive Schick reaction might recover without treatment, possibly on account of an antibacterial immunity. This supposition overlooks the possibility of the patient having undergone a slight degree of immunisation sufficient to cause a "secondary response" upon infection and consequently an antitoxic immunity in spite of an absence of demonstrable antitoxin at the time of infection (Glenny and Allen (2)1922).

Solis-Cohen, Heist and Solis-Cohen (3)(1920) also demonstrated an antimicrobic quality of the whole blood against diphtheria bacilli, which, however, did not run parallel with "immunity" as demonstrated by the Schick test, although in a general way some correspondence was established.

(1) Am. J. Pub. Health 6,431
(2) J. Hyg., 21, 100 and 104
(3) Tr. Ass. Am. Physicians, 25,263

The possibility of phagocytosis playing a part in the establishment of immunity received some attention during the first decade of this century, when Wright's work on opsonins stimulated active research on this factor in immunity. This new method was employed in the case of diphtheria by Reque[1] (1906) who showed that C. diphtheria was very susceptible to phagocytosis but could not find any definite alteration of phagocytic power as a result of the disease; Royer, Weston and Clark[2] (1908), who stated that diphtheria patients showed definite rise in phagocytic power on the fourth day of the disease, and Tunnicliff[3] (1916), whose work tended to confirm the results of the latter. The last-named worker found, working with three acute cases and ten carriers, that the opsonic index rose as the diphtheria bacilli disappeared from the throat and also, that it was high in the majority of the carriers. More detailed mention of these investigations will be made later.

It does not appear in the accounts of these researches just mentioned why the work was undertaken but, judging from the dates of the experiments, one would conclude that the investigations were stimulated by the almost contemporary work of Wright and his colleagues, and that the new method was applied to diphtheria as it was to many other diseases at that time. Some points in connection with clinical diphtheria, however, must raise certain questions in the clinician's mind, questions which are not altogether answered by the generally accepted ideas that the immunity in diphtheria is purely antitoxic. For example, one has noted frequently in fairly severe early cases of diphtheria, when the local manifestations are very obvious, that the membrane spreads very decidedly after serum has been administered, in some cases for as long as 24 - 48 hours, before/

(1) J. Infect. Diseases 3,440
(2) J.Med.Res.18,107
(3) J. Infect. Diseases 19,97

before the ultimate limits are reached and separation begins; this, too, in cases adequately treated with antiserum. Again, patients are also frequently seen who, when they first come under observation, show signs of undergoing spontaneous cure. It would appear therefore that the administration of therapeutic antiserum does not of itself establish the cure of the disease, a point which, of course, is obvious from the fact that the patient, in convalescence and for the remainder of his lifetime, usually remains Schick negative. Again, of recent date,(1) observations have been recorded showing that some cases do not benefit from adequate doses of serum. That latter observation, however, has not been my experience, though it is well known that the types of diphtheria prevalent in different communities vary greatly in their virulence. However, the point does emerge that the patient's own reaction to the disease is the factor which establishes the permanent immunity established as a result of infection and, from the lack of immediate effect on the spread of the membrane, as stated above, by adequate doses of serum, also must be a very important, if not essential, factor in immediate cure. It is a remarkable fact that, irrespective of the dose of serum, however great that may be, second attacks of diphtheria are of rare occurrence, and it is an almost universal practice now to treat cases with larger rather than with smaller doses of antitoxin. In other words, the antitoxin administered, no matter how great the dosage, does not dispense with the patient's own tissue response to the action of antigen. It is possible that the administration of the serum only protects the body tissues during the time when they are most vulnerable to the action of the toxin and probably produces many of its undoubtedly beneficial effects indirectly in this way.

(1) Robinson and Marshall: Jn. Path. & Bact. Jan. 1934.

It is of interest to note that in connection with scarlet fever, in the treatment of which antitoxin also now-a-days has a place, Burton and Balmain[1] have shown that serum in scarlet fever tends to increase the number of Dick positive reactors during convalescence. Yet, though the general dosage of serum, one would gather, is higher in the treatment of diphtheria than of scarlet fever, no such statement could justifiably be made in connection with the former disease. However, such a comparison cannot justly be made on account of our lack of knowledge of the agent in scarlet fever, and what we know rather tends to show that that disease may be due to many strains of streptococci serologically different, though they are indistinguishable by the Dick test.

OBJECT OF PRESENT INVESTIGATION

The question which determined the line of this investigation arose out of such points as the foregoing, and an attempt was made to find out whether, by the study of some particular phase of the immunity mechanism other than the production of antitoxin, this method being rendered useless by the immediate production of a Schick negative reaction on the administration of serum, indications of the progress of the patient's own bodily response in the establishment of cure and immunity to the disease could be studied. It was further desired to find out if possible to what extent the patient's own response contributed to clinical improvement and cure.

These objectives necessitated the investigation of some phase or manifestation of the immunity mechanism other than the antitoxic because of the use of antitoxic serum in treatment. Some previous work had been done on the activity of/

(1) Ker's Infect. Diseases p.112. - 3rd Ed.

of opsonins in diphtheria, as already mentioned, but that work was scanty and the results tended to be contradictory. Therefore this field appeared to offer scope for the particular object of this work and in the present investigation the activity of opsonins in diphtheritic infection was made the object of more extended study than had previously been attempted.

To determine whether opsonins play any part in connection with diphtheritic infection and if so, what the extent and significance of that part may be, form, therefore, the general purposesof this work which, however, includes in addition, many experiments the objects of which are of a closely related nature. The details of these experiments will be discussed later and more opportunely.

"OPSONINS" AND "TROPINS"

That phagocytosis plays a very important part in immunity has for long been an established fact and research into this phenomenon has shown it to be a process of much complexity. Round it, Metchnikoff built up his theory of immunity and, while subsequent work has not wholly substantiated his views, it has not minimised, but rather served to confirm the importance of phagocytosis as part of the immunity mechanism. A close parallel has been established between this phenomenon as it occurs in in vivo and in vitro experiments, and it has been shown beyond doubt that protection in antibacterial immunity depends more on the ingestion of the organisms by the phagocytes than on any direct bactericidal effect of the serum.

Metchnikoff regarded the activity of the phagocytes as the sole factor in the process of phagocytosis but this view became/

became untenable with the discovery of an antibacterial serum which could confer passive immunity. However, Metchnikoff attributed this property of the antiserum to its effect on the phagocytes in virtue of its "stimulines" and still postulated the activity of the cell as the most important and immediate factor in phagocytosis. The obvious objection to this view was that the immunity conferred by an antibacterial serum is specific. As a result of Denys' and Leclef's work in 1895,(1) it was clearly demonstrated that the important factor was a property of, or a substance contained in, the serum and Marchand (1898)(2) concluded from his researches that the antiserum produced some physical change in the organisms which made them susceptible to ingestion by the phagocytes. Wright and Douglas (1903 - 04)(3) confirmed the importance of the serum and showed that normal serum could prepare a great variety of organisms for ingestion. This property or substance, they termed "opsonin" and demonstrated its thermolability, showing that it was destroyed on being heated at a temperature of 55 ^{o}C.

The question of the actual part played by the phagocytes was then, and still remains, an open one. Whether the leucocytes of a normal serum differ from those of an immune serum, not only in activity but specificity, still remains doubtful; for, though no specific effect of leucocytic activity has been shown, the leucocytes from an infected individual phagocytose, not only the particular infecting organism, but other organisms also, much more actively than the leucocytes of a healthy subject. This point was demonstrated by Rosenow (1910)(4) in the case of pneumonia.

(1) Cellule 11,177.
(2) Arch. Med. Exp. 10,253.
(3) Proc. Roy. Soc. 72,357.
(4) M. R. C. A System of Bact. Vol. 6.
"Opsonic Action: Tropins" (R. Muir.)

Moreover leucocytes from different sources show differences in phagocytic activity and the temperature of the person from whom the leucocytes are obtained, even in cases of pyrexia, is found to be the optimum for the corresponding leucocytes.[1]

Wright and Douglas (1903 - 04) demonstrated their "opsonin" as already stated and introduced the technique for the estimation of the opsonic content of a serum, the results being expressed in the form of an index. This "opsonic index" was shown by Wright to be low in infections, high in immunity and increased by vaccines. For a time, this work enjoyed a popularity among bacteriologists and clinicians alike, a popularity, however, which did not for long survive.

Further studies were directed towards establishing the nature of "opsonin" and its relationship to other immune bodies. Neufeld, Rimpau and others (1904 - 05)[2] showed that increase in the phagocytic property of a serum in the establishment of immunity was not associated with an increase in the thermolabile "opsonin" of Wright but with the appearance of a thermostable body which was specific in action and different from other recognised antibodies. To this substance they gave the name "Bacteriotropin" or "Tropin". This work was confirmed by the researches of Leishman (1905)[3] in experiments carried out with B. typhosus and B. melitensis and of Dean[4] in the same year. The latter, in addition to confirming the difference between the thermostability of Wright's "opsonin" and Neufeld's "tropin", showed that when normal serum was added to diluted immune serum/

(1),(2) & (3) M.R.C. A System of Bact. Vol. 6 "Opsonic Action: Tropins" (R. Muir).

(4) Proc. Roy. Soc. B. 76,506.

serum, the resultant opsonic power was greater than the sum of the two acting separately,i.e., that complementing had occurred.[1] The "tropins", however, have conclusively been shown to possess the power of opsonic action independent of complement and this, in very high degree. In immunity however, the opsonic effect of the "tropins" may be enhanced by the presence of specific immune body binding normal complement - opsonin and indeed it may be that the two modes of action, viz., direct action on the organism and indirect action through the medium of combined complement, may be possessed by the same substance . However, the presence of opsonic activity due to immune -body and complement cannot always be demonstrated in immune sera.

Normal "opsonin" is very closely related to, if not quite identical with, complement. Dean came to this conclusion as a result of his researches and Muir and Martin (1906),[2] treating a normal serum with powerful "complement absorbers" e.g. sensitised bacteria, showed that the opsonic power could be as effectively abolished in this manner as by heating the serum to 55° C. Similar treatment of an immune serum had little or no effect on its opsonic activity. This very close relationship between "opsonin" and complement has been further borne out by the work of Levaditi and Inman (1907),[3] Sachs and Terunchi (1907)[4] and C.H. Browning (1908).[5]

The question of the specificity of normal "opsonin" has been a subject of much controversy and the results of the many investigations into this matter have been contradictory, some workers finding that they could absorb all the normal "opsonin"/

(1) Proc. Roy. Soc. B.76,399
(2), (3)&(4) M. R. C. A System of Bact. Vol. 6. "Opsonic Action: Tropins" (R. Muir.)
(5) J. Med. Res. 19,201

"opsonin" from a serum with one particular organism, and others that they could remove only the "opsonin" for the particular organism used.[1] R. Muir, after a review of the results concludes that specific normal opsonin in small amount may be demonstrated in normal sera, in some more readily than in others, and that thermolabile, non-specific "opsonin" can always be found in much more considerable quantity. The findings of Gordon and Wormall (1928)[2] and Dunlop (1928)[3] working on the nature of natural antibody in respect of recent knowledge of "complement-components" or fractions into which complement can be split support the view that normal serum contains amboceptor of relatively non-specific type capable of acting as sensitisor in conjunction with complement. The complementing of natural immune bodies has been demonstrated further by the fact that heated normal serum may have its opsonic power restored by the addition of diluted unheated serum.

There is some evidence that complement can unite directly with the organisms and opsonise them just as organic and inorganic particles absorb complement from a normal serum and are taken up by the phagocytes in consequence. This view is supported by experiments in which a normal serum was treated with a certain amount of a particular bacterial suspension (e.g. B. Coli) and lost some of its opsonic power by absorption as a result. It was then shown that this loss could be increased in one of two ways, viz., by increasing the amount of the bacterial suspension used or by adding small quantities of B. Coli antiserum, leading to increased binding of complement. By either method the opsonic action could be reduced to nil, indicating that absorption through the medium of immune-body can be replaced by the direct absorption of complement by the bacteria.[4]

(1) M. R. C. A System of Bact. Vol. 6.
"Opsonic Action : Tropins" (R. Muir).

(2) & (3) M. R. C. A System of Bact. Vol. 6
"Opsonins and Tropins of Normal Serum.".
(J. C. G. Ledingham)

(4) M. R. C. A System of Bact. Vol. 6.
"Opsonic Action: Tropins" (R. Muir).

Some normal sera have also been shown to possess the homologue of "tropin". Thus, when such a serum is heated to 55°C., a residual amount, usually small, of opsonic power remains and this has further been shown by Dean (1905) to be identical with the "tropin" of an immune serum. Some of these normal "tropins" are probably more susceptible to heat than the "tropins" of immune serum.

From the foregoing it will be readily seen that the phenomenon of phagocytosis may be brought about in a variety of ways and that the subject is full of complexities. It may however be stated that the most important variations in the opsonic content of the serum as a result of infection depend on the development of specific immune-opsonins ("tropins") though the presence of immune body may play a part in raising the index, by leading to the union of more normal-complement-opsonin.(1)

OPSONINS IN DIPHTHERIA - PREVIOUS STUDIES.

Reference might now conveniently be made to previous work done on the subject of phagocytosis in diphtheria.

Wright and Douglas, as already stated, showed that normal "opsonin" acted in a general way against many organisms but failed to find any evidence of this in the case of the diphtheria bacillus. Tunnicliff (1908)(2), Lindemann (1910-11)(3), and Ohkubo (1910)(4), however, showed that the latter organism was susceptible to phagocytosis and this observation has been confirmed by the present investigation.

This discrepancy in the results of these workers may partly be explained by differences in the virulence of the organisms/

(1) Muir & Ritchie. Manual of Bact. p.202. -(8th Ed.)

(2), (3),&(4) Opsonic Action: Tropins (R. Muir) M.R.C. System of Bact. Vol. 6. Section on "Opsonic Action of Normal Serum."

organisms employed in the tests; for, it has been shown conclusively that virulent strains of various organisms are not so susceptible to phagocytosis as avirulent strains of the same organisms. Thus Marchand (1)(1898) showed that easily phagocytosed, avirulent streptococci became very resistant to opsonin when their virulence was heightened by passage. Ohkubo (2) noted similar differences in the case of virulent and avirulent strains of C. diphtheria.

Reque (3)(1906), in an extended study of this subject, also demonstrated the susceptibility of the diphtheria bacillus to phagocytosis but he was unable to find any conclusive evidence that this was altered as a result of the disease.

Royer, Weston and Clark (4)(1908), working in the Municipal Hospital, Philadelphia, carried out some investigations on phagocytosis in diphtheria. Their observations were made on fifteen acute cases, before and after serum was administered; one case of acute non-specific tonsillitis; two healthy children who had had diphtheria seven and twelve years respectively before the time of the experiments ; eight healthy children who had never suffered from diphtheria; four healthy children, before and after the injection of diphtheria antitoxin; and three children who had chronic otorrhoea with diphtheria bacilli present in the discharge, two of whom were treated with antitoxin and one with small immunising doses of diphtheria toxin. In their investigations, these workers used the whole blood of the patients and estimated the percentage p h a g o cytosis by enumerating the number of leucocytes out of consecutive series of fifty showing ingestion. From their results they concluded that, - (1) the blood of normal individuals shows only slight phagocytosis of diphtheria bacilli;

(1) & (2) Opsonic Action: Tropins (R. Muir) M.R.C. System of Bact. Vol. 6 Section on "Opsonic Action of Normal Serum."
(3) J. Infect. Dis. 3,440. 1906.
(4) J.M.R. 18, 1908.

(2) the blood of patients suffering from diphtheria shows very active phagocytosis of the infecting organism; (3) the phagocytic activity of the blood reaches its maximum about the fourth day of the illness in cases untreated with antitoxic serum; and (4) the degree of phagocytosis is uninfluenced by the dosage of serum administered or the age of the patient.

In the same year, Tunnicliff[1] also made a study of the activity of opsonins in diphtheria, using Wright's method of estimating the opsonic potency of sera and expressing her results in terms of the "index". The observations in this study were made on fourteen acute cases of diphtheria and the opsonic index was estimated daily. The results largely confirmed those of the previous workers and active phagocytosis of diphtheria bacilli was again demonstrated. In this investigation also, it was further shown that the opsonic index was low at the beginning of the disease, rose as the membrane separated and returned to normal in from two to nine days later.

A subsequent study of a similar nature was made by the same worker[2] in connection with the carrier state from the results of which she concluded that the opsonic index in this condition was definitely above normal.

Conflicting statements have been made regarding the effect of antitoxic serum on phagocytosis. In the researches of Royer, Weston and Clark (1908)[3] and of Tunnicliff (1905)[4] mentioned above, this problem was investigated and these workers concluded that the injection of diphtheria antitoxic serum/

(1) & (4) J. Infec. Dis. 5. 14, 1908.
(2) J.Infec. Dis. 19, 1916.
(3) J. Med. Research. 18, 1908.

serum had no effect on phagocytosis. Ohkubo,[1] (1919,) Lindemann (1910)[2] and Baecher and Laub (1911)[3] on the other hand found antitoxic serum exerted a stimulating action on phagocytosis and this effect was shown to be associated with the specific nature of the serum. To such a property is often attributed the power of antitoxin to expedite the shedding of the diphtheritic membrane.

ACCOUNT OF PRESENT INVESTIGATION.

1. Scope.

The present study, of which I now proceed to give an account, relates primarily to an investigation of cases of diphtheria, admitted as such, to Heathfield Hospital, Ayr, during the period September to December, 1933, and of certain other admissions used as controls. The latter group comprised cases in the acute and convalescent stages of infections other than diphtheria and many, selected from among the admissions to the scarlet fever wards, had received anti-scarlet therapeutic serum prior to the carrying out of opsonic tests on samples of blood collected from them. These cases total in all, one hundred and fourteen and form the chief group, - hereafter referred to as Group I, - examined for the purposes of this investigation.

Further collateral experiments were made on a few cases admitted in the early months of 1934 but these experiments were, for the most part, modifications of those carried out on the earlier and larger group and will be recorded separately. These additional cases, nine in number, form Group II of the study.

(1), (2) & (3) M.R.C. Monograph, "Diphtheria" 1923. p. 169.

This investigation, therefore, entailed the examination of one hundred and twenty three cases, representing the total of both groups and the results are based on no fewer than five hundred and forty opsonic estimations. These figures do not include the many preliminary tests which were performed with a view to finding whether opsonins were developed as part of the immunity mechanism in diphtheria and to obtain efficiency in the technique involved in such an investigation, before any serious study was attempted.

The cases comprising Group I were classified in four sub-groups, distributed and designated as follows:-

Class IA. cases of diphtheria in which the clinical diagnosis was confirmed bacteriologically. Each case had many opsonic estimations of the serum made throughout the period of hospitalisation and forty-four of the total fifty of this class had a test before the administration of serum, the first sample of blood being withdrawn immediately after admission. The observations on the opsonic potency of the serum in each case of this class were sufficiently numerous to justify the investigation being regarded as "complete".

Class IB. comprises seventeen cases in which also the diagnosis of diphtheria was based on both clinical and bacteriological grounds, but which, forming chiefly the first lot of cases examined and being at various stages in their convalescence when the investigation was first started, were regarded as "incomplete" in respect of their records of progress as based on opsonic estimations of their sera. The tests performed on the individual members of this group were much fewer on the average than in the cases of Class A., but were more evenly distributed throughout their periods of stay in hospital, differing in this respect again from Class A cases in which most of the observations were made in the earlier, acute stages of the illness when, it was found, the opsonic readings fluctuated most.

Class IC. includes cases with positive throat swabs but with doubtful or no clinical manifestations of diphtheria. In two cases the opsonic tests were carried out from the first day of admission and these are regarded as "complete" in the same respects as the cases in Class A. In four other cases only one or very few observations on the opsonic activity of the sera were made and two of these were ambulatory carriers. This sub-group therefore is represented by six cases in all.

Class ID. includes all the cases used as controls and is composed of two cases "complete" in the sense defined above and thirty-nine cases on which, with a few exceptions, only one opsonic estimation was made. Among the latter are twenty-six cases of scarlet fever on whose sera the tests were made at various intervals after the administration of scarlet-fever antitoxin.

The two "complete" cases were one case of scarlet fever whose faucial appearances gave rise to a suspicion of diphtheria also and who was given 32,000 units of diphtheria antitoxin pending the result of the swab examination which proved to be negative, and one case admitted with a febrile condition accompanied by epistaxis but in which neither clinically nor bacteriologically was the diagnosis of diphtheria confirmed. These two cases were examined in respect of the opsonic activity of their sera on the same lines as those of Class A., and the former constituted a valuable control from the viewpoint of finding the effect of anti-diphtheritic serum on the opsonic readings.

The cases of Group II were employed in the carrying out of certain subsidiary and collateral tests to be described in a later section.

2. Method.

Various methods have, from time to time, been described and employed to estimate the opsonic content of a serum. That of Leishman[1] consisted simply of mixing equal quantities of the patient's whole blood and bacterial emulsion and incubating a drop of the mixture, placed on a glass slide under a cover-slip, in an incubator for fifteen minutes at a temperature of 37°C. At the end of this period, the film was dried and stained and the preparation compared with a similarly prepared control in which the blood of a normal subject was used. The number of bacteria in fifty leucocytes was then counted and an average struck.

Later Wright introduced his method which is too well-known to require description here. It differed from Leishman's in that he used only the serum of the patient, preparing the leucocytes from the blood of a normal subject and also, in the method of recording his results, which he expressed in the form of an index. Many modifications of this method have been devised, only those of Simon[2] and of Klein[3] requiring mention here since these were investigated and employed in this work.

Simon estimated, not the number of organisms ingested, but the number of cells out of any given amount showing ingestion and the results obtained, after comparison with a normal, he expressed as a "percentage index". He claimed that the results closely corresponded with those obtained by Wright's method.

Similar claims were advanced by Klein for the method he established, a method which is still employed in the estimation of/

(1) Muir & Ritchie: Manual of Bact. 8th Ed. p.128.
(2) Ibidem. p.129.
(3) John Hopkins Hospt. Bulletin. 1907. 18,245.

of the potency of certain therapeutic sera, notably antimeningococcal. The method consists in making dilutions of the serum and employing these dilutions in the test in place of the undiluted serum as in the other methods. In this way an attempt is made to find that dilution of the serum with which phatocytosis fails, or almost fails, to occur. Clark and Simonds (1) working with B. Typhosus compared the methods of Wright and Klein and found a close correspondence in the results. The method was tried out in the case of diphtheria by Tunnicliff (2) who found in the case of this organism also a close correspondence between the results obtained by Wright's and Klein's methods. The number of tests carried out on this point by that worker, however, are too few to warrant a dogmatic statement. She found in two cases examined that the opsonic activity of the serum disappeared with dilutions of one in forty-eight and one in ninety-six respectively.

Dean, (3) working with this same method, but using staphlococci and tubercle bacilli, could find no direct proportion between the serum concentrations and the degrees of phagocytosis, his figures showing as much activity even with one eighth dilutions as with serum of full strength. Fleming (4) also indicates that the dilution of the serum is not a serious factor in the production of variations of the opsonic index as estimated by Wright's method.

The dilution method of Klein was applied to many of the earlier cases in this series, dilutions up to one in three hundred and twenty being tested, but even with these large dilutions of serum, phagocytic activity was found in as high degree/

(1) Jn. Infect. Dis. 1908. Vol. 5. 1.
(2) Ibidem. vol. 5. 14.
(3) Proc. Roy. Soc. 1907. B. 79. p.399.
(4) Practitioner. Vol. 80. No. 5. p.611.

degree as with undiluted serum. The method therefore was soon abandoned but was investigated again at a later stage, when 1.5% saline solutions were substituted for the"normal" solutions used in the earlier tests in order to abolish the possibility of interference by spontaneous phagocytosis.(1) (An account of this experiment is given later.) The results of these later tests, shown in Table I, are seen however to corroborate the earlier findings.

TABLE I.

Results obtained by Klein's Dilution Method.

Serum Samples	Dilutions of Serum. 1/10	1/20	1/40	1/80	1/160	1/320
1	22	17	16	21	16	27
2	19	17	16	13	10	15
Case A. 3 (Diphtheria)	38	28	38	35	36	33
4	7	4	10	7	3	10
5	8	10	9	9	9	9
6	21	27	30	26	28	25
7	4	6	7	5	5	4
Case B. 8	10	12	9	7	7	9
(Convalescent Pneumonia) 9	5	10	11	9	9	7
10	7	10	12	10	6	6

The samples of blood from each case were taken off on different days.

A modification of Simon's method was chosen as standard in this investigation and that for very good reasons. Wright's method of estimation has fallen into disrepute and is now practically abandoned. That is no matter for surprise; for the results obtained by this method can be influenced by too many variable factors and are therefore unreliable. Further, in the case of the diphtheria bacillus, the irregul - arity of the staining makes it difficult in cases where ingestion/

(1) Wright & Reid, Proc. Roy. Soc. B. 1906, 77, 211.

ingestion of large numbers of organisms has occurred, to enumerate individual bacilli with any degree of accuracy. In this work, one in ten dilutions of the sera were used in order to diminish the possibilities of interference by bacterial agglutinins and haemagglutinins, the presence of which, as has been shown by Fleming,(1) lead to erroneous results. Also, the bacterial emulsion was made of such a density as would ensure that all the leucocytes in the mixtures would have equal opportunities for ingesting the organisms. The percentage of leucocytes, - one hundred being counted in every preparation, - showing any degree of phagocytosis was estimated and this figure is recorded in the results as the "percentage phagocytosis".

3. Technique.

(a) Preparation of Materials.

The technique employed in the present investigation necessitated the usual desiderata for the estimation of opsonins, - viz, sera from the patients and controls, emulsions of bacteria and leucocytes, Wright's pipettes, solutions of saline and saline with sodium citrate, clean glass slides and a rubber teat. These various materials and articles were prepared in the following manner:-

Sera.

Blood was withdrawn with a thoroughly clean syringe and needle from a vein in the antecubital fossa of the patient in the usual way and was kept until the time of the actual investigation in tightly - stoppered, sterile tubes, at low temperatures. These were stored in a cool, dark receptacle for periods varying from a few hours to a few days, a practice which was necessitated by circumstances, immediate investigation being often impossible, the tests being carried out/

(1) Practitioner 1907. Vol. 80, No.5 pp. 614 & 620.

out in convenient intervals between other duties. Eight samples only were stored for a period of seven days, all the others being tested within four days of collection.(1) Fleming states that storage for such periods, provided air is carefully excluded from the specimens and these are kept at low temperatures, does not result in deterioration of the opsonic power of the sera. This observation was confirmed on the serum of a convalescent pneumonia used as one of the controls in this work. Four samples of blood were collected from this patient on different days and all were stored under the above conditions until the day of the test. The results are shown in Table II.

TABLE II.

To show keeping properties of Serum in in respect of "Opsonin".

Serum Sample	Date of Collection	Opsonic Reading.
1	10: 5: 34	14%
2	11: 5: 34	9%
3	13: 5:34	17%
4	15: 5:34	8%

*

No similar test was made on an "abnormal" serum, unfortunately, but Fleming(2) investigated this point and came to the conclusion that "normal" serum can be stored for seven days and "abnormal" serum for a few days less, without ill effect. No hard and fast rule can be laid down however, since different sera tend to vary somewhat in their keeping properties.

The conditions mentioned above were observed in the storing of specimens for this investigation and consequently all the specimens may be regarded as "fresh" in respect of opsonic content.

(1) Practitioner 1907. Vol. 80, No. 5. pp. 614 & 620.
(2) Ibidem. pp. 622 et seq.

* Tests performed on 15:5:34.

Leucocytes.

The leucocytes were prepared in the following manner:- Blood from a normal, apyrexial subject was taken off into a syringe containing a solution of .85% NaCl and 1.5% sodium citrate at 37° C. and ejected into a centrifuge tube placed in a vessel containing water at body temperature. The corpuscles were sedimented by centrifuging the specimen and the supernatant fluid was withdrawn. The corpuscles were then washed with normal saline solution at 37° C. to remove the sodium citrate. The corpuscles were washed a second time and after removal of the saline, the centrifuge tube was placed at a few degrees from the horizontal in the incubator to keep the corpuscles at body temperature. This sloping of the tube provided a large leucocytic layer from which the leucocytes were "creamed" by means of a fine pipette. Any remaining traces of saline were removed at this stage after the tube was given a final spin in the centrifuge.

Fleming (1) investigated the efficiency of the leucocytes from various sources, using normal subjects and patients suffering from various conditions. He found no alteration in the phagocytic power of the leucocytes from these different donors, and the results of a similar experiment, carried out in the course of this investigation, serve to confirm his findings. The results of that experiment are given in Table III.

TABLE III.

Results obtained by using leucocytes from various sources.

Source of Corpuscles.	Opsonic Reading.
1. Convalescent pneumonia	36
2. " erysipelas	30
3. " "	36
4. Diphtheria carrier & measles	32
5. Diphtheria patient	33
6. " "	33

(1) Practitioner. Vol. 80. No. 5. p.618.

Bacterial Emulsion.

Swab cultures were made from the throats of acute cases of diphtheria on Loeffler's blood serum and from these, the C. Diphtheria was isolated on Smith's telluric acid medium. The isolated organism was then again sown on the blood serum medium and incubated for twenty-four hours prior to the tests. These pure cultures were sub-cultured, on an average, every forty-eight hours and twenty-four hour sub-cultures were used in the tests. At frequent intervals during the period over which the investigations were conducted, fresh cultures, obtained by the above method, were substituted. This was done to ensure as far as possible that the cultures used would be of uniform virulence. Unfortunately, however, guinea-pig inoculations were not employed as a check on the virulence but a scrutiny of the results would indicate that any change in virulence the cultures might have undergone as a result of sub-culturing has not materially affected the experiments. This statement applies only to the investigation as carried out with the cases comprising Group I. In the case of Group II, the experiments were carried through with cultures whose virulence was established by guinea-pig inoculations.

In making the emulsions, loopfuls of the twenty-four hour cultures were mixed with very small quantities of the saline on the side of the suspension tube and just above the level of the suspending fluid until a thick, uniform emulsion was obtained, care being taken to break down all bacterial clumps. Only then was the main body of the saline allowed to participate in the emulsification. In this way, very satisfactory suspensions of the organisms were obtained and with practice, one could judge fairly accurately the degree of opacity required in order to ensure that the preparations would be sufficiently charged with bacteria so that all the phagocytes would have equal opportunities for ingesting organisms

Pipettes.

Wright's pipettes as supplied by the makers were made more efficient by heating the fine ends in a Bunsen flame, drawing them out to a fine capillary bore and making a new end by breaking them across at the attenuated part. Each pipette then had a very fine point which facilitated the complete aspiration of the leucocytes, organisms and serum after these had been mixed on clean glass slides.

Teat.

At the lower, open end of the teat, a small hole was made by piercing the rubber with a hot needle. The advantages of a teat prepared in this way were found to be of great service; for, by means of the thumb of the hand controlling the teat, the entry of air into and out of, the teat could be controlled without disturbing the column of fluid in the stem of the pipette and also at will, the movements of the latter could easily be controlled by the same method.

(b) Procedure.

The procedure here described was followed in all the investigations made in connection with the principal object of this work - i.e. in all the cases comprising Group I. Certain modifications were introduced in the subsidiary tests and these will be referred to when the related experiments are described.

Dilutions of one in ten of each specimen of serum was made in Widal tubes, using Dreyer's dropping method. Care was taken to avoid contamination of the serum with corpuscles from the associated clot in each case since admixture with corpuscles, according to Fleming[1], leads to erroneous results. Normal saline was used as the diluting fluid and thorough mixing of the serum and saline was ensured in each case by drawing up the mixture into a pipette and expelling it into the Widal tube several times.

(1) Practitioner, May 1908. p.621.

The prepared dilutions of the sera, leucocytes and bacterial emulsion were placed on the bench conveniently before the operator. A carefully cleaned glass slide was marked off into six squares by means of a grease pencil. The teat was fitted on to the end of a Wright's pipette, at the lower, attenuated end of which a mark was made with a grease pencil about 2 mm. from the tip. Bacillary emulsion was drawn up to the mark on the stem of the pipette, then a small column of air was sucked up. The same quantity of the leucocytic emulsion was drawn up and this again was followed by a short column of air. Finally, the diluted serum to be tested was drawn up to the mark. Equal volumes of the three constituents of the opsonic mixtures, separated from each other by 'air locks', were, in this way collected in the stem of the pipette. Mixing these in one of the squares marked on the slide was the next step and this was done by expressing the column of serum, and then after raising the pipette and expelling the column of air to avoid causing bubbles in the serum, the leucocytes were mixed with the serum. After expelling the second column of air with the same precaution as before, the bacillary emulsion was mixed with the serum and leucocytes on the slide. Thorough mixing of all three components was ensured by aspirating and expelling the mixture in turn several times, the mixture being finally drawn up into the stem of the pipette in an unbroken column. During the mixing process care was taken to avoid blowing air into the mixture and leading to interruption of the final column in the pipette. This was done by carefully keeping the fine point of the pipette always under the surface of the mixture on the slide.

The mixture having been finally drawn up into the middle of the capillary stem of the pipette, the end of the latter was sealed off in a Bunsen flame and the pipette placed upright in/

in the incubator for 15 mins exactly at 37°C. At the end of that time, the tip of the pipette was broken off, the teat refitted to the other end and the mixture expelled on to the end of a clean slide. A film was then prepared in the usual way and, after being allowed to dry at room temperature, was stained with Jenner's Eosin-Methylene Blue. This stain was found to be very highly satisfactory for the organisms as well as for the cells, and very fine pictures were obtained under the microscope.

(c) Counting.

As already stated, one hundred leucocytes were counted consecutively and the number showing ingestion of organisms noted, the result being expressed as a "percentage phagocytosis." Certain precautions were, however, observed. The leucocytes were found most abundantly at one end of the preparation due to their being carried along the slide by the spreader. In counting, care was taken to avoid closely grouped masses of cells and organisms and also thick parts of the film where it might be difficult to be sure whether the organisms were in, or merely above, the cell. Isolated cells and small groups of cells only were counted and in this way truly representative counts were obtained.

4. Account of Experiments and Discussion of Results.

This work is essentially made up of a series of experiments the objects of which all have a bearing, direct or indirect, on the main subject of the study. The reason for this method of approach is due chiefly to the nature of the study, since new problems presented themselves as the work progressed and these were investigated as they arose. It will therefore perhaps be most convenient to describe the investigation as a series of experiments and discuss the results of each separately in the first instance.

The study is concerned primarily with the cases constituting Group I which were investigated by the method described above. The specimens of serum were examined under the same conditions and the method employed was the same throughout. The cases of the group were separated into four classes, designated IA, IB, IC, and ID, according to the scheme described on pp. 17 - 18 and to facilitate comparison of the results, the cases of class IA, i.e. the cases of acute diphtheria, were further subdivided according to the clinical condition. For this purpose, the degree of toxaemia, the presence and gravity of complications and the extent of the membrane, where possible, were noted, the severity of each case being assessed on these points which are indicated in Table IV by the letters T, C, and E respectively.

The cases of this group were examined over twenty-three experimental days and the results are shown in Tables IV to VIII inclusive. Table IV is divided into two sub-sections, a and b, the former of which comprises the results obtained in all the cases of class IA, i.e., the cases of acute diphtheria and the latter is made up of results obtained in the investigation of the three "complete" cases of class ID. The first of these three control cases was a scarlet fever patient who was given both antiscarlet and antidiphtheritic serum on admission/

admission although he was later proved by swab examination to be free from diphtheria. The results in this case constitute a valuable control to the cases in class IA and also show the absence of effect on the opsonins by specific antiserum. The second and third controls were less completely investigated but the results confirm those obtained in the first case. The results in these three control cases are incorporated in Table IV for the purpose of convenient comparison with the results obtained in the cases of acute diphtheria.

In this same table the results of swab examinations made during the convalescence of the patients are shown. The swabs are taken as a routine at times when it may reasonably be expected that the patient is free from infection and with a view to his/her discharge. According to the practice of this hospital two sets of successive swabs must be negative before the patient is dismissed. The results shown therefore indicate the dates of the last positive and first negative swabs. Where only one date is shown, it is indicated that the patient's swabs taken during convalescence were all negative.

Table V shows the results obtained in the cases of class IB and are supplementary to those in Table IVa.

The results of the investigations in connection with the cases of class IC, cases from which positive swabs were obtained but in which clinical signs of infection were absent, are shown in Table VI. The notes relating to each case are conveniently included in the table. In four of the cases Schick tests were carried out and the results of these tests are also shown.

The cases used as controls, with the exception of the three/

three cases included for convenience in Table IVb, are all contained in Table VII. Twenty-nine of these cases were given therapeutic serum on admission and the opsonic potency of their sera was estimated at varying intervals after the injection. They serve both as ordinary controls and as controls from the point of view of studying the effects of specific and non-specific therapeutic sera on the opsonins.

On six occasions tests were made using saline in place of serum and the counts obtained varied from nil to eleven per cent.

Additional experiments were carried out on the sera from five cases in class IA and from three controls to discover the effect of heating the sera at 55° C. for 20 minutes. The results are shown in Table VIII.

The experiments carried out with the cases of Group I were designed to investigate the chief problems of this study and to provide answers, if possible, to such questions as the following:-

1. Are opsonins elaborated in the serum of diphtheria patients?
2. If so, what part do these opsonins play?
3. Is that part an important one (a) in the establishment of immediate cure and (b) in immunity generally?
4. At what time or times are the opsonins developed in the disease?
5. What is the nature of the opsonins, and,
6. Does the administration of therapeutic serum play any part in their development?

An attempt will now be made to answer these questions in the light of the results shown in Tables IV - VIII.

In the introductory part of this thesis, it was shown, by reference to other studies, that the whole subject of

opsonins is a very complicated one and that complexity has to be borne in mind when experimental results are studied with a view to accurate interpretation. For the same reason, conclusions must necessarily be drawn in a general way only, and exceptions to any apparent rule, may be, not unexpectedly, numerous. However, the cases investigated in this study are sufficiently numerous and representative of infection of all degrees of severity, to justify certain general conclusions, some more apparent from a scrutiny of the results than others.

The first and very clear result of this investigation has been to show that opsonins are developed in the serum during the course of diphtheria and may be present in abnormal degree in the serum of carriers (see Table VI).

What part these opsonins may play in immunity however and of what importance that part may be, are questions much more difficult to answer. From a study of the results, however, one perhaps is justified in concluding that they do play a part in the establishment of cure and to some extent also, in immunity and further, that that part is probably an important one. Evidence in support of such conclusions will be found in Tables IV to VIII.

From a study of the results shown in Table IV, it will be readily seen that decided increase in the opsonic potency of the patient's serum during diphtheria infection does occur and that increase arises, for the most part during the first 10 - 14 days of the disease. Especially is this found to be the case in patients with an infection of mild or average severity and in which recovery is uneventful or attended by minor complications only. In the more severe cases in which recovery took place, e.g. Nos. 46, 47 and 48, the opsonic increase occurred definitely later. Case 49 in Table IV, which/

Abbreviations - Tables IVa and IVb.

T_1 - Mild degree of toxaemia: T_2 - Moderate degree of toxaemia: T_3 - Toxaema marked:

T_4 - Severe toxaemia: T_5 - Pronounced toxaemia.

C_o - No complications: C_m - Mild complications, e.g. albuminuria, otorrhoea:

C_s - Severe complications, e.g. paralysis, cardiac involvement, nephritis.

E_1 - Follicular exudate or patch on one tonsil only: E_2 - Both tonsils patched:

E_3 - Extensive membrane on both tonsils and slight on palate or uvula:

E_4 - Very extensive membrane on both tonsils and involving palate and other parts extensively.

I.M. - Intramuscular. I.V. - Intravenously.

B.S. - Before administration of serum.

Table IV a.

Case. Age.	Clinical condition.	Amount & route of injection.	estimated Day of illness.	Opsonic Estimations in Percentage Phagocytosis. Days after Serum. B.S.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	Later Results	Swab Examinations.	Remarks.
1.J.M. 2	T_2 C_9 E_1	16,000 I.M.	5th	25									**45**						21st day 18 29th " **62** 50th " **53**	37th day pos. 39th " neg.	Persistent +ve swabs in convalescence.
2.A.A. 6½	T_2 C_o E_1	24,000 I.M.	2nd	29	**43**	51	38						21						17th" 36 26th" 28 33rd" **43**	8th " neg.	
3.W.McL.6	"	16,000 I.M.	3rd	**35**		23			25				28						16th" 32	26th " pos. 33rd " neg.	Positive swabs in convalescence.
4.W.C. 6	"	16,000 I.M.	3rd	**70**	**62**		**49**		**55**		**67**								16th" 10	11th " neg.	
5.B.M. 2½	"	18,000 I.M.	2nd	33			**64**		**78**		**34**			35					17th" 15	27th " pos. 33rd " neg.	Positive swabs in convalescence.
6.M.K. 6	"	18,000 I.M.	4th	**75**		**47**		**57**		**46**			36						15th" 14	11th " neg.	
7.S.M. 11	"	24,000 I.M.	3rd	34	49		**65**		**57**		26								16th" 21	5th " neg.	
8.J.G. 6	"	24,000 I.M.	3rd	29	**49**		**49**		**55**		30								16th" 28	5th " pos. 12th " neg.	
9.M.N. 6	"	24,000 I.M.	4th	20	15		25		31		**43**								20th" 31	3rd " neg.	
10.H.M.13½	"	24,000 I.M.	3rd	**41**		**52**		55			68							8		21st " neg.	

Case.	Age.	C.C.	Amt. & Route of injection	Day of illness	B.S.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	Later Results	Swab Examinations.	Remarks.
11.	A.A.	8	T_3 C_o E_1	16,000 I.M.	3rd	33		23							44						21st day 19 29th " 42	9th day neg.	
12.	M.S.	6	T_3 C_m E_1	24,000 I.M.	2nd	36	11	25				35		42							18th "29	25th " pos. 30th " neg.	Positive swabs in convalescence.
13.	A.M.	7	T_2 C_m E_1	16,000 I.M.	3rd	48	52		39			23				42					17th "22 31st "37	23rd " pos. 27th " neg.	Appeared to be undergoing spontaneous cure on admission. Positive swabs in convalescence.
14.	M.H.	5	T_3 C_m E_1	24,000 I.M.	7th	43	40			21				30							15th "18 29th "50	19th " neg.	
15.	W.T.	9	"	24,000 I.M.	4th	40	18		31				21		45					29		17th " neg.	
16.	I.M.	10	"	24,000 I.M.	8th	17	56		63		75		64							62	20th "18	35th " pos. 39th " neg.	Positive swabs in convalescence.
17.	N.M.K.	6	"	24,000 I.M.	4th	21		81			59			48			59				19th "23	20th " pos. 27th " neg.	Positive swabs in convalescence.
18.	G.M.	8	"	24,000 I.M.	6th	15	41		46		41		42		48						15th "23	10th " neg.	
19.	D.D.	8	T_3 C_m	32,000 I.M.	8th		23		73		71			69							15th "13	22nd " pos. 31st " neg.	Laryngeal. Tracheotomy performed. No exudate in throat.
20.	W.L.	8	T_2 C_m E_1	24,000 I.M.	5th	21	31		70		69			47							15th "12	22nd " neg.	
21.	M.L.	10	T_2 C_o E_2	24,000 I.M.	3rd	6		42		35		64		48							16th "24 23rd "40	12th " neg.	

Case.	Age.	C.C.	Amt. & Route of injection	Day of illness	B.S.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	Later Results.	Swab Examinations.	Remarks.
22.AMcC.	7	T_2 C_o E_2	16,000 I.M.	4th		29		67		41		18		33							34th day pos. 72nd " neg.	Vaginal & Faucial diphtheria.
23.H.C.	5½	"	24,000 I.M.	3rd	33	50		58		38		48							48		14th " pos. 20th " neg.	
24.N.B.	7	"	32,000 I.M.	5th	58		61		40		60			47						17th day 23	13th " neg.	
25.M.McB.	12	"	24,000 I.M.	2nd	54		63		62		34			59						17th " 14	15th " neg.	
26.M.McC	12	"	20,000 I.M.	7th		70		77		20		35		50						15th " 28	16th " pos. 23rd " neg.	
27.E.J.	7	"	24,000 I.M.	3rd	77	51		31		43		50						16			15th " pos. 30th " neg.	
28.I.B.	11	"	24,000 I.M.	3rd	57		36		58		40		15							17th " 22	54th " pos. 56th " neg.	Positive swabs in late convalescence.
29.S.D.	10	"	24,000 I.M.	3rd		38		27		50			22							22nd " 16	18th " neg.	
30.F.H.	8	T_2 C_m E_2	24,000 I.M.	3rd	42		23						57						22	22nd " 46 31st " 52 41st " 30	30th " pos. 32nd " neg.	Positive swabs in convalescence.
31.McC.	7	T_2 C_o E_2	16,000 I.M.	4th		29		67		41		18		33								Case 22 repeated by mistake.
32.R.P.	9	T_3 C_o E_2	32,000 I.M.	4th	50	56	40	39						11						17th " 35 26th " 20 33rd " 23	5th " neg.	
33.J.McD.	7	T_3 C_m E_2	16,000 I.M.	3rd		14	51	40	45	52			49		30					23rd " 36 37th " 40	29th " pos. 33rd " neg.	Positive swabs in convalescence.

Case.	Age.	C.C.	Amt. & Route of injection.	Day of illness.	B.S.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	Later Results.	Swab Examinations.	Remarks.
34.W.B.	10	T_3 C_m E_2	24,000 I.M.	2nd	19		26		4		58		38					30		31st day 48	16th day pos. 19th " neg.	
35.C.C.	13	T_3 C_m E_2	32,000 I.M.	4th	34	19		25			32		23							23rd " 88	20th " pos. 27th " neg.	
36.C.F.	12	"	16,000 I.M.	2nd	19	40		33		53		55								15th " 43 22nd " 32	11th " neg.	
37.P.D.	3	"	24,000 I.M.	3rd	54		72	47			40			51						17th " 22	12th " pos. 18th " neg.	
38.J.C.	1 8/12	"	16,000 I.M.	2nd	53		42		56		48									17th " 26	6th " neg.	
39.M.S.	12 4/12	T_2 C_o E_3	40,000 I.M.	3rd	59	32		61		16		26							53		10th " neg.	
40.H.McC.	7	T_3 C_o E_3	32,000 I.M.	3rd	52		34		67		42		16							17th " 22	13th " pos. 30th " neg.	
41.P.G.	6½	"	24,000 I.M.	3rd	34			23						38						15th " 6 23rd " 38 30th " 21	11th " neg.	
42.H.McC.	11	T_3 C_m E_3	32,000 I.M.	4th		22	25		26		52		68							16th " 58 23rd " 53	21st " pos. 27th " neg.	Serum administered before admission.
43.E.McK.	7	"	24,000 I.M.	2nd	41	17				28		35								17th " 40	24th " pos. 29th " neg.	Pos. swabs in convalescence.
44.A.McM.	5	"	40,000 I.M.	3rd	45		14		13			26		19						26th " 69	14th " neg.	
45.J.N.	9	T_4 C_m E_3	40,000 I.M.	3rd	32	62		50		76		37					55			16th " 74 20th " 17	15th " pos. 21st " neg.	

Case.	Age.	C.C.	Amt. & route of injection.	Day of illness.	B.S.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	Later Results.	Swab Examinations.	Remarks.
46. M.P.	10	T_4 C_m E_4	8,000 I.V. 32,000 I.M.	2nd	20	11	21	30	23	22	28			50						18th day 57 39th " 80	6th day pos. 17th " neg.	Membrane continued to spread for 2 days after serum.
47. M.J.	11	"	56,000 I.M.	3rd	34		5		5		22				29				25	24th " 42	14th " neg.	
48. M.F.	13	T_4 C_s E_4	16,000 I.V. 24,000 I.M.	3rd	32				15		23		28		51					17th " 50 27th " 60	43rd " pos. 50th " neg.	
49. T.L.	9½	T_5 C_s E_4	70,000 I.M. 5 c.c. A.S.S. I.M.	2nd	44	16		65		65			55							15th " 13	103rd " neg.	
50. B.McG.	13/12	T_s	32,000 I.M.	7th	67		28		45												11th " pos.	Profuse post-natal & faucial discharge with little membrane formation. Died.
51. J.C.	5½	"	20,000 I.V. 20,000 I.M.	3rd	23																	Laryngeal. Trach - eotomy. Died on day after admission.

Table IV b. - Controls.

Case.	Age.	Amt. & route of injection.	Day of illness.	B.S.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	Later Results.	Remarks.
1. D.R.	4	16,000 I.M.	4th	14		23		24		30					24					Case of scarlet fever with suspicious throat. Swabs proved neg
2. I.H.	4	16,000 I.M.	5th	25			16			21		33							16th day 22	Febrile illness. Diagnosed as diphtheria on account of epistaxis. Not confirmed clinically nor bacteriologically.
3. J.C.	13	24,000 I.M.	2nd	10									12							Acute non-specific tonsillitis.

Table V - Class IB Cases.

Case.	Age.	Clinical condition.	Amt. & route of injection.	Day of illness.	Opsonic Estimations. Day.	Reading.	Swab Examinations.	Remarks.
1.T.R.	7	T_2 C_o E_1	24,000 I.M.	1st	6th 32nd 41st 47th	**64** 24 37 20	38th + 41st -	Pos. swabs late in convalescence.
2.C.G.	9½	T_3 C_o E_1	20,000 I.M.	3rd	7th 16th 28th 36th 43rd	21 32 13 37 15	16th -	
3.G.B.	14	T_2 C_o E_2	32,000 I.M.	3rd	25th	21	14th -	
4.M.G.	41	"	32,000 I.M.	1st	22nd	29	12th -	
5.C.G.	10	T_3 C_o E_2	49,000 I.M.	1st	17th	27	30th + 34th -	Pos. swabs in convalescence.
6.J.D.	3	T_3 C_m E_2	16,000 I.M.	4th	45th	25	23rd + 26th -	
7.I.B.	9	"	24,000 I.M.	8th	17th	20	8th + 26th -	
8.I.K.	5½	"	32,000 I.M.	4th	1st 15th 32nd 44th	23 32 26 38	15th -	
9.C.R.	7	"	32,000 I.M.	5th	3rd 5th 11th 19th 28th	35 **44** 11 38 36	24th -	
10.A.B.	7	T_2 C_o E_3	16,000 I.M.	1st	32nd	**47**	32nd + 38th -	Pos. swabs late in convalescence.
11.D.B.	3	"	16,000 I.M.	1st	27th	**55**	13th -	
12.W.B.	5	T_3 C_o E_3	32,000 I.M.	5th	33rd	**42**	33rd + 38th -	Pos. swabs late in convalescence.
13.W.L.	4	"	8,000 I.V. 24,000 I.M.	8th	7th 33rd 42nd	26 10 37	84th + 88th -	Pos. swabs late in convalescence.
14.I.C.	9	T_3 C_m E_3	32,000 I.M.	4th	7th 21st	31 38	21st + 25th -	
15.M.B.	10	T_3 C_s E_3	48,000 I.M.	4th	35th 61st 71st	20 28 34	26th -	
16.W.C.	1½	T_4 C_m E_3	32,000 I.M.	8th	29th	**45**	29th + 35th -	Pos. swabs late in convalescence.
17.R.S.	5	T_5 C_s	40,000 I.M.	4th	6th	24	6th + 46th -	

which is one of the most severe I have seen during four years' experience of diphtheria, is especially interesting. This patient's brother (No. 20) was attended by the family doctor on account of a sore throat and as a result of the diagnosis, this patient was examined as a contact. His fauces was extensively covered with membrane although his infection was quite unsuspected and it is probable, from the faucial appearances on admission to hospital that that infection was of much longer standing than is indicated in the Table. So severe indeed was the case, that a very bad prognosis was given but the boy made a very good recovery after months in hospital, although he developed a very marked paralysis of his soft palate and left leg during convalescence. It is a question to what extent the early appearance of opsonins in his serum affected the ultimate result.

Mention of case No 46 might also be made, since it also presented interesting features. The membrane in this case continued to spread for forty-eight hours after the administration of serum and this phenomenon was associated with very low opsonic readings. This case would appear, therefore, to indicate that antitoxin is not the only factor in the arrest of the local inflammatory process and that some other part of the immunity mechanism, possibly the opsonins, are important in the limitation of the spread of the membrane and such a conclusion is supported by the fact, already noted, that, in the less toxic cases, the opsonins show early increase, and that increase corresponds generally with the time of separation and shedding of the membrane.

The possibility of such a local effect of the opsonins is also suggested by the fact that in many cases, where negative swabs are not obtained until late in convalescence, correspondingly/

correspondingly late increases in the opsonic potency of the sera are also often, though not invariably, noted.

Whether or not the opsonins owe their importance, as the results suggest, if not actually prove, to their effects on the local manifestations of the disease, that importance none the less appears to be established, and is further indicated by the fact that during the progress of the disease, there is a decided increase not only in the total opsonic potency of the serum of the patient, but also in the "tropins" or thermostable specific opsonin. This will be readily seen by reference to Table VIII and the accompanying graph. The greater potency of the unheated serum may well be due to the action of immune-body and complement, an effect which sometimes does occur in immunity as stated earlier in this thesis.

Table VIII.

To Demonstrate effect of heating Serum at 55° C.

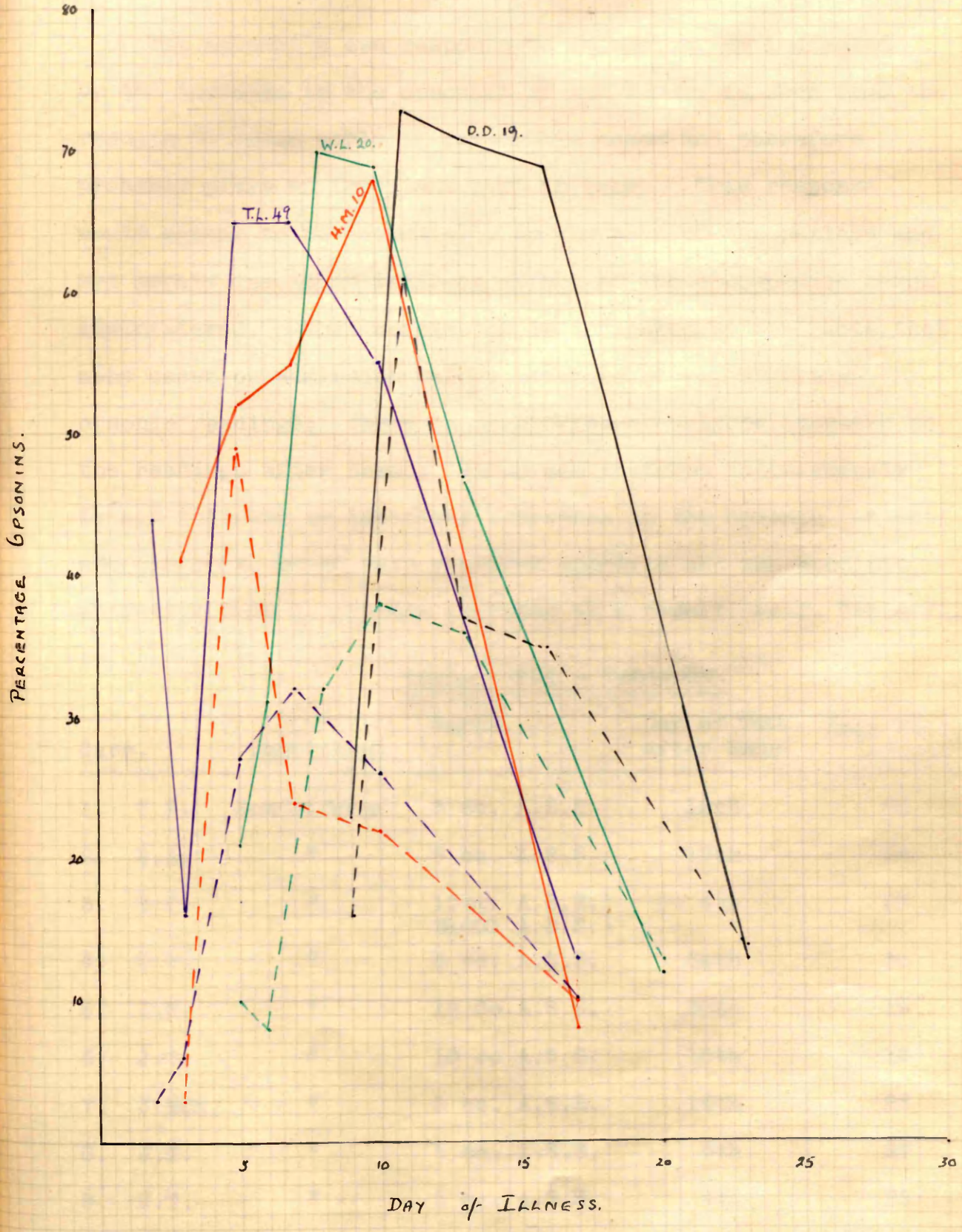
Case.	Bs.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10. H.M.	41 3		52 49		55 24			68 22							8 10	
20. W.L.	21 10	31 8		70 32		69 38			47 36							12 13
49. T.L.	44 3	16 6		65 27		65 32			55 26							13 10
19. D.D.		23 16		73 61		71 37			69 35							13 14
51. J.C.	23 8															

Normal. (1) 23 / 6

Convalescent Pneumonias. (2) 5 / 3 (3) 12 / 5

Unheated specimens - Figures in red.

Graph Showing Effect of Heating Serum.



Each Case is Shown in Distinctive Colour : Continuous Line Represents Unheated Specimens + Broken Line, Heated Specimens

The time at which maximum opsonic increase occurs cannot be related to any particular day of the disease and this is not surprising since cases differ so much from one another in severity; but, from what has been said, it would appear to occur earlier in cases where the prognosis is more favourable.

The patient's own response to infection, as indicated by the increase in the opsonins of the serum, is seen from the results to occur early in favourable cases and therefore probably plays an important part in cure. This response would appear to be essentially on the part of the patient and not merely due to an indirect effect of the therapeutic serum administered. This conclusion is indicated by the facts that many cases on admission before serum is given, show high opsonic readings; there is an absence of uniform increase in the readings after serum; serum administered intravenously is not followed by immediate increases in the opsonic content; and controls, which have received specific and non-specific antiserum show no opsonic increase as a result. (c.f. Table VII)

Table VII - Controls.

Case.	Condition.	Serum.	Day of Test after Serum.	%age Phagocytosis.
1. E.I.	Scarlet fever	5 cc. A.S.S	12th	30
2. W.A.	"	5 cc. A.S.S.	15th	24
3. M.K.	"	10 cc. A.S.S.) 32,000 A.D.S.)	4th	27
4: D.G.	"	5 cc. A.S.S.	34th	30
5. J.S.	"	10 Cc. A.S.S.	30th	19
6. J.C.	"	10 cc. A.S.S.	10th	18
7. J.McM.	"	5 cc. A.S.S.	14th	23
8. J.T.	"	5 cc. A.S.S.	6th	27
9. M.T.	"	5 cc. A.S.S.	5th	28
10. W.B.	"	5 cc. A.S.S.	18th	28
11. P.McG.	"	5 cc. A.S.S.	10th	23
12. G.T.	"	5 cc. A.S.S.	17th	25

Table VII (Contd.)

Case.	Condition.	Serum.	Day of Test after Serum.	%age Phagocytosis.
13. N.M.	Scarlet fever.	5 cc. A.S.S.	16th	24
14. M.M.	"	5 cc. A.S.S.	2nd	27
15. M.M.	"	5 cc. A.S.S.	1st	18
16. I.B.	"	5 cc. A.S.S.	19th	19
17. J.R.	"	5 cc. A.S.S.	3rd	7
18. E.S.	"	10 cc.A.S.S.	3rd	22
19. M.H.	"	5 cc. A.S.S.	1st	24
20. J.C.	"	5 cc. A.S.S.	19th	17
21. J.G.	"	5 cc. A.S.S.	8th	24
22. W.R.	"	5 cc. A.S.S.) 24,000 A.D.S.)	1st	21
23. H.McK.	"	10 cc.A.S.S.	3rd	18
24. J.McI.	"	5 cc. A.S.S.	3rd	10
25. H.W.	"	5 cc. A.S.S.	3rd	19
26. J.B.	"	5 cc. A.S.S.	1st	12
27. J.H.	"	10 cc.A.S.S.	20th	8
28. J.S.	Tonsillitis	8,000 A.D.S.	8th	9
29. J.F.	Puerperal Fever	20 cc.A.S.S.	10th	19
30. E.M.	Convalescent pneumonia			5
31. A.A.	Enteric fever			8, 23, 14.
32. A.B.	Gonorrhoea & Syphilis			16
33. M.S.	Normal			4
34. J.R.	Gonorrhoea			20
35. M.M.	"			21, 30, 31.
36. J.B.	"			17
37. J.K.	"			12
38. D.C.	"			19
39. J.W.	Convalescent pneumonia			5
40. S.	"			12

A.S.S. - Antiscarlet Serum.
A.D.S. - Antidiphtheritic Serum.
Six saline controls gave readings of 0, 8, 7, 6, 11, 3.

The few cases included in Table Vi form a very interesting group in this study, and the results, though too incomplete to be conclusive, are at least suggestive. Cases 1, 2, 3 and 4 of this series were probably all cases which had undergone spontaneous cure. Nos. 1 and 2 were associated with virulent cases occurring in their respective homes but their own symptoms were very mild. Cases Nos. 5 and 6 are of especial interest, in that they were both ambulatory carriers occurring in the same household and associated with virulent cases. J.G. is the father of M.G. and they were examined as contacts to three other acute cases in the same household, one of which, an infant, died. From the results in these two cases and from a consideration of their Schick test results, it would appear that J.G. had no antitoxic immunity but the opsonic potency of his serum was above normal and this is possibly the factor which prevented his becoming infected. The results, at least, would suggest this. The daughter M.G. however, did possess an antitoxic immunity and the opsonic power of her serum was also above normal.

Table VI.

"Class IC" Cases.

Case.	Age.	Serum.	Day of illness (if history)	Opsonic Readings. B.S.	After Serum. Day		Schick Test.	History.
1. A.L.	5½	16,000I.M.	15th	20	5th 7th 34th	50% 25% 66%	Not done	Infant brother died of laryngeal diphtheria. Patient discovered as contact and gave history of sore throat 15 days previously. No exudate. Albuminuria and elevation of temperature only signs.
2. P.McK.	5	16,000I.M.	1st	46	2nd 5th 9th 16th 30th	48% 21% 34% 30% 51%	Not done	Brother of 43 E.McK. - Table IVa. No exudate. Throat swab yielded pure culture of K.L.B. Complaint of sore throat.
3. N.L.	4	8,000 I.M.	2nd		26th	25% (26th day (after serum	neg.	
4. M.F.	33	-	7th	35			Neg. on admission.	History of sore throat 7 days before admission & febrile disturbance. Throat clear on admission.

Table VI.(Contd.)

Case	Age	Serum.	Day of illness (if history)	B.S.	After serum. Day		Schick Test.	History.
5.J.G.	41	8,000 I.M.			6th	40%	13th day after serum pos.	Father of no. 6 M.G. below. Wife & other 2 children clinical cases - 1 died. Patient had no symptoms. Found as contact. Organisms proved virulent by guinea-pig inoculation.
6.M.G.	7	-			42		Negative.	Not admitted to hospital. Daughter of 5 J.G. above. Discovered as contact.

Readings over 40 shown in Red.

B.S. - Before serum administered.

Reference to Tables IVb and VII, i.e. to the control cases will show that the highest reading obtained in this group was 31. For this reason, estimations of 40 and over can be regarded as definitely above normal, after leaving a generous margin of 10% for any experimental and other errors, and for convenience, these are indicated in colour.

During the early part of 1934, the few cases comprising Group II of this study were investigated in some modified experiments of a supplementary nature to those carried out on the cases of Group I.

In the first place, it was thought that the earlier results obtained by Klein's dilution method of opsonic estimation might have been influenced by spontaneous phagocytosis. Consequently, the method was re-investigated but this time the organisms were emulsified in, and the serum diluted with, 1.5% saline solution in place of the usual 0.85%.

By this means, the possibility of spontaneous phagocytosis affecting the results was excluded.(1) A case of diphtheria of mild severity and a patient convalescing from pneumonia used as control, were investigated in this experiment.

The results obtained by this modified method confirmed the earlier unrecorded findings and are shown in Table I (p.21) from which it will be seen that diluting the serum even to 1 in 320 did not result in any diminution of its opsonic potency.

Two other subsidiary experiments carried out in connection with this group were those designed to test the keeping properties of serum in respect of opsonins and to find whether leucocytes from different sources showed differences in their ability to phagocytose organisms. These experiments were carried out with the same modifications of the original technique as mentioned above and the results are shown on pages 23 and 24 respectively, having already been quoted.

Another interesting experiment was made on a patient in this group who was admitted suffering from measles. During convalescence he had a swab examination of his throat made on account of his having been in contact with another case which developed diphtheria following measles. The swab was found to be positive and remained so for a period of several weeks in spite of treatment with antitoxin. Leucocytes were prepared from his blood in the manner previously described and these were used in a test with a 1 in 10 dilution of his serum and a mixture of pure cultures of diphtheria of proved virulence. His leucocytes were also used in a similar test in which "normal" serum was substituted. Finally, his serum was tested with leucocytes from a normal individual against the same mixture of organisms. The results of these three tests, carried out at the same time and under identical conditions/

(1) Wright & Reid. Proc. Roy. Soc. B. 1906, 77,211.

conditions were as follows:-

										% age Phagocytosis.
Patient's Leucocytes:	1/10 dilution of	patient's serum:	Mixture of	K.L.R.						45
"	"	:	"	"	"	normal	"	:	"	"	"	29
Normal	"	:	"	"	"	patient's	"	:	"	"	"	35

These results would indicate that the combined effect of the patient's own leucocytes and serum is to increase to some extent the degree of phagocytosis. They also confirm the earlier result obtained in the case of carriers in Group I, namely that the opsonic potency of the serum in carriers is above normal. The virulence of the organism isolated from this case, it may be noted, was proved by guinea-pig inoculation.

The serum of another case was investigated in a further experiment to see whether the actual organism isolated from the patient was as actively opsonised as organisms from other sources by the patient's serum. To investigate this point a very toxic case was selected and the serum tested within the first few days of the disease. In a dilution of 1 in 10, the serum was tested against the organism isolated from the patient's throat and then against a mixture of diphtheria bacilli of proved virulence, isolated from other cases. The results of this experiment were:-

1. Using organism isolated from patient - 4%.
2. Using mixed culture - 15%.

A final experimental test was made on a sample of therapeutic diphtheritic antitoxin to see whether it possessed any opsonic activity. The reading obtained in this case was 8%, indicating that the serum had no opsonic value.

5. Summary and Conclusion.

An account of an investigation of some eighty cases of diphtheria and of about forty control cases has been given. That investigation had for its immediate purpose the study of the immunity mechanism in diphtheria as evidenced by the appearance and development of opsonins in the serum of patients and it was hoped that this work would contribute indirectly something also to our knowledge of immunity in general. Subsidiary experiments, carried out in the course of the work, were concerned with the nature of opsonins themselves and with various methods for their study.

The results set out in the Tables, therefore, might best be considered under headings indicated by the various objects of the investigation.

A general survey of the results shows clearly that opsonins are developed in the sera of diphtheria patients and this is in accordance with the findings of previous workers, which were reviewed in an earlier section of this thesis. The elaboration of opsonins in diphtheria is seen to be a regular event in the course of the disease and their appearance generally coincides with the limitation and separation of the membrane.

The increase in the opsonic potency of the patient's serum in diphtheria appears to occur chiefly in the acute stages of the disease and this is noted particularly in cases of average or moderate severity, in which recovery is normal and uneventful. In the more severe types of cases , e.g. Nos. 46,47 and 48, Table IVa, the opsonic increase tends to appear later and sometimes this delayed increase is associated with positive swabs late in convalescence (c.f. Nos. 3, 12, and 35 Table IVa). The latter, however, is not an invariable result, some cases showing

late/

late positive swabs although opsonins appeared early and in marked degree in the serum. Many cases (Tables IV and V) show a later rise in the opsonins about the time when negative swabs are first obtained, confirming an observation previously made by Tunnicliff.

The times at which the opsonins appear in the serum, would suggest that the part played by these antibodies is concerned with the local processes of the infection and probably achieve their beneficial effects by expediting the disappearance of the membrane. They certainly do not appear to have any power to prevent directly the development of remote complications, these probably being purely toxic in origin. This is borne out by the results shown in Table IVa.

Further evidence of the association between high opsonic content of the serum and the local condition is shown by the results of the investigation of three "carriers". (c.f. Table IV Nos. 5 and 6, and case quoted on pp. 46 and 47). These results would at least suggest that the opsonins not only play a part in effecting cure by expediting the disappearance of the membrane in acute cases, but also are important in the immunity of carriers. One of the cases, as already pointed out, did not possess an antitoxic immunity as shown by the result of the Schick test, but he did not develop the disease although virulent diphtheria bacilli were found in his throat. The opsonic potency of his serum, however, was above normal.

The results of additional experiments carried out on a few cases of Group IA, in which the specimens of sera were heated at 55° C. for 20 mins. prior to the tests, show that the opsonic increase during infection is largely due to the thermostable moiety, or "tropin". It will be observed, however, that the tropin is not the only factor on which the increase/

increase in opsonic activity of the serum depends and this is in keeping with the results of previous work on the nature of opsonins in general, as already described earlier in this thesis. The total opsonic potency of the serum in diphtheria, therefore, probably depends on a summation of effects. (See Table VIII and graph).

From the point of view of prognosis in diphtheria, it is possible that some value may be attached to opsonic estimations. This is suggested by the features and progress of case No. 49, already quoted (see p.32) and also by the results generally, shown in Table IV a, where, it will be seen that many of the cases already showed opsonic increase when they first came under treatment. Case No. 50 may be thought to contradict this suggestion but it may be stated that this case showed features which indicated that the fatal issue was due to causes other than diphtheria. The child was very ill on admission and the only local signs were profuse nasal and faucial discharge without membrane formation. The swabs gave a profuse flora on culture and only the later ones showed the presence of diphtheria bacilli.

Another point to be considered in this general discussion of the results, is the effect of the administration of therapeutic serum on the opsonic readings. For reasons already stated, it must be concluded so far as the results of this work are concerned, that specific and non-specific therapeutic sera have no influence on the opsonins and diphtheria antitoxic serum itself possesses no opsonic value.

The remainder of the experimental results of this research are concerned with the technical side of opsonic estimations and, for the most part, they confirm the results obtained by other workers.

As already stated (c.f. Table I. p.21) Klein's dilution method of estimating opsonins was investigated and it was found that dilutions of 1 in 320 did not result in any appreciable diminution of the opsonic power of the serum. This finding is contrary to the results obtained by Tunnicliff.

Experiments made to determine the keeping properties of serum in respect of opsonins and the effect of using leucocytes from different sources were performed and the results have already been quoted (pp. 23 and 24). These confirmed the observations already made by A. Fleming.(1) The serum was found to lose none of its opsonic activity after being stored in tightly-stoppered tubes in a cool, dark place (see Table II p.23) and the leucocytes from different sources produced little or no variation in the results of the opsonic tests.

Another experiment, quoted on P.46, was made to determine the effect of using the patient's own leucocytes and serum together in an opsonic test. The result tended to indicate that phagocytosis was increased to some extent by this method. No definite statement, however, can be based on one observation, but the result tends to ascribe some importance to the part played by the patient's own phagocytes.

Inconclusive, but also suggestive, is the result obtained by testing the patient's serum against the actual organism responsible for the infection as compared with the result obtained by using organisms from other sources. This experiment is described on p.47.

(1) Practitioner 1907. Vol.80. No.5.

From a general consideration of the results of this research, it would appear that the immunity in diphtheria is not purely antitoxic as is generally supposed. Indeed, it is not only possible, but probable that opsonins play a not unimportant , though unsuspected, part in the processes concerned in the establishment of immunity in that disease. That phagocytosis should play any part at all in a disease, which, from the eminently satisfactory results obtained by antitoxic serum in treatment, we have come to regard as being purely in the nature of an intoxication, must give us cause for reflexion. One possibility that suggests itself is that a part of the immunity mechanism may be directed against the establishment of the infection and another against the inroads of that infection once it has become established.

If the opsonins can be regarded as an indication of the development of the patient's own response to infection, as well they might, then it is seen that the cases in which the disease is quickly brought under control are those in which that response occurs early. The amount of serum given and the route by which it is administered, even when, by intravenous injection it is rendered immediately available to the tissues of the body generally, do not influence directly the time and extent of that response. This is readily seen by reference to the cases, already quoted, where the opsonic rise was delayed. All these cases were of unusual severity and were treated with liberal doses of antitoxin. Thus it would appear that the patient's own reaction to infection is a very important factor in cure and immunity. In other words the administration of therapeutic serum, containing ready-formed antibodies, is not alone responsible for the successful overthrow of infection. In infections such as diphtheria, however, where much of the damage to the organism is accomplished

through/

through the medium of exotoxin, antitoxic serum is of considerable importance, but it has little effect in infections where exotoxins play little or no part.

On these grounds one might make bold to suggest that investigation into methods whereby an early response on the part of the patient's active defences might be brought about, would provide a profitable field for further research and, any contribution to our knowledge in this connection would doubtless be of far-reaching and fundamental importance. It is probable indeed, that antitoxic serum in diphtheria produces its beneficial effects indirectly by protecting the organism over a period during which its own natural defences are being mobilised. Thus, it is possible that, in cases where adequate doses of antitoxin appear to fail, that failure is due rather to lack of response on the part of the patient himself.

As a preliminary to any such investigation, a study of the part played by the leucocytes might well prove valuable. That part, one feels, cannot but be of primary importance and Metchinkoff's views might not be so much erroneous as incomplete. We know that even the fixed cells of a tissue may play an important part in pathological processes and the cells of that "fluid tissue", the blood, in view of their mobility, can hardly be of any less importance in the body defences.

It may be said in conclusion, that the success which has attended previous research on the subject of diphtheria is probably largely attributable to very exhaustive and exclusive studies of the diphtheria bacillus. Perhaps even greater success will reward us if we but turn our attention now to the patient and study him with the same care and patience.