BACTERIOLOGIST:
[illegible]
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COUNTY LABORATORY,
PUBLIC HEALTH OFFICE,
COUNTY BUILDINGS, DUMFRIES.

5th April, 1940.

The Dean of the Faculty of Medicine.
University New Buildings,
Teviot Place,
EDINBURGH.

Dear Sir,

I beg to submit a thesis entitled "Natural Immunity and Natural Antibody Reactions" for the degree of Doctor of Medicine.

I certify that the context of this thesis and its compilation are my own work.

In section 6, however, I have acknowledged collaboration with W. M. Arnott, M.D. and G. D. Mather, M.D., F.R.C.S.Ed. For the work of this section clinical material was collected by my colleagues for my examination and a correlated study was made jointly on the blood complement of Acute Glomerulo-nephritis and pregnancy conditions. The clinical material for the other conditions studied in section 6 was collected by me personally.

Yours faithfully,

ST/EB.

Ackg^d.
22-12-6 asked for - also date of passing Clin. exam.
M.B. 1933

NATURAL IMMUNITY,

and

NATURAL ANTIBODY REACTIONS.

A thesis submitted for the degree of

Doctor of Medicine.

by

Scott Thomson.

1940.

PREFACE.

This thesis records the results of investigations into certain phenomena in natural immunity and natural antibody reactions.

Many of the experiments were exceedingly complex and the tables of results are also, therefore, complex. Typical examples of the various phenomena encountered are expressed only once in order that the thesis be not overweighted with tables. Every step has been taken towards simplification of the expression of results.

For this reason also, the thesis does not include any protocols of the experiments.

ACKNOWLEDGEMENTS.

It is a pleasure to acknowledge my indebtedness to Professor T. J. Mackie for his encouragement, advice and helpful criticism.

My thanks are due also to Major Wood for his permission to do Schick tests on militia-men and to Dr. A. Joe for his permission to withdraw samples of blood from patients in the City of Edinburgh Infectious Diseases hospital.

The work was done in the Department of Bacteriology, University of Edinburgh over a period of four years and the expenses were defrayed by grants from the Earl of Moray Fund and from the Lewis Cameron Fund.

CONTENTS.

GENERAL INTRODUCTION.

Immunity.

The term "immunity" means resistance against infective disease. The term is a little misleading because in the study of resistance against bacterial infection there are recognised all grades of resis-:tance varying from complete insusceptibility to a grade of resistance so low that a few bacteria may be able to invade and bring about the death of their host. The term "immunity" is used to cover all these degrees and not merely the high degree of insusceptibility.

Resistance of an absolute degree to a pathogenic bacterium exists where there is a complete insuscep-:tibility of a given animal species to infection by a given bacterial species. Such resistance is, of course, found in all the members of the animal species. For example, measles and chicken-pox are diseases peculiar to the human subject, while the viruses of rinderpest and distemper never attack human beings. Fowls are apparently absolutely resistant/

resistant to experimental infection by the pneumo-:coccus. This absolute degree of resistance exhibi-:ted by an animal species to a particular bacterial species is a section of immunity which does not attract a great deal of attention except to record where it occurs. There appears to be little to be learned from it. The subject of immunity is more concerned with the study of the interplay between a bacterial parasite and a host which can be infected by that parasite. This study is not confined to one particular member of the host species, because one of the essential points of interest is to note the great differences in reaction and result which manifest themselves between a given parasite and many different members of the host species.

Between a parasite and its host there is always an interplay of factors which determine the final result. In the case of the bacteria these reactions have been given extensive study. It is clear to the clinician that, as a rule, infections leave behind them a resistance to future infection by the same bacterium, i.e. compared with other members of the same host species. This raised resistance may be of a very slight order as, for example, in human beings following an attack of coryza or tonsillitis. On the/

the other hand certain diseases due to filterable viruses, e.g. measles and chicken-pox, leave behind them a resistance which must be considered as absolute. For the most part, however, an intermediate degree is met, as for example in scarlet fever, which leaves the patient with a greatly raised resistance but by no means absolute.

In considering the interplay of forces between a host and a parasite there are many aspects to be considered. In the first place the pathogenic bacteria compared with other parasitic forms of life are highly infectious. Large numbers of the host population can be infected in a very short time. Many are highly virulent and can cause the death of the host in a very short time. The existence of parasites of such infectivity and virulence demands a state of resistance in the host varying in degree from individual to individual of the host species, otherwise an unstable state would exist in nature. If all human beings developed a resistance of a very high degree so as to resist not only disease production by the bacterium but also infection by the bacterium, then that particular bacterium would die out as a species, provided of course that it were one of the strictly parasitic species. If all human beings/

beings had a uniformly low resistance to a particular infection the race would be in danger of being wiped out by that infection. Parasites of great infec-:tivity exist, e.g. influenza virus, which can infect the whole human race in a pandemic, and if such parasites are to survive as a species it is essential that all the hosts are not killed by the infection. The equilibrium between a bacterial species and the human race as opposed to the individual case must therefore also be considered in the study of immunity.

Evidence of Immunity.

The evidence for the existence of immunity is collected from many varied sources. The original source was, of course, clinical observation. From the earliest of times there are writings to show that physicians were aware of the resistance left behind by many infective conditions, e.g. small-pox, mumps. Such observations were easy with those infective diseases which leave behind them a very high degree of immunity. A small number of cases would be sufficient to reveal the fact to any one observer. Where the degree of resistance was less, a greater number of cases would be necessary to reveal the presence/

presence of the altered state, and few men would get this experience. If the alteration of resistance was slight it would require the combined experience of many observers to reveal it, and for such condi-:tions we require epidemiological evidence.

The agreement between clinical observation and the experience of bacteriology is close, but not always so simple as would at first appear. For example, a clinical entity or syndrome like lobar pneumonia, is not an entity to the bacteriologist because there are many different types of pneumo-:coccus. Conversely one bacterial species represen-:ting an entity to the bacteriologist may produce several clinical syndromes. For example, the haemo-:lytic streptococcus is responsible for scarlet fever, tonsillitis, otitis media, mastoiditis, wound infec-:tion, lymphangitis, erysipelas, puerperal fever and secondary pneumonia, and we can readily see some obstacles which disturb the sympathy between the clinical observer and the student of immunity.

Other evidence is obtained from field observa-:tions. In such circumstances we can observe the attack rate or death rate of a disease in two or more different groups to ascertain any significant differ-:ence between them.

Finally/

Finally, laboratory experiments have been devised to analyse the nature of this resistance and to measure its degree. The analysis of its nature has presented many difficulties. The relative parts played by cellular activity and humoral antibodies at one time occupied a great deal of attention, which has now waned. The significance of the circulating antibodies is mentioned here only briefly as it will be referred to again as it is the basis of this work. The measurement of the degree of immunity in labora-:tory study is a most difficult problem. On very few occasions is it possible to measure the exact amount of a bacterial toxin, or the exact amount of an infecting dose of live bacteria required to pro-:duce a given effect in one animal. If we succeed in producing the required effect we do not know how much less would have done, and if we do not succeed it is fallacious to repeat the experiment because the former contact with the specific organism has increased the host's resistance. It is no longer the same animal. For experiments of the above nature, therefore, we have to resort to the use of large numbers of animals and average the results.

Results/

Results of Infection.

The end result of any bacterial infection depends upon two groups of factors. One is the virulence of the organism, the other the resistance of the host, and it is the latter which will be studied here.

When an individual comes in contact with an infecting agent a great variety of possible results may ensue. The organism may not, of course, succeed in establishing itself in or on the tissues of the individual. When, however, the bacterium does succeed in becoming established on the host the result may be a genuine clinical illness (with all its grades of severity) or a carrier state. There is no sharp demarcation between the two. Gradations are found from the severe case down to the very mild clinical case, and even below this to the atypical mild infec-:tion or latent infection, and the pure carrier state. There are also many gradations in the term "carrier state", because an individual may harbour a pathogenic bacterium for a short time only or he may "carry" the bacterium in large numbers over a prolonged period. Now all such contacts whether appearing as a clinical case or merely a latent infection or carrier state increase/

increase the resistance of the individual against that infection, and it is therefore not easy to dis-:tinguish between immunity which is acquired and immunity which is natural.

The other factor determining the end result of an infection is the virulence of the infecting organ-:ism, and it is customary to regard virulence as being dependent upon two factors, viz. the invasive-:ness and the toxicity of the organism. It is possible to recognise all types of infective disease ranging from a pure toxaemia where the bacteria do not invade the tissues of the host, as for example in botulism and diphtheria, to a disease which is charac-:terised by the invasiveness of the bacterium, as for example in anthrax, where the toxic factor appears to be of minor importance. Some bacteria exist - the haemolytic streptococcus is one - which are highly invasive and produce potent toxins. The bacterial toxins have been extensively studied because they are responsible for the damage done to the host. They stimulate the production of antibodies, i.e. they are antigenic. Certain of them - the exotoxins - stimulate the production of antibodies - the anti-:toxins - which neutralise their toxic action. The majority, however, stimulate the production of antibodies/

antibodies which although they interact with the bacterial antigens do not neutralise their toxic action.

Certain animal species are almost immune to some of the bacterial exotoxins and this resistance is not due to the presence of neutralising antitoxin in the animal's blood. These animal tissues are insuscep-:tible to the specific actions of the toxins. If, however, a certain animal species is susceptible to a particular exotoxin we find that the varying degrees of immunity exhibited by the individual members of that species can be directly correlated with the presence of circulating antitoxin. In the case of those bacteria whose pathogenic action is not <u>via</u> an exotoxin the immunity is due to an increased ability to prevent the infecting agent from becoming established in the tissues. This is designated antibacterial immunity as opposed to the former, which is antitoxic immunity. It is convenient to make this subdivision for reasons which will be seen later, and the study of the mechanisms of these two forms of resistance are the aims of this work. Particular reference will be paid to natural immunity and natural serological reactions, and especially to enquire into their/

their origin and means of alteration by non-specific means.

Natural Immunity.

Mention has already been made of the two types of immunity, natural and acquired. An acquired immunity can always be traced back directly or indirectly to the infecting agent. If the immunity is actively acquired then the individual himself has reacted to the organism or its products and has built up his own resistance. If the immunity is passively acquired then the antiserum used to immunise the individual was obtained from some other individual who was actively immune. An acquired immunity may even be congenital, as in the case of immunity to measles in the first six months of life - a congenital passive, acquired immunity due to the fact that the mother is actively immune.

Natural immunity embraces all the mechanisms which a host exposes to an infecting agent and which are not dependent upon previous association with the infecting agent. It is not always possible, however, to draw an absolute distinction between the two because of the existence of latent infections which pass/

pass unnoticed and produce no clinical illness.

The evidence for the existence of natural immunity has been obtained from diverse sources. There can be clearly recognised degrees of resistance varying greatly between different animal species. For example, anthrax is primarily a disease of herbivora but man is also affected. In man, the disease is not so virulent but even within the human species there are various degrees of resistance. The varying degrees of susceptibility shown towards an infecting agent by different members of the host species is not always, of course, due to natural immunity. In the particular case of anthrax in the human subject it probably is. The same may be admitted in the human diseases, small-pox, chicken-pox, measles and mumps, etc. where the first contact with the infecting agent usually results in clinical disease. Any differences in resistance shown by different individuals is due to natural resistance.

There exist also many examples of an absolute degree of resistance between certain animals and bacteria. Fowls are immune to pneumococcal infection; animals are insusceptible to leprosy, and human beings are not susceptible to dog distemper.

Among the different races of the human species there are/

are varying degrees of resistance to the same infec-
:tion. Examples of this are easily found in human medicine. For instance, uncivilised races possess a resistance to tuberculosis of a very low order compared with civilised races. The death rate from tuberculosis amongst the Red Indians towards the end of last century was ten times that of the white popu-
:lation. The incidence of tuberculosis among the Senegalese troops brought to France during the war years was appalling. The susceptibility of tropical races to measles and of Europeans to yellow fever are other examples of the same principle. As a general rule a race will become more resistant to an infection to which it is constantly exposed. Various factors contribute to this as, for example, natural selection and congenital transmission of immunity which, of course, is an acquired immunity. From this it will be appreciated that it is difficult to draw a sharp differentiation between natural and acquired immunity.

PARTICULAR INTRODUCTION.

The Existence and Significance of Antibodies.

Following infection by a bacterium or after specific active artificial immunisation, there appear in the circulating blood, specific antibodies which are closely associated with the serum globulin. This phenomenon is part of a general rule that anti-:bodies appear in the blood following the parenteral introduction of a foreign protein into the tissues of an animal. At one time there was a great controversy as to the importance of these antibodies. One school of opinion held that body resistance was a property of certain cells and that the presence of circulating antibodies was incidental, while the other school of opinion held that body resistance in the actively immune was closely inter-related with the circulating antibodies. A justifiable position of compromise has been accepted, as one of the activities of anti-:bodies is to "sensitise" the bacteria and render them able to be phagocytosed by the cells, and it has also been shown that the degree of immunity to an exotoxin is directly related to the amount of antitoxin in the blood. The transference of the immune state in passive/

passive immunisation and the therapeutic use of antisera also show the value of the circulating antibodies. In the particular case of those bacteria which have little or no invasive properties and rely entirely on their exotoxins for their pathogenic action, this circulating antitoxin will determine the degree of resistance to the disease and to the infec-:tion as well as to the exotoxin itself. In such a category is the diphtheria bacillus and the disease diphtheria has accordingly been extensively studied in this connection.

In the apparent absence, however, of any specific stimulus there are present in the blood antibodies which have the same activities as the antibodies which result from specific immunisation. These have been designated "natural antibodies", and it is the object of this work to consider the nature of these sub-:stances, their origin, their specificity, their relationship to the specific antibodies and the possible alteration of their activity by non-specific means.

The Nature, Specificity and Origin of the Natural Antibodies.

The blood serum of animals may contain a large number/

number of antibodies which will react with bacterial and other antigens. The question at once arises of their identity with the antibodies which result from specific immunisation. They are present in smaller amounts than are found after immunisation, and Browning (1927) speaks of them as prototypes of the specific antibodies. Normal serum may contain antibodies against a large number of bacteria and other antigenic substances, as for example red blood corpuscles of other animal species. These antibodies are absent from the blood of newly born animals and appear after a few weeks of life. This has been shown for the normal bacterial agglutinins by Gibson (1930), Lovell (1932, 1934), Kraus and Low (1899), for normal complement fixing antibodies by Mackie and Finkelstein (1928,), and for the normal haemo-:lysins by Hirszfeld (1926) and Ehrlich and Morgenroth (1899, 1 and 2; 1900, 1 and 2).

Normal serum may react with a large number of antigens and the question arises if these owe their existence to multiple specific stimuli, and further if they represent multiple antibodies or a few "antibody-like substances" each capable of reacting with many different antigens. Gibson (1930) and Lovell/

Lovell (1932, 1934) concluded that the natural bacterial agglutinins were specific and multiple, and the evidence obtained by Lovell is all the more striking because he found multiple specific agglu-:tinins against the several species of one bacterial genus (Salmonella). Mackie and Finkelstein (1931) found a high degree of specificity in the natural bactericidins, but Gordon and Carter (1932) and Gordon (1933) concluded that the antibody-like substance responsible for natural bactericidal activity was to a large degree non-specific. In the case of the normal opsonins the evidence for their specificity has not been unequivocal.

As regards the origin of these natural anti-:bodies there are two schools of thought, but it is probable that both schools are correct in particular instances. One school of opinion maintains that these antibodies appear in the circulation as the result of a physiological process. That they are not present at birth, is not upsetting to this view which maintains that their formation is the result of the maturation of a physiological process occurring after birth. Furthermore, the globulin content of the serum of newly born animals is low, and it is perhaps/

perhaps noteworthy that antibodies are always associated with the globulin of the serum (Howe, 1922).

The best evidence to support the latter view is obtained from the study of those antibodies which do not react with bacteria but with other substances. The haemolysins and haemagglutinins cannot conceivably arise from specific stimulus by the red cells of another animal species, yet they are frequently found in serum and are specific (Ehrlich and Morgenroth, 1899, 1 and 2; 1900, 1 and 2). In human beings, of particular interest are the isohaemagglutinins, i.e. antibodies against the red cells of other human beings. These are the antibodies which determine blood groups and it is possible to divide humans into four groups by testing for the presence of these antibodies in the serum and the antigens on the corpuscles. The blood group of an individual is determined by heredity and, in fact, blood group is a characteristic which follows Mendelian laws.

As regards those antibodies which react with bacteria or their products the superficial evidence would appear to favour the view that they also develop as the result of some physiological activity. It is difficult to appreciate how an animal may come in contact with and react to so many different bacteria, but/

but although unlikely, this is not impossible. It is admitted that the specificity of antibodies pro-:duced by antigenic stimulation depends upon the chemical structure of the antigen. It is recognised that there are a large number of bacteria, e.g. species of the same genus, which show cross reactions in their antigen:antibody reactions, and it may be that these cross reactions of a low order are much more common than is at present thought. Furthermore, antibody production was formerly thought to be dependent upon the parenteral introduction of the antigen into the animal tissues. This definition has been relaxed because it is recognised that antibodies can be pro-:duced in small amount to antigens taken orally and this is especially so in the early days of life, or following some disturbance of the alimentary canal. (Pijper and Dau, 1930; Greenwood, Topley and Wilson, 1931; Pfeiffer and Lubinski, 1930; Ross, 1930, 1931; Kanai, 1921).

The importance of these facts cannot be over-:looked and although not directly producing evidence that all of the antibacterial antibodies are produced in this way it is conceivable that some of them at least owe their origin to this process.

Evidence/

Evidence to favour the view that some of the antibodies to bacteria and their products might be produced as a normal physiological process in the absence of stimulation was produced by Hirszfeld, Hirszfeld and Brookman (1924), who maintained that there was a connection between blood group trans-:mission and the reaction to the Schick test. They claimed that the positive or negative reaction of a young person was determined by the Schick reaction and the blood groups of the parents, but this has been challenged by Rosling (1928).

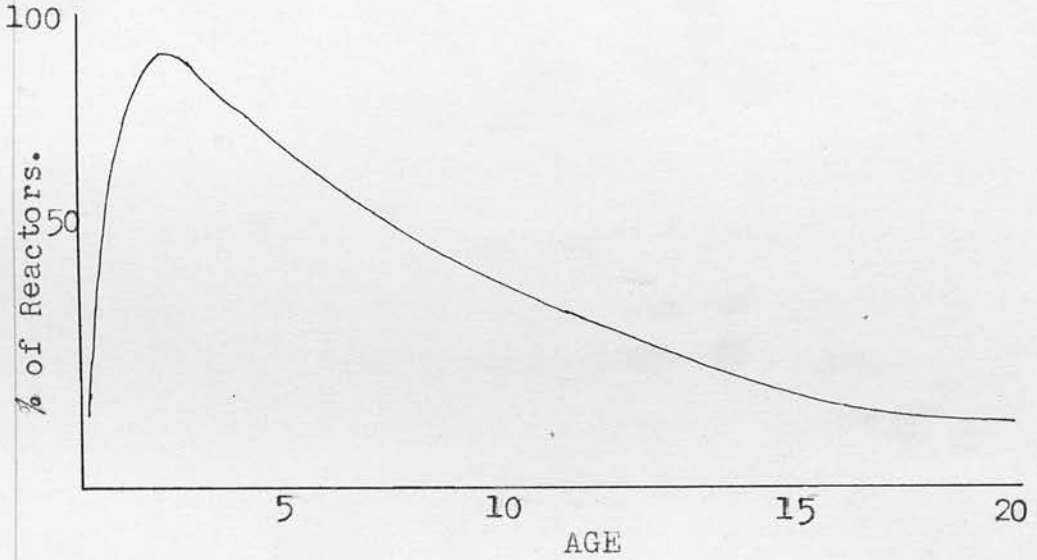
The study of the Schick and Dick reactions has thrown much light on this problem. The Schick and Dick reactions reveal the presence or absence of circulating antitoxin and it is convenient to separate the antitoxins from the antibacterial antibodies already considered. An antitoxin not only interacts with its corresponding toxin but neutralises its effect and although the antibacterial antibodies interact with the bacteria they do not neutralise any toxic action. The antibacterial antibodies act by preven-:ting a bacterium from gaining a foothold in the tissues, i.e. they prevent infection. In the case of the antitoxins there is a close relationship between the amount of antitoxin in the blood and the degree

of immunity of the individual. Although it is known that there are a large number of antibacterial anti-:bodies in any serum, the exact relationship of this to immunity is not known. It is not known if the presence of a small amount of an agglutinin, etc. necessarily means a state of increased resistance compared with those individuals who have no such antibody. With the particular case of the antitoxins this point has been settled because the presence of a circulating antitoxin means resistance to the parti-:cular toxin. This does not necessarily mean a state of resistance to infection by the bacterium producing the exotoxin because the bacterium, e.g. the haemo-:lytic streptococcus, may have other toxic products and be able to invade even although its exotoxin is being neutralised. The Dick test, therefore, does not indicate directly a degree of resistance to infection by the haemolytic streptococcus. In the case of diphtheria and the Schick test, however,, we have to deal with an organism whose whole pathogenic action is produced by its exotoxin and the presence of a neutralising antitoxin is directly responsible for a state of immunity not merely to the artificial injection of the toxin, but also to clinical disease.

The/

The test can be used, therefore, to determine a state of immunity to a disease and its importance is exhibited in many ways. In the first place it is a very reliable test, its reliability having been shown by making an actual measurement of the amount of antitoxin in the blood at the same time as doing the Schick test. It has been found that the Schick negative reaction is determined by a certain critical amount of antitoxin in the blood. A Schick positive individual has less than 1/100th unit of antitoxin per c.c. of blood and it is very rare to find diphtheria occurring in a known Schick negative individual and there is no evidence that the test has been made too exacting as all Schick positive individuals must be considered as liable to an attack of diphtheria. Secondly it is an exception to the general rule that it is not possible to measure the resistance of one individual to a disease, a point which has been already discussed. Since the disease is a pure toxaemia and the circulating antitoxin can be measured because of its neutralising properties, the degree of resistance of one individual can be accurately gauged. The intradermal Schick test is a simplification of this to determine whether this amount is above or below a certain critical level. Thirdly the test is easily done /-

done and finally although it is a test which determines the state of resistance of an individual it is the test which has supplied most information on herd immunity or immunity of communities and races as opposed to the individual. This would appear to be something of a paradox. It is possible to find the number of susceptibles (Schick positive) at various ages in the total population and in this country the distribution is as follows.



The low incidence of reactors in the first few months of life is due to a congenital passive immunity acquired in utero from the mother and from the colestrom in the early days of life. This passive immunity declines and the maximum incidence of reactors occurs in the age group $1\frac{1}{2}$ - 4 years and this coincides with the ages of maximum incidence of the actual /-

actual disease. From this point onwards the number of reactors decreases until at age 20 some 10% are still Schick positive. The decrease in the number of reactors from age 3 onwards is due to an active immunity and this is not dependant upon the fact that the non-reactors have had an attack of clinically recognisable diphtheria. If this is so, the fact that resistance increases with age can be due to one of two possible processes. Either it is specific being dependant upon subclinical attacks of the infection or it is due to a physiological process i.e. some general property of growth. Superficially, it would appear an easy matter to settle this point, but the evidence is conflicting. Smits (1926) examined the Schick reaction of the natives of Java and this work was paralleled by Heinbecker and Irvine-Jones (1928) among the Eskimos and by Kleine and Kroo (1930) among the East African natives. In all of these countries, diphtheria is rare yet all these workers found few positive reactors among the adults. The low incidence of diphtheria in Java, however, has been challenged by Kirschner (1929).

It is interesting to compare these results with those obtained on examining different sections of the same race. Zingher (1923) examined the reaction

to /-

to the Schick test in American school children and found markedly fewer reactors amongst the less fortunate social classes than among the more fortunate. It is difficult to see how such differences could arise in sections of the same race if the development of diphtheria antitoxin is due to a physiological process, independant of specific contact with the diphtheria bacillus. Zingher examined children up to age 15 and in section X of this work are shown the results of Schick and Dick tests on young adults of different social classes. In this connection it is admitted that specific contact with the diphtheria bacillus can cause a stimulation of immunity (Dudley 1923) and the point at issue is, can this process be accepted as the only one at work? In section 7 evidence was obtained to show how active this specific immunisation may be and that there is no need to postulate the action of a physiological process in the absence of specific contact.

In the case of the antitoxins (for the same principles hold true for the Dick Test) the weight of evidence favours the view that these antibodies result from specific stimulus by the bacterium concerned. It is not justifiable, however, to argue from the particular /-

particular case of the antitoxins to a general rule as regards the natural antibacterial antibodies. There are several differences. In the first place, it is known that there is a direct association between the presence of antitoxin in the blood and a state of immunity and it has yet to be shown that the presence of the natural agglutinins, bactericidins, etc. is associated with a relatively immune state. In the second place, as already stated, there exist antibodies against non bacterial antigens (haemolysins etc.) and these can not be the result of specific antigenic stimulation, and some, - the isohaemagglutinins - are inherited according to Mendelian law.

In the present state of knowledge it would be safe to say that as far as the antitoxins are concerned, the weight of evidence favours the view that they appear as the result of specific antigenic stimulation and that the natural antibacterial antibodies apparently develop in the absence of such stimulus. The latter show resemblances to the antibodies to non-bacterial substances.

Non-Specific Alteration of Antibodies.

The injection of certain substances, organic and inorganic has been said to have an effect on the production of antibodies. The investigation into this phenomenon has followed two different lines. Firstly there are those observations made to record the output of antibodies following the injection of one or other of these substances during the course of immunisation with an antigen. It was claimed that these substances caused an increased production of antibody as compared with controls which received injections of antigen alone. Secondly there is evidence that these same substances may produce an alteration of the natural antibodies in the absence of any injection of antigen.

The removal of blood during the latent period of immunisation may lead to an increased antibody output. (Friedberger and Dorner 1905: Hektoen and Carlson 1910). Injections of metallic salts, e.g. manganese chloride have been said to produce a similar effect, (Walburn 1921, 1925, 1926) but O'Brien (1924) failed to confirm this. Alum has been used by Glenny, Pope, Waddington and Wallace (1926) and/

and by Glenny and Waddington (1928) to cause an increased production of diphtheria antitoxin when immunising with diphtheria toxoid. Turpentine has been used by Glenny and Waddington (loc. cit) to produce the same result. The addition of tapioca to diphtheria or tetanus toxoid has been used by Ramon (1926) and Schmidt (1928) to produce a larger amount of antitoxin. Steabben (1925, 1926) found an increased amount of antibody if certain colloids were injected along with the antigen. Piloccipine has been used by Salomonsen and Madsen (1898) for the production of diphtheria antitoxin and salvarsan has been claimed by Walker (1920) to stimulate the production of agglutinins but this has been challenged by McIntosh and Kingsbury (1924).

All of the above observations were made on animals which were being injected with an antigen simultaneously with the chemical under study. Attempts have been made, however, to increase the natural antibodies by such non-specific means, i.e. in the absence of any injection of specific antigen.

Mackie (1925) found that injections of many metallic salts in rabbits produced an increase of the natural haemolysin for sheep's red cells. Bedson (1914) /-

(1914) produced an increase of opsonic power by injecting nuclein and this was independant of the leucocytosis produced. An increase in bactericidal activity of whole blood has been described by Fleming (1926, 1928) following injections of metallic salts.

Sections 2 and 4 of the work described here show the effect of certain sera and other organic substances on natural bactericidal activity.

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THE INFLUENCE OF THE INJECTION OF FOREIGN SERUM AND OTHER ANTIGENS ON NORMAL BACTERICIDAL ACTIVITY OF SERUM.

INTRODUCTION.

Although the natural bactericidal power of animal sera *in vitro* is not a true reflection of the *in vivo* defences, the study of this reaction has revealed much interesting information on some aspects of immunity. In recent years the natural bactericidal mechanisms of serum have been given extensive study by Pettersson (1926, 1928) and by Mackie and Finkelstein (1930, 1931). Pettersson recognised two types of mechanism designated α and β lysins. The α lysin, he defined as the mechanism involving the combined lytic activity of serum complement and an antibody and therefore labile at 55°C in virtue of the thermolability of complement. The β lysin on the other hand is heat-stable being able to withstand 60°C for half an hour. According to Pettersson the β lysin is of complex structure and can be divided into an "activable" fraction and an "activating" fraction, but the latter is not serum complement as it is stable at 60°C. The β lysin is not influenced by immunisation and this affords another difference from the α lysin.

The question has arisen as to the specificity of the natural bactericidal antibodies as compared with the antibodies which result from specific immunisation. It /

It is argued that the only essential difference is quantitative. There is not, however, complete agreement on this question. Browning (1927) suggested that prototypes of immune bodies might exist normally in blood plasma. Gordon and Wormall (1928) found that the bactericidal activity of guinea-pig serum on B.dysenteriae Flexner-Y depends on the dual action of a heat-labile factor (complement) and a heat-stable factor which could be absorbed out by the organism. Gordon and Carter (1932), however, reported their inability to demonstrate specific absorption effects and considered that the heat-stable factor was not specific. On the other hand Mackie and Finkelstein (1930) concluded that there were present in normal serum, multiple specific antibody-like principles, and this view has been supported by other workers who have clearly demonstrated the specificity of natural agglutinins (Gibson, 1930; Lovell, 1932, 1934).

It is noteworthy that in general, Gram-positive bacteria are sensitive to the β lysin and Gram-negative bacteria to the α lysin. β lytic activity is always of a low order and bactericidal activity against Gram-positive bacteria is best demonstrated by the action of whole blood. Methods for estimating the bactericidal /

bactericidal power of whole blood have been devised by Wright et al. (1923) but in this study the activity of serum only has been investigated.

The influence of non-specific factors on antibody production has been studied by Walbum (1921) and Madsen (1923) who injected organic and inorganic substances along with an antigen and claimed to have produced a greater output of antibodies to the antigen as compared with controls. Steabbin (1925) found that the simultaneous injection of an antigen and certain colloids yielded increased antibody production. These findings have not been confirmed by subsequent workers.

In the absence of any specific immunisation, the above substances injected alone can at times influence natural immunity mechanisms. Fleming (1928) obtained an increased bactericidal power of whole blood following injections of nuclein. Mackie (1925) found definite changes in titre of the natural haemolytic antibody of the rabbit for sheep's red blood corpuscles, following injections of certain metallic salts such as those used by Walbum. Kalinin, Schereschewskaji and Selikowa (1935) injected normal serum along with another antigen (B. typhosus) and obtained increased output of antibodies against the organism as compared with controls.

In /

In the following work pronounced changes in the α lytic activity of rabbit serum are recorded, following the injection of foreign sera and egg albumin.

METHODS.

Bactericidal Tests.

The technique adopted was that described by Mackie and Finkelstein (1931) and is briefly described as follows.

A constant volume, 0.25 c.c., of fresh serum was pipetted into each of six sterile tubes. The serum was used either undiluted or in low dilution 1:1 with normal saline. In another six tubes decimal dilutions of a suspension of the organism under test were prepared in saline and added in 0.1 c.c. amounts to the serum. In making the serial dilutions of the organism a fresh pipette was used for each dilution. This procedure gave a series of six tubes containing a constant amount of serum but varying amounts of bacteria. The mixtures were plated on agar immediately by a single stroke of a standard loop and again after four hours' incubation at 37°C. The density of bacterial emulsion from which /-

which the serial dilutions were made, was chosen by trial to be that standard which on the immediate plating described above, gave an end-point within the range. Adjustment was made so that the sixth dilution on plating would give no growth. The bactericidal activity was estimated by comparing the end-points of growth in the immediate plating and the plating after four hours' incubation. For example, a series which showed growth in all but the sixth tube on immediate plating and in all but the fourth, fifth and sixth tubes after incubation, was taken to have a bactericidal titre of 2, i.e. 6 - 4, the difference in the two end-points.

The following illustrates the results of a test in which the bactericidal power was recorded as 2.

Tubes	1	2	3	4	5	6
Immediate	++++	+++	++	+	f.c.	-
After 4 hours' incubation	++	+	f.c.	-	-	-

The amount of growth is indicated by "+" marks; f.c. = a few colonies only.

Complement Titration.

A 3 per cent suspension of washed sheep's red cells was sensitised with 7 M.H.D. of haemolytic immune body from a rabbit. This immune body is referred to as "R. v S. I.B.". In some experiments the sheep's cells /

cells were sensitised with an immune body from a horse. This is referred to as "H. v S. I.B."

The following range of quantities of serum was tested for complement with 0.5 c.c. of a 3 per cent suspension of cells: 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.075, 0.1, 0.2, c.c.

The mixtures were incubated at 37°C for 1½ hours and the amount of lysis noted. The haemolytic activity was expressed as the reciprocal of the smallest fraction of a c.c. of serum which produced complete lysis of the cells.

Precipitin Tests.

Precipitins for horse serum proteins in the rabbit were detected by a "ring" test in which varying dilutions of horse serum were layered on a constant amount of undiluted rabbit serum. The highest dilution of horse serum producing a precipitate was recorded but no claim is made to regard this "titre" as a strict estimate of the antibody content of the rabbit serum.

RESULTS.

Experiments with Horse and Ox Serum.

Following subcutaneous injections in rabbits of horse serum there was a fall in the bactericidal activity of the rabbit's serum towards B. typhosus and Vibrio cholerae. Usually this was a decline to zero by the technique used. This fall of α lytic activity was unaccompanied by any change in the β lysin as tested with Staphylococcus aureus. At the same time there occurred a fall, also complete in most cases, in the haemolytic activity of the complement when sheep's red cells sensitised with an H.v S. I.B. were used, but no change in haemolytic complement was found with an R.v S. I.B.

A typical result is shown in table I which details the results obtained in a rabbit which was injected subcutaneously on two occasions with 10 c.c. of horse serum. The second injection was given six days after the first.

TABLE I.

Experiment 638.

Day of Experiment.	Horse Serum Injected.	Bactericidal Activity. B. typhosus	Bactericidal Activity. V. cholerae	Complement R.vS. I.B.	Complement H.vS. I.B.	Precipitins for Horse Serum.
1	10 c.c.	3	2	6	13	0
7	10 c.c.					
8		0	0	20	0	512
15		2	4	13	0	4096
38		3	3	13	16	256

On /

On the 8th day, the day after the second subcutaneous injection of horse serum, the bactericidal activity against B.typhosus and V.cholerae had completely disappeared and the serum had lost its ability to act as a haemolytic complement with H. v S. I.B. There was no loss of haemolytic activity with R.v S. I.B., and although a rise in titre from 6 to 20 was obtained this was not regarded as significant since differences as large as this are not infrequently obtained on testing the haemolytic action of the same complement against different samples of red blood corpuscles sensitised with the same immune body. Precipitins at this stage had reached a titre of 512.

A second retest was made a week later by which time the bactericidal activity of the serum towards B.typhosus and V.cholerae had returned to the former levels but there was no recovery in the haemolytic activity with H.v S. I.B. Precipitins had increased in titre to 4096.

Three weeks later with the exception that precipitins were still present to a titre of 256, the rabbit's serum had returned to its original state.

The data shown in table I are typical of results that were readily obtained in a large number of animals but since table I shows no titration of β lysin, table II /

II is included to show the results of three experiments where both α lytic and β lytic activity were titrated with B. typhosus and Staphylococcus aureus respectively.

TABLE II.

Day of Experiment.	Horse Serum Injected.	Bactericidal activity. B. typhosus	Bactericidal activity. S. aureus	Complement. H. v S. I.B.
		Experiment 858.		
1	20 c.c.	2	1	30
3		3	1	13
6		1	1	5
10		0	1	<3
17		3	1	16
33		3	1	20
		Experiment 860.		
1	20 c.c.	2	1	20
3		2	1	10
6		1	1	13
8	5 c.c.			
10		0	1	<3
17		2	1	0
26		3	1	0
		Experiment 842.		
1	5 c.c.	3	1	10
3		3	1	13
6		1	1	5
8	5 c.c.			
10		0	1	0
18		0	1	0

Exactly similar results were obtained following the injection of ox serum and a typical result is shown in Table III.

TABLE /

TABLE III.

Experiment 865.

Day of Experiment.	Ox Serum Injected.	Bactericidal Activity. B. typhosus	S. aureus.	Complement. H. v S. I.B.
1	20 c.c.	3	2	13
3		2	2	13
7		1	1	10
11		0	2	< 3 zone
18		0	2	5
26		3	2	20

The fall in bactericidal activity towards B. typhosus was as marked with ox serum as with horse serum but generally the fall in haemolytic activity when using an H. v S. I.B. was not so marked. The latter, however, was quite definite and was not due to differences in the various specimens of sheep cells which were used. In experiment 865 (Table III) on the eleventh day there was a marked depression of haemolytic activity as shown not only by the titre <3 but also by the presence of a zone. In this zonal phenomenon the greatest amount of lysis was produced by volumes of serum in the middle of the range tested. That is, there was an increase in the amount of lysis up to a certain point in the range but beyond this there was a decrease. In none of the tubes, however, was complete lysis produced. The presence of this zone indicated some interference with lysis.

An experiment was designed to investigate the period of onset and duration of the fall in lytic activity. A number of rabbits were given a single subcutaneous /

subcutaneous injection of horse or ox serum with results shown in table IV.

TABLE IV.

Experiment 859.						
Day of Experiment		1	4	7	11	18
Horse serum injected		20 c.c.				
Bactericidal activity	(B. typhosus	2	2	1	0	2
	(S. aureus	1	1	1	1	1
Experiment 858.						
Day of Experiment		1	3	6	10	17	33
Horse serum injected		20 c.c.					
Bactericidal activity	B. typhosus	2	3	1	0	3	3
Experiment 864.							
Day of Experiment		1	4	7	12	14	26
Horse serum injected		10 c.c.					
Bactericidal activity	B. typhosus	3	3	0	0	1	2
Experiment 866.							
Day of Experiment		1	4	7	12	19	40
Ox serum injected		10 c.c.					
Bactericidal activity	(B. typhosus	4	3	0	0	1	2
	(S. aureus	1	2	2	1	1	1
Experiment 857.							
Day of Experiment		1	2	3	5	9	17
Ox Serum injected		10 c.c.					
Bactericidal activity	(B. typhosus	3	3	3	3	1	2
	(S. aureus.	1	1	1	2	1	2
Experiment 854.							
Day of Experiment		1	2	3	5	9	17
Ox serum injected		10 c.c.					
Bactericidal activity	(B. typhosus	2	2	2	2	0	3
	(S. aureus	1	1	2	1	1	1
Experiment 865.							
Day of Experiment		1	4	7	11	18	26
Ox Serum injected		20 c.c.					
Bactericidal activity	B. typhosus	3	2	1	0	0	3

The depression occurred about the 6th to 9th day following a single subcutaneous injection of serum and lasted /

lasted for about a week on the average but frequently lasted much longer, as for example in Experiment 865, where bactericidal activity towards B.typhosus was completely absent on the 18th day.

A review of the preceding tables shows the following points which will be examined and discussed:

(1) A pronounced disturbance of bactericidal activity towards B.typhosus and V.cholerae.

(2) No associated fall of bactericidal activity towards S. aureus.

(3) A marked loss of the activity of the rabbit serum to act as haemolytic complement when tested against sheep red cells sensitised with an H.V S. I.B. This loss was usually complete following injections of horse serum, and a marked decline, though often incomplete, was found following injections of ox serum.

(4) No loss in haemolytic activity with a R.v S. I.B.

(5) Precipitins for the injected serum were invariably present when the phenomena were detectable and persisted even in high titre after these effects had disappeared.

Since the bactericidal activity of normal serum towards B.typhosus and V.cholerae.depends on the dual mechanism of a heat-stable factor and heat-labile complement /

complement it seemed possible that the fall in bactericidal activity for these organisms and the fall in haemolytic activity with a H.v S. I.B. might have been due to the same cause.

Thus, the complement concerned in α lytic activity is closely allied to, if not identical with, haemolytic complement. Presumably, the fall in activity of the α lysins must have been due to an interference with the heat-stable factor or with complement. In view of the loss of complement activity with H.v S. I.B. the latter possibility was examined first.

At a casual examination the two phenomena would appear to be closely associated because of their simultaneous appearance but at the same time there was no loss in complementing activity with R.v S. I.B. The fall in haemolytic activity in a rabbit injected with horse serum on testing with H.v S. I.B. might have been due to an <u>in vitro</u> fixation of complement. Thus, in the test for complement, rabbit serum which contained precipitins for horse serum was added to red cells sensitised with a haemolytic antiserum from a horse. Thus

1. /

1. Sheep red cells
2. Specific I.B. against sheep red cells in
3. Horse Serum

) Sensitised cells.

\+

4. Complement
5. Precipitins for horse serum

) in rabbit serum.

Fixation of complement might occur because of the interaction of the horse serum of the immune body (3) and its homologous antibody (5) present in the serum of the rabbit.

Since haemolytic complement was disturbed only when acting with H.v S. I.B. and not with an R.v S. I.B. the above was thought to be the correct interpretation.

The fall in haemolytic activity with an H.v S. I.B. also occurred following injections of ox serum but it is well established that there are cross precipitation reactions between ox and horse serum (Kolmer, 1925) and Experiment 643 illustrates this.

Experiment 643.

Day of Experiment	1	7	8	17
Ox serum injected	10 c.c.	10 c.c.		
Titre of precipitins against Horse serum			128	256

Since haemolytic activity was disturbed only in the special case where a H.v S. I.B. was used, it was regarded as a separate phenomenon to the fall in α lytic activity and will be described in detail in a subsequent section. (part 2).

Although the two phenomena are regarded as distinct, the fall in α lytic activity might still have /

have been due to an interference with complement. Dean (1931) has shown that the freshly separated serum of a rabbit which has been previously injected with horse serum may show at a certain stage when antibodies are beginning to appear, a fine precipitate due to the interaction of antibodies with horse serum which has not disappeared from the circulation. This phenomenon is demonstrable only at a certain stage following the injection of horse serum and is dependent on the co-existence in the animal of an antigen and its homologous antibody. In the particular case considered here it is possible that antibodies to the injected horse serum were appearing before the injected serum proteins had been completely eliminated from the rabbit's system. The interaction between these two may have fixed or absorbed complement with a resultant fall in α lytic activity of the serum.

The experiment quoted as table I might seem to support this explanation, the absence of α lytic activity being noted on the day following the second injection of horse serum. It might be argued that the second dose of antigen (horse serum) was interacting with antibodies produced by the injection of the first dose. On the other hand in experiment 865, table III, and experiment 864, table V (vide infra), the fall was noted on the 18th and 19th days respectively, following a /

a single injection of horse serum.

TABLE V.

Experiment 864.

Day of Experiment.	Horse serum injected.	Bactericidal activity. B.typhosus	S.aureus	Complement H.v S. I.B.
1	10 c.c.	3	2	13
4		3	2	10
7		0	1	5
12		0	2	0
19		1	1	0
26		2	1	16
33		2	1	13

Against the acceptance of the above explanation is the fact that haemolytic complement as estimated with an R.v S. I.B. maintained its activity. It might be supposed that an antigen:antibody reaction should have fixed the haemolytic complement as well as the bactericidal complement. Thus the antigen:antibody reaction which gives rise to a visible precipitate, as is assumed to be the case here, is particularly active in absorbing haemolytic complement.

On the other hand there may have been some selective absorption of bactericidal complement. Neufeld and Haendel (1908) using an anticholera serum and V.cholerae, obtained a selective absorption of bactericidal complement at 0°C. Muir and Browning (1909) treated normal serum with graded amounts of bacteria and found that bactericidal complement was absorbed before /

before haemolytic complement. Larger amounts of bacterial emulsion absorbed both bactericidal and haemolytic complement.

In all of the above experiments to separate bactericidal and haemolytic complement, quantitative estimations were made and any procedure which interfered with one was able to interfere with the other if the same measure was carried to a further degree. Furthermore, Mackie and Finkelstein (1931) and Finkelstein (1933) have shown that cultures of bacteria readily yield a substance which is inhibitory to bactericidal action. These workers found in absorption tests with bacterial cultures that carefully washed growths had to be used because of this inhibitory substance. Some of the previous work on the separation of bactericidal and haemolytic complement would require to be reviewed in view of these findings.

If, however, an <u>in vivo</u> fixation of bactericidal complement occurs then the phenomenon should be present for as long as antigen remains in the circulation. Ionesco-Mihaiesti (1911) detected horse serum in the circulation for 10 days following the last of a series of immunising injections. Opie (1923) found that horse serum was detectable in the blood for 7 to 9 days following /

following an injection, but in certain cases where precipitins appeared slowly and to a low titre, the antigen might persist for as long as 19 days.

An antigen is removed more quickly from the circulation of an immune animal than from normal animals (Culbertson, 1935). Accordingly, the phenomenon might have been expected to be more transient following the second or third injections of serum. Table VI shows the results of experiments where more than one injection of serum was given

TABLE VI.

Experiment 847.

Day of Experiment	Horse serum injected.	Bactericidal activity. B. typhosus	S. aureus	B. diphtheriae
1	5 c.c.	3	1	1
7	5 c.c.	0	2	1
10	5 c.c.	0	2	1
14		0	2	1
25		0	2	1
39		3	2	1

Experiment 862.

Day of Experiment	Horse serum injected.	Bactericidal activity B. typhosus
1	5 c.c.	3
3		3
6		1
8	5 c.c.	
10		0
18		0

Experiment /

Experiment 634.

Day of Experiment.	Horse Serum Injected.	Bactericidal activity. B. typhosus	V. cholerae	Complement R.v	S.I.B.	Precipitins
1	10 c.c.	3	2	13		0
6		1	3	10		0
7	10 c.c.					
13		0	4	25		1024
27		3	2	20		16000
36		2	3	25		4096

Experiment 635.

Day of Experiment.	Horse Serum Injected.	Bactericidal activity. B. typhosus	V. cholerae	Complement. R.v	S.I.B.	Precipitins.
1	10 c.c.	3	2	10		0
6		4	3	50		64
7	10 c.c.					
13		0	3	16		128
17		2	4	10		256
28		3	4	20		1024
36		2	4	16		512

In experiment 847, no bactericidal power for B. typhosus was present on the 13th day following the 3rd subcutaneous injection of horse serum. Experiments 862, 634, 635, showed no bactericidal activity towards B. typhosus for a week to ten days following the second injection of horse serum. Ionesco-Mihaiesti showed that on occasions an antigen could be detected in the circulation up to the 19th day after the final of a series of injections.

It might also be expected that, assuming the above explanation to be the true one, the phenomenon would either not appear or would last for a short time only, following intravenous injections of serum since the injected /

injected serum would disappear from the circulation more rapidly than after subcutaneous injection. Table VII shows the results of two experiments where the horse serum was injected intravenously.

TABLE VII.

Day of Experiment.	Horse serum Injected I.V.	Bactericidal activity. B. typhosus	V. cholerae	Precipitins.
		Experiment 632.		
1	5 c.c.	3	2	0
5		2	3	0
6	5 c.c.			
9		0	1	512
15		3	4	1024
		Experiment 633.		
1	5 c.c.	4	3	0
6	5 c.c.			
7	5 c.c.	0	1	256
12		2	3	2048
21		0	3	8192
35		4	3	256

The effect was definitely obtained and in the case of experiment 632, was present three days after the second intravenous injection of serum. The same effect was demonstrated in 633 on the day following the second injection. Four days later recovery had occurred but after a further nine days, i.e. on the fifteenth day after the second intravenous injection of serum, there was a renewed effect. This second depression of activity might have been due to antibodies produced /

produced by the second injection of serum, but in both of the animals the result was more definite than would have been expected if the reasons advanced are correct.

Sufficient results have been given to show that although the bactericidal activity against S. aureus is of a low order, the phenomenon did not extend to include it. This bactericidal activity depends on the β lysin and is independent of the action of complement.

The study of precipitins gave very little information on the phenomenon except that precipitins were always present to some degree when the fall in α lysin appeared.

Experiments with Other Sera.

Reference has already been made to the existence of cross reactions between ox and horse sera. In order to examine whether or not the depression of α lytic activity is a general change following the injection of serum, small groups of rabbits (4 or 5) were injected with sheep, guinea-pig, fowl and duck sera. Cross reactions between sheep serum and anti-horse serum have been described but the author never found any in his experiments. Guinea-pig, fowl and duck sera /

sera show no cross reactions with horse serum.

The results with sheep serum are shown in Table VIII.

TABLE VIII.

Day of Experiment.	Sheep serum	Bactericidal Titres. B. typhosus	B. aertrycke	S. aureus	Complement H.v S.I.B.	Precipitins v. Horse Serum.
A.78						
1	20	3	3	2	5	0
9		0	2	2	5	0
309.						
1	20	3	2	2	13	0
11		1	1		13	0
15		0	2	2	10	0
313.						
1	20	3	2	2	10	0
8		4	4	2	16	0
13		4	3	2	16	0
332.						
1	5	3	2	2	17	0
6	5					
8		0	0	2	5	0
370.						
1	10	4	3	1	5	0
7	10					
8		0	2	2	13	0
11		1	2		13	0
15		2	4	3	13	0

Although the results were not so constant the same depression of α lytic activity did appear. A fall in bactericidal activity against B. aertrycke was also demonstrated, although not so uniformly or distinctly. There was no fall in β lytic activity as far as the bactericidal /

bactericidal activity against S. aureus revealed and no fall in haemolytic complement when tested against sheep red cells sensitised with H.v S. I.B. No precipitins for horse serum were demonstrated.

Results with Duck Serum.

Four rabbits received two injections of 10 c.c. of duck serum subcutaneously, with an interval of a week between them. Three of the rabbits revealed no depression of α lysin but the fourth showed a complete absence of α lytic activity against B.typhosus on the 3rd day after the second injection.

Results with Fowl and Guinea-pig Serum.

No depression of α lytic activity was found following injections of fowl and guinea-pig serum. Four rabbits were tested with each serum.

Experiments with Egg Albumin.

In all of the previous experiments the antigen injected consisted of a foreign serum. The depression was most marked with horse and ox serum, less marked with sheep and duck serum and was not found following injections of guinea-pig or fowl serum but of course only small groups of rabbits were used. Duck, fowl and guinea-pig sera were included in the investigation to determine whether or not the depression could be produced /

produced by sera which showed no cross serological reactions with horse serum. As stated above, it was found that duck serum could produce the phenomenon.

It was obviously of importance to extend this to discover if the phenomenon was a general one following the injection of any antigen. For this purpose 4 rabbits were injected subcutaneously with egg albumin and the results are shown below in table IX.

TABLE IX.

	Day of Experiment.	Volume of 10per cent Egg Albumin Injected.	Bactericidal activity towards B.typhosus.
Expt.93	1	10 c.c.	4
	7	10 c.c.	
	10		0
Expt.94	1	10 c.c.	4
	7	10 c.c.	
	10		0
Expt.95	1	10 c.c.	4
	7	10 c.c.	
	10		1
Expt.97	1	10 c.c.	4
	10		4

The depression of α lytic activity therefore was again definitely produced and would appear to be a general change following the parenteral injection of a soluble protein.

Analysis of the Depression.

In the previous experiments no evidence was obtained

to /

to indicate that a disturbance of bactericidal complement was responsible for the depression of α lytic activity because the haemolytic complement remained active.

Accordingly other possibilities were considered, viz. an interference with the heat-stable antibody-like factor or an inhibitory process preventing the action of thermolabile complement on the sensitised bacterium.

An experimental rabbit X was injected with horse serum producing a depression of α lytic activity for B. typhosus from 3 to 0. A fresh rabbit Y had a bactericidal activity of 4 and some of the serum of this rabbit was heated to 55°C for half an hour to inactivate it. This inactivated serum was able to restore bactericidal activity to the serum of X. Furthermore, the addition of serum X did not reduce the bactericidal activity of serum Y when added either fresh or after heating to 55°C for half an hour.

Thus, on the third day following the second injection of horse serum the following results were obtained, on testing against B. typhosus.

TABLE /

TABLE X.

	Bactericidal Activity.
1. Y (fresh)	4
2. Y (55°C)	0
3. X (fresh)	0
4. X (55°C)	0
5. X (fresh) + Y (55°C)	4
6. X (fresh) + Y (fresh)	4
7. X (55°C) + Y (fresh)	4

In tests 1-4 the sera were diluted 1:1 with sterile saline, and in tests 5-7 the sera were mixed in equal volumes. In any test, therefore, any given serum was present in 1:1 dilution whether with saline or with another serum. The only uncontrolled factor was the varying viability of B.typhosus in pure serum or serum diluted with an equal volume of saline. This latter factor from other experiments performed was known not to play any interfering part as B.typhosus during the time of the experiment is as viable in pure serum heated to 55°C for half an hour as it is in saline.

These results were readily obtained if the fresh serum Y had a strong α lytic activity as in the above case but sometimes did not occur if the α lytic power was of a lower order, e.g. 2. Nevertheless it is obvious that the phenomenon is due to some interference with a heat-stable component or components. In α lytic activity /

activity there is a dual mechanism of a heat-stable antibody-like factor interacting with thermolabile complement but it must be remembered that complement has not a simple structure and is itself composed of thermostable and thermolabile fractions. The thermolability of complement as a whole is due to destruction by heat of certain fractions only.

Referring to the above experiments the following conclusions are justifiable. From test No. 6 it would appear that the phenomenon is not due to the presence of some substance which interferes with bactericidal activity in vitro or there would have been a greater or less reduction in killing power of serum Y. It would appear that the lack of bactericidal power in the test animal is due to the absence of a heat-stable substance which can be supplied by adding heated serum from a fresh rabbit (test 5). The restoration of $\propto$ lytic activity by inactivated normal serum could only have been due to the addition of either (a) the heat-stable antibody-like factor, (b) the heat-stable components of complement. The various fractions of complement have been separated on the basis of haemolytic tests and any disturbance of these components should have produced a pronounced effect on the haemolytic complement /

complement. This has been shown not to occur (with an R. v S. I.B.) and the restoration of bactericidal activity by heated serum must have been due to the addition of the heat-stable antibody-like principle concerned in ∝ bacteriolysis.

In order to test this further, experiments were planned on the following lines. A rabbit (A) was injected with horse serum so as to depress the bactericidal activity of its serum against B.typhosus from 3 or 4 to 0. As before, serum from a normal rabbit (B) was inactivated by heating to 55°C for half an hour (designated "B 55°C"). Fresh serum from rabbit B was absorbed with B.typhosus to remove the heat-stable antibody-like factor. For this absorption the growth from 6 agar plates was killed by heating to 65°C for 1 hour. The emulsion was washed twice in 5 c.c. of saline. After centrifuging, the deposited growth was cooled to 0°C before being resuspended in 3 c.c. of serum B, which had been previously cooled to 0°C. Absorption was allowed to continue for 2 hours at 0°C before centrifuging and the supernatant serum was designated "B absorbed". This procedure removes the antibody principle but does not absorb complement /

complement if the correct amounts are used. Four experiments all with different pairs of animals are quoted thus.

TABLE XI.

Experiment	1	2	3	4
B Fresh	2	3	3	2
B "55°C"	0	0	0	0
B "absorbed"	0	1	0	0
B "(55°C)" + "B absorbed"	2	2	1	2
A (serum of rabbit previously injected with horse serum)	0	0	0	0
A + "B absorbed"	0	1	0	0
A + "B 55°C"	1	3	1	2

The absorbed serum presumably containing only complement was as a rule quite unable to restore the bactericidal activity to the serum of the test rabbit. Occasionally a very slight partial restoration was obtained (experiment No.2). If the phenomenon is due to an interference with the heat-stable components of complement, the addition of absorbed serum should have restored the bactericidal effect as regularly and successfully as did the heated serum. This it failed to do.

The successful separation of the fresh serum B into the absorbed fraction (complement only) and heat inactivated /

inactivated fraction (antibody-like factor + heat-stable components of complement) was always demonstrated by mixing the two to show that although each individually had no activity the two combined had a bactericidal power. Successful separation is very difficult and reconstitution was not always complete.

These results, however, taken as a whole can only be interpreted by inducing that the depression of α lytic activity occurring after injections of horse serum was due to an interference with the heat-stable antibody-like component of normal serum. This interference was apparently a depression of the factor rather than due to the production of some factor inhibiting its action.

DISCUSSION.

In the rabbit a marked interference with the normal α lytic activity of serum is produced by the parenteral injection of certain foreign sera and egg albumin. This depression regularly followed injections of horse and ox serum but occurred also after injections of sheep and duck serum. The phenomenon was not demonstrated with fowl or guinea-pig serum but only a small number of rabbits were tested with these two sera. From the experiments, however, it would appear that some sera regularly produce the depression, others less regularly, and others either seldom or not at all. The effect was also marked following injections of egg albumin.

Experiments were carried out which indicated that the depression was due to some change in the heat-stable antibody-like component of the serum. Other possibilities, however, were considered. An interference with complement was considered first but this was not found to be the case because haemolytic complement (as estimated with sheep cells sensitised with a R.v S.I.B.) remained active and because the α lytic activity was restored by the addition of heated serum from a fresh rabbit. This left only one somewhat theoretical objection /

objection to the acceptance of the view that the influence was on the antibody-like component of normal serum, viz. that the effect might have been due to an interference with the heat-stable components of bactericidal complement, assuming this to be different from haemolytic complement. This possibility was also excluded as absorbed fresh serum was unable to restore activity to the serum showing the depression. Thus, the effect might have been due to a temporarily diminished production of the antibody or to some inhibitory effect which in vitro was able to prevent the proper interaction between bacteria, antibody and complement. The addition of the serum under test, however, produced no inhibition of a fresh normal serum. Admittedly in this the greater quantities of the reagents might interfere with such inhibitory influence though some partial inhibition might still be expected to occur. This was never found to be so as the test serum when mixed with the heated or unheated serum from a control rabbit gave the full activity of the control rabbit serum. The conclusion was reached, therefore, that the injected proteins produced a temporarily diminished production of the heat-stable antibody-like factor of the α lysin.

CONCLUSIONS.

1. Parenteral injection of foreign sera and egg albumin produces in the rabbit a complete depression of the α lytic activity of the animal's serum.

2. The effect was easily demonstrated with horse and ox sera, less easily with sheep and duck sera but not demonstrated with fowl and guinea-pig sera.

3. The depression was easily produced with egg albumin.

4. The effect appears about the seventh day following a single injection of serum and lasts on the average for a week.

5. There was no depression of haemolytic complement.

6. The depression of α lytic activity was considered to be due to some depression of the antibody-like principle which is responsible for normal α lytic activity.

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APPENDIX TO SECTION I.

The results of experiments shown in Section I were obtained in the course of a wider investigation into the non specific alteration of natural antibodies. Many substances other than antigens were used and this appendix is entered to record the negative results obtained.

Small groups of rabbits (4 to 6) were tested with each of the following measures:-

Injections of Manganous chloride, Collosal manganese, Sodium nucleinate, Aleuronat, Pituitrin, Thyroxin, Adrenaline, Neoarsphenamine, Potassium Iodide, but no alterations were produced in any of the following natural antibody reactions:-

(1) Bactericidal activity towards B. Typhosus, V. Cholerae, Staph. Aureus, C. Diphtheriae.

(2) Complement.

(3) Natural haemolysin against sheep's red cells.

(4) Wassermann reaction.

(5) Sachs Georgi reaction.

THE EFFECT OF INJECTIONS OF NORMAL SERUM ON THE ACTIVITY OF HAEMOLYTIC COMPLEMENT, WITH PARTICULAR REFERENCE TO THE ANTI-IMMUNE-BODY EFFECT.

INTRODUCTION.

In the previous section the depressant effect of the parenteral injection of normal sera on the natural bactericidal activity of rabbit's serum was described.

About a week after the subcutaneous injection of horse serum in a rabbit, the rabbit's serum loses its α bacteriolytic activity and at the same time loses its ability to lyse sheep's red cells sensitised by the addition of a haemolytic immune body prepared in a horse. The α lytic activity was tested by examining the bactericidal power of rabbit's serum towards B. typhosus, V. cholerae and B. aertrycke. The bactericidal activity of normal serum can be divided into two types designated α lysin and β lysin (Pettersson, 1926) the former requiring complement in its action while the latter is independent of complement. The β lysin did not appear to be depressed by the injection of a foreign serum. The loss of α lytic activity and the apparent fall in activity of complement did not appear to be related because the rabbit's serum did not lose its ability to lyse sheep's cells sensitised by the addition of an immune body prepared in a rabbit. The fall /

fall in lytic activity was considered to be due to a depression of the heat stable antibody-like factor of normal serum which is responsible for ∝ lytic activity along with complement. The apparent loss of haemolytic complement when tested against sheep cells sensitised with an immune body from a horse was considered to be due to an in vitro antigen:antibody reaction.

The question was raised whether the fall in bactericidal activity might have been due to an"anti-immune-body" effect. There is as yet no unequivocal evidence that an antibody to an immune body can be produced but Bordet (1904) was the first to claim that he could demonstrate such an effect. He examined the lysis of a haemolytic system consisting of ox red cells sensitised with an immune body prepared in a rabbit, and the "anti-immune-body" was the heated serum of a guinea-pig previously injected with rabbit serum. A suspension of ox red cells was sensitised by the addition of immune body and after a period of contact for fixation, washed free of any excess immune-body and non-specific proteins of the antibody containing serum. The suspension of cells was reconstituted and the addition of"anti-immune-body" prevented the lysis of these cells by a fresh complement. Control experiments /

experiments with heated normal guinea-pig serum resulted in lysis. Bordet stated further, that the later addition to the test experiment of more haemolytic immune-body resulted in lysis, indicating that complement was present in the original mixture but prevented from uniting with and lysing the sensitised cells. Muir and Browning (1909) also came to the latter conclusion.

The experiments described in this paper were designed to study Bordet's "anti-immune-body" effect and its relationship to the phenomenon already stated, viz. the inability of rabbit's serum to lyse red cells sensitised with a haemolytic immune-body prepared in a horse if the rabbit has been previously injected with horse serum.

Nomenclature.

Haemolytic immune-body prepared by injecting sheep's red cells into a horse is referred to as "horse versus sheep cells immune-body" or abbreviated "H.v S. I.B." Similarly a haemolytic immune-body for sheep cells prepared in a rabbit is "R. v.S. I.B." and one for ox cells prepared in a rabbit is "R.v.O. I.B."

A suspension of red cells sensitised with haemolytic immune body is referred to as a"haemolytic system".

METHODS.

1. Complement estimation.

Complement was titrated by adding varying amounts of rabbit's serum to 0.5 c.c. of a 3 per cent suspension of thrice washed sheep's red cells sensitised by the addition of an excess of H.v.S. I.B. Other titrations were made using R.v.S. I.B. The immune-body and red cells were incubated at 37°C for half an hour at least to allow of fixation before adding the complement. The range of complement tested was 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.075, 0.1, 0.15 and 0.2, giving reciprocals of 100, 50, 33, 25, 20, 16, 13, 10, 6 and 5. The mixtures were incubated for 1½ hours at 37°C and the activity or "titre" was taken as the reciprocal of the smallest amount of rabbit serum producing complete lysis of the test amount of cells.

2. Precipitins.

The presence of precipitins for horse serum was detected by means of a "ring" test. Varying dilutions of horse serum (antigen) were layered on top of undiluted rabbit serum (antibody) and the highest dilution of antigen producing a precipitate was recorded. No claim is made to consider this end-point as a strict estimate of the antibody content of the rabbit serum.

RESULTS.

The serum of a rabbit which has been previously injected with horse serum loses its ability to lyse sheep's red cells sensitised with an immune body prepared in a horse, but maintains its ability to lyse the same cells sensitised with an immune body prepared in a rabbit. Table I shows the type or result which was regularly obtained when a rabbit was injected subcutaneously on two occasions with 10 c.c. of horse serum.

TABLE I.

Day	Horse Serum (c.c.)	Complement. H. v S. I.B.	Complement. R. v S. I.B.
1	10	20	13
6		<5	30
7	10		
13		0	25
27		0	20
36		10	25

From the 13th to the 27th day the rabbit's serum could not lyse sheep cells sensitised with H.v S. I.B. but retained its activity if R.v S. I.B. was used.

The /

The rabbits which showed this phenomenon had presumably developed antibodies to horse serum and the effect could be explained in terms of a fixation of complement due to the interaction of these antibodies with the horse serum of the sensitising immune body. In the test for complement using H.v S. I.B. there were mixed together

(1) Sheep's red cells
(2) Specific sensitising immune body in
(3) Horse serum

} H.v S. I.B. } Haemolytic system

\+

(4) Complement
(5) Antibodies to horse serum

} Rabbit serum

and it would appear that the interaction of horse serum (3) and its antibody (5) fixed complement so rapidly that none was left uncombined to lyse the sensitised cells (1) and (2).

The phenomenon must be considered to be the same as the "anti-immune-body" effect of Bordet which was that the antiserum to the serum of species "A" would inhibit the lysis by complement of cells sensitised with an immune body prepared in species "A". The effect described here merely simplifies this into a demonstration that the fresh antiserum to the serum of species "A" cannot lyse red cells sensitised with an immune /

immune body prepared in species "A", i.e. the anti-serum to species "A" and the complement were one and the same serum. Bordet explained his results by stating that the serum of species "A" contained a natural haemolytic immune body against the red cells he was using. An antibody to this serum contained an "anti-immune-body" which would prevent the action of even an artificially prepared haemolytic immune body from that same species. Bordet considered that complement was kept out of combination with the sensitised cells but the results are more simply explained in terms of complement fixation by a second antigen:antibody reaction as described above.

It may be stated at this point that the samples of horse serum used in these experiments were examined for the presence of a natural haemolysin for sheep's cells and none was ever found. The method of testing consisted of the addition of varying amounts of heated horse serum (55°C for 30 minutes) to a constant amount (0.5 c.c.) of a 3 per cent suspension of sheep's red cells along with a constant excess of complement in the form of guinea-pig serum from which any natural haemolysin against sheep's red cells was removed by absorption with an equal volume of washed deposited sheep's red cells for 1 hour at 0°C.

The phenomenon appeared about a week after a single injection /

injection of horse serum and persisted for a varying period usually a little more than a week. Table II shows a result obtained following the subcutaneous injection of 20 c.c. of horse serum.

TABLE II.

Day of Experiment	1	3	6	10	11	13	19
Horse serum injected 20 c.c.							
Titre of Complement	10	10	<5	0	0	0	10

The period of onset of the effect would support the view that it was dependent upon the production of antibodies to the injected horse serum but the phenomenon did not persist for as long as circulating antibodies were detectable.

Table III shows the result in an animal which had been injected with horse serum intravenously on two occasions with an interval of 5 days between them.

TABLE III.

Day of Experiment.	Horse serum injected in c.c.	Complement H.v S. I.B.	Complement R.v S. I.B.	Precipitins for Horse serum.
1	5 c.c.	13	10	0
5		16	10	0
6	5 c.c.			
9		0	10	512
15		13	13	1024

Not /

Not infrequently a zonal phenomenon was obtained as is shown in Table IV.

TABLE IV.

Day of Experiment.	Horse serum injected.	Precipitins for horse serum.	Complement added (in fractions of 1 c. .01	.02	.03	.04	.05	.06	.075	.1	.15	.2
1	5 c.c.	0	0	0	1	2	4	5	5	5	5	5
6	5 c.c.											
7		256	0	0	1	1	2	4	4	4	4	4
12		2048	0	1	2	2	3	4	4	4	3	2
21		8192	0	1	2	3	4	5	5	5	5	5
35		256	0	0	1	1	2	2	3	3	5	5

0 = no lysis
1 = trace of lysis
2 = distinct lysis
3 = marked lysis
4 = very marked lysis
5 = Complete lysis.

There was an obvious zonal effect present on the 12th day and the table also shows how precipitins were still present to a high concentration on the 21st. day by which time the complementary activity had returned to its former state. It must be made clear that this zonal phenomenon did not always appear and where a serum is stated to have lost its complementary activity there /

there was no lysis with any of the amounts of the range tested. In the above table the fall in complement with H. v S. I.B. between the 21st and 35th day is not regarded as significant but merely due to differences which appear when a serum is titrated against different samples of sheep cells.

Repeated injections of serum could maintain the phenomenon over long periods. Thus

TABLE V.

Day of Experiment.	1	7	8	14	16	21	24	28	30
Serum Injected.	5c.c.	5c.c.		5c.c.		5c.c.			
Complement.	16	0	0	0	0	0	0	0	0

A serum which would not lyse sheep's cells sensitised with H.v S. I.B. would inhibit the lysis by an active complement. In Table VI, row (1) shows the result obtained in the titration for complement of a normal rabbit 621. Row (2) shows the absence of lysis by the same range of quantities of the serum of rabbit 612 which had been injected previously with horse serum. Row (3) shows the result of adding 0.1 c.c. of serum 612 to each of the tubes of row (1).

TABLE /

TABLE VI.

	.01	.02	.03	.04	.05	.06	.075	.1	.15	.2 c.c.
(1) 621 in amounts indicated	0	1	4	5	5	5	5	5	5	5
(2) 612 in amounts indicated	0	0	0	0	0	0	0	0	0	0
(3) 621 + 0.1 c.c. of 612	0	0	0	0	0	0	0	0	0	0

Obviously therefore the addition of 0.1 c.c. of the serum 612 was able to fix or deviate 5 times the M.H.D. of complement 621 but this degree of fixation was much greater than the average result obtained where it was frequently necessary to record the inhibition of lysis of fractions of 1 M.H.D. of the fresh complement. The above experiment is similar to that of Bordet except that the sensitised cells were not washed free of any excess immune body and non-specific elements of the sensitising haemolytic serum, i.e. an antibody to the serum of species "A" (rabbit serum containing antibodies for horse serum) was able to prevent the lysis by complement (621) of red cells sensitised with an immune body from species "A" (H.v S. I.B.).

The serum of a rabbit such as 612 contained an active haemolytic complement when tested with R.v S. I.B. but complementary activity could be demonstrated indirectly even when using H.v S. I.B. This was done by /

by studying the power of such a serum to inhibit a fresh complement when added fresh as in Table VI and after heating to 55°C for half an hour to destroy its own complement. Thus in Table VII,

Row (1) shows the result of a titration of a normal rabbit's serum (621).

Row (2) shows the absence of lysis by the same amounts of serum 603 from a rabbit previously injected with horse serum.

Row (3) shows the results of adding 0.1 c.c. of serum 603 (fresh) to each of the tubes of row (1).

Row (4) as for row (3) except that serum 603 was heated to 55°C for half an hour.

TABLE VII.

	.01	.02	.03	.04	.05	.06	.075	.1	.2 c.c.
(1) 621 in amounts indicated	0	1	2	3	5	5	5	5	5
(2) 603 in amounts indicated	0	0	0	0	0	0	0	0	0
(3) 621 + 0.1 c.c. of 603 fresh	0	0	1	1	1	2	3	4	5
(4) 621 + 0.1 c.c. of 603 heated.	0	0	0	0	0	0	0	2	5

The inhibitory power of serum 603 was greater after heating to 55°C since there was more inhibition of lysis in row (4) than in row (3). This would indicate that some of the lysis in row (3) was due to the complement of serum 603 even although this serum had no haemolytic activity when acting alone on the sensitised,/

sensitised cells (row 2). This type of result was regularly obtained.

Since haemolytic immune body "fixes" directly to the red cells a 3 per cent suspension of red cells was sensitised by the addition of 5 M.H.D. of H.v S. I.B. as before. The mixture was incubated for an hour at 37°C to allow fixation to occur and then centrifuged and washed three times in normal saline before being reconstituted into a 3 per cent suspension. The serum of a rabbit previously injected with horse serum was titrated for complement against the washed and unwashed suspension of sensitised cells. The result is shown in Table VIII.

TABLE VIII.

	.01	.02	.03	.04	.05	.06	.075	.1	.15	.2 c.c.
Using unwashed sensitised cells.	0	0	1	1	1	1	0	0	0	0
Using washed sensitised cells.	0	1	1	1	2	2	3	4	5	5

Clearly therefore the inability of the serum of a rabbit previously injected with horse serum to lyse the haemolytic system was due to the presence of horse serum in the sensitising immune body. When a crude serum containing a haemolytic antibody is added to its homologous antigen the antibody becomes directly fixed to the antigen. The non-specific proteins of the antibody containing serum will remain free in the mixture and can be washed away with saline. Nevertheless /

Nevertheless although more lysis could be obtained with a serum such as that quoted, the result was not always so marked. This raised the controversial subject of the relationship between an antibody and the globulin in which it is contained. It might be that the specific antibody molecules still behave as serum globulin and therefore it would be difficult to wash the sensitised cells free of the proteins responsible for the fixation of complement already mentioned. This point will be raised later in this section.

The ability of the heated serum of a rabbit previously injected with horse serum to inhibit the lytic action of a fresh complement was examined, using cells which had been washed free of any excess unfixed immune body and non-specific proteins of the crude antiserum. The results of an experiment are shown in Table IX.

TABLE IX.

	.01	.02	.03	.04	.05	.06	.075	.1	.15	.2 c.c.
Row (1) 621	0	1	1	2	3	4	5	5	5	5
Row (2) 621 + 0.03 of 603	0	0	0	0	0	0	0	0	4	5
Row (3) 621 + 0.03 of 603 (sensitised cells washed)	0	1	1	2	2	3	5	5	5	5

Row (1) shows the results of a titration of a normal rabbit /

rabbit serum 621. Row (2) shows the inhibition which was produced by the addition of 0.03 of serum 603 to each tube of row (1). Rabbit 603 had received an injection of horse serum.
Row (3) as for row (2) except that the suspension of sensitised red cells was washed as described.

The result shows that the inhibitory effect was completely removed when the non-specific proteins of the crude H.v S. I.B. were washed away thus confirming the opinion reached in the previous experiment. The inhibition of lysis in the above experiment and the absence of lysis of cells sensitised by H.V S. I.B. by a complement containing antibodies to horse serum were therefore due to complement fixation between the horse serum of the crude H.v S. I.B. and its antibody. Results, however, were not usually so definite as that quoted above (Table IX) showing the difficulty of washing the cells free from horse serum. Bordet, in his experiments quoted, washed the cells after sensitising with haemolytic immune body but this washing was probably not sufficient to wash away completely all the non-specific proteins.

The titre of complement as estimated with R.v S. I.B. did not fall and if the above conclusions are correct it was to be expected that the addition of horse serum to such a titration would interfere with lysis. /

lysis. Table X shows the result of an experiment designed to show this.

TABLE X.

Amount of serum added.	.01	.02	.03	.04	.05	.06	.075	.1	.15	.2 c.c.
1. Using H.v S. I.B.	0	0	1	1	1	1	0	0	0	0
2. Using R.v S. I.B.	0	1	1	2	2	3	3	4	5	5
3. Using H.v S. + R.v S. I.B.	0	0	1	2	2	2	1	0	0	0
4. Using R.v S. I.B. + normal horse serum.	0	0	1	1	1	1	0	0	0	0

Row 1. Using cells sensitised with H.v S. I.B. (7 M.H.D.)

Row 2. Using cells sensitised with R.v S. I.B. (7 M.H.D.)

Row 3. Using cells sensitised with 7 M.H.D. of H.v S.I.B. +7 M.H.D. of R.v S. I.B.

Row 4. Using cells sensitised with 7 M.H.D. of R.v S.I.B. + normal horse serum to give the same concentration as in 1 and 3.

There was practically the same degree of inhibition of lysis by the addition of normal horse serum as by the addition of H.v S. I.B. whether the latter was used alone to sensitise the cells or along with R.v S. I.B. showing that the inhibition was dependent upon the non-specific proteins of the horse serum of the crude haemolytic antiserum. Again, however, although obtained in most cases the above result was by no means regular as for example in Table XI.

TABLE /

TABLE XI.

Amount of serum added.	.01	.02	.03	.04	.05	.06	.075	.1	.15	.2 c.c.
1. Using H.v S. I.B.	0	0	0	0	0	0	0	0	0	0
2. Using R.v S. I.B.	1	2	4	5	5	5	5	5	5	5
3. Using R.v S. I.B. + normal horse serum.	2	4	5	5	5	5	5	5	5	5

The part played by the non-specific proteins of the crude haemolytic antiserum in fixing complement was further studied by mixing the various reagents in different orders. Since there is fixation of complement between horse serum and its antibody present in the rabbit serum which is being titrated for complement, one would expect the degree of fixation to be greater if the horse serum (antigen) and the rabbit serum (containing antibodies) were mixed and allowed to stand for an interval before the addition of the sensitised cells. This would allow the precipitin reaction to proceed and fix complement before the sensitised cells were added. This experiment was carried out using crude horse serum and H.v S. I.B. and the results are shown in Table XII.

TABLE /

TABLE XII.

Amount of serum added.	.01	.02	.03	.04	.05	.06	.075	.1	.15	.2 c.c.
1.	0	1	3	4	5	5	5	5	5	5
2.	0	0	1	1	2	2	1	1	0	0
3.	0	0	0	0	0	0	0	0	0	0
4.	0	0	0	0	0	0	0	0	0	0
5.	1	2	4	5	5	5	5	5	5	5
6.	0	1	2	2	2	3	1	0	0	0

Row 1. Titration of serum 632 using R.v S. I.B.

Row 2. Titration of serum 632 using H.v S. I.B.

Row 3. Titration of serum 632 with normal horse serum added to each tube and allowed to stand for half an hour before the addition of cells sensitised with R.v S. I.B.

Row 4. as for 3 using H.v S. I.B. instead of normal horse serum.

Row 5. Titration of serum 632 using cells sensitised with H.v S. I.B. + R.v S. I.B.

Row 6. as for row 5, using normal horse serum in place of H.v S. I.B.

The concentration of horse serum when used whether as crude serum or as H.v S. I.B. was kept constant.

There was clearly less lysis when horse serum either normal as (3) or in the form of H.v S. I.B. as in (4) was added to the complement before the addition of sensitised cells than when added along with the sensitised /

sensitised cells. That is, there was less lysis in 3 and 4 than in 5 and 6. The discrepancy in the readings of rows 5 and 6 is not disturbing because serum 632 was able to produce some lysis of cells sensitised with H.v S. I.B. (row 2).

Although the phenomenon has been explained in terms of complement fixation between the horse serum of the sensitising immune body and its antibody in the rabbit serum it does not follow that this would be obtained regularly no matter what quantity of sensitising immune body was used. This was found to be the case and a result is shown in table XIII where lysis was obtained when using larger amounts of H.v S. I.B. Serum 603 was titrated for complement using (1) 3 M.H.D. of H.v S. I.B., (2) 5 M.H.D., (3) 15 M.H.D.

TABLE XIII.

Amount of serum added	.01	.02	.03	.04	.05	.06	.075	.1	.15	.2 c.c.
1. 603 using 3 M.H.D.	0	0	0	0	0	0	0	0	0	0
2. 603 using 5 M.H.D.	0	0	1	1	1	1	2	2	1	0
3. 603 using 15 M.H.D.	0	1	2	2	2	3	4	4	5	5

An excess of H.v S. I.B. therefore could be used to reveal the presence of complement in the rabbit serum. /

serum. This result was not unexpected because Dean (1911, 1 & 2) has emphasised that complement is fixed during the course of formation of a precipitate. If a fine precipitate is formed slowly then much complement is fixed and the total amount of complement fixed does not depend upon the total amount of precipitate but more upon the size of the particles of a precipitate. A heavy precipitate formed quickly is less active in fixing complement. Further, the rate of formation of a precipitate depends upon the relative amounts of antigen and antibody and in the experiment above, using 15 M.H.D. of H.v S. I.B., the rate of formation of precipitate may have been slowed because of a relative antigen excess. During the course of the experiment, therefore, much of the complement would be left free to be taken up by the sensitised cells and thus effect their lysis.

Reference has already been made to the difficulty of washing sensitised cells free of the horse serum proteins responsible for the fixation of complement. This was further examined in an experiment where 1 c.c. of a serum which would not lyse sheep cells with H.v S. I.B. was added to 5 c.c. of a 3 per cent suspension of such a haemolytic system. After an hour the cells were centrifuged and washed four times in a gross /

gross excess of saline and reconstituted into a 3 per cent suspension. The fresh serum of a normal rabbit was titrated for complement using (1) the suspension treated as above, and (2) a suspension of cells similarly sensitised and washed but untreated with the serum of a rabbit previously injected with horse serum. The following result was obtained.

TABLE XIV.

Amount of serum added.	.01	.02	.03	.04	.05	.06	.075	.1	.15	.2 c.c.
(1) Test suspension	0	0	0	0	0	0	1	2	5	5
(2) Control "	0	0	1	1	2	4	5	5	5	5

This would indicate that it is difficult to wash away the fine precipitate which has resulted from an antigen:antibody reaction unless Bordet's explanation is to be accepted, viz. that there is an anti-immune body which becomes fixed to the sensitised cells and prevents fresh complement from acting. Bordet stated further, however, that the complement which was kept out of union with the sensitised cells was left free in the mixture but this finding was quite unconfirmed by the writer. For example the supernatent fluid from the several tubes of a complement titration with a serum producing no lysis with H.v S. I.B. could not lyse /

lyse cells sensitised with R.v S. I.B. although such amounts were quite active with R.v S. I.B. in the first instance. This shows that complement was absorbed and not merely kept out of union with the sensitised cells. Thus a titration of serum 634 against cells sensitised with H.v S. I.B. showed no lysis.

TABLE XV.

Serum 634	.01	.02	.03	.04	.05	.06	.075	.1	.15	.2 c.c.
Lysis	0	0	0	0	0	0	0	0	0	0

Each tube was centrifuged and the supernatent added to 0.5 c.c. of a 3 per cent suspension of cells sensitised with R.v S. I.B. There was no lysis in any tube.

In many of his experiments Bordet suspended the sensitised cells in heated guinea-pig serum instead of saline as the effect was more readily demonstrable. In his typical experiment he adopted the following technique. Ox cells were sensitised with R.v O. I.B. To these sensitised cells he added anti-immune body which was the heated serum of a guinea-pig previously injected with rabbit serum. After a period of incubation the cells were centrifuged and washed in saline and resuspended finally in normal heated guinea-pig serum (55°C for half an hour). On adding complement there /

there was no lysis, but lysis was obtained in a control experiment where the cells were treated with normal heated guinea-pig serum. An experiment similar to this except that the sensitised cells were finally suspended in saline has been quoted in Table XIV, and commented upon. Bordet's technique was followed in the experiment below except that the sensitised cells were washed before adding the "anti-immune body". A 3 per cent suspension of cells was sensitised with 5 M.H.D. of H.v S. I.B. and incubated at 37°C for an hour to allow fixation of the immune body to the cells. The suspension was washed three times and finally re-suspended in the heated serum of a rabbit previously injected with horse serum. After a period of contact the cells were again centrifuged and washed. After the final washing the 3 per cent suspension was re-constituted, one lot in heated normal guinea-pig serum and the other in saline. Control suspensions were made as above except that heated normal rabbit's serum was used instead of serum from a rabbit previously injected with horse serum. Complement in the form of fresh guinea-pig serum was titrated against these four suspensions and the results are shown in Tables XVI and XVII.

TABLE /

TABLE XVI.

Cells suspended in saline.

Complement in cc.'s	.002	.004	.006	.008	.01	.015	.02
Test.	1	2	3	5	5	5	5
Control.	1	2	3	5	5	5	5

TABLE XVII.

Cells suspended in heated guinea-pig serum.

Complement in cc.'s.	.004	.008	.016	.024	.032	.048	.064	.08	.09	.1
Test.	0	0	0	0	1	1	2	3	4	5
Control.	0	0	0	0	1	1	2	3	4	5

There was therefore no difference between test and control suspensions and this was irrespective of the medium in which the cells were finally suspended. It will be noted that the experiment quoted as Table XVI is somewhat similar to that quoted as Table XIV and yet a different result was obtained. In the experiment in Table XVI the sensitised cells were washed free of the non-specific proteins of the crude H.v S. I.B. before adding the rabbit serum containing antibodies to horse serum. In the earlier experiment the cells were unwashed after sensitisation so that an antigen:antibody reaction could occur and comment was made upon the apparent difficulty of washing the cells free /

free of the antigen:antibody precipitate. The complement dose required to produce complete lysis of cells suspended in serum was 14 times the amount required if the cells were suspended in saline. A ratio of this order was regularly obtained and wasmuch greater than the ratio found by Muir and Browning (1909). The complement used in the above experiments as well as the sera from the test and control rabbits contained no natural haemolysin for sheep's cells. This was tested by the technique already described.

In order to test more conclusively that there was no union or interaction between the haemolytic immune body proper and some substance in the serum of the rabbits previously injected with horse serum an absorption experiment was carried out by mixing such a serum with an equal volume of <u>washed</u> deposited sensitised cells (H.v S. I.B.). The mixture was centrifuged after a period of incubation and the inhibitory power of this absorbed serum was compared with an unabsorbed fraction according to the technique of Table VI and no change was found. There could have been little union, therefore, between the immune body proper and some factor which Bordet labelled "anti-immune-body". Emphasis has already been placed, however, on the difficulty of washing sensitised cells free of the non-specific proteins of the sensitising immune body.

<u>Results</u> /

Results of Experiments with Ox Serum.

Injections of ox serum in rabbits produce antibodies which react with horse serum and vice versa (Kolmer, 1925; and P.46 of this work) and injections of ox serum were found to produce a fall in haemolytic complementary activity when using cells sensitised with an H.v S. I.B. but no change when using cells sensitised with R.v S. I.B. The effect was less marked than with horse serum but was quite definite. Two results are quoted in Table XVIII.

TABLE XVIII.

A	Day.	1		8		12	23
	Ox serum injected.	5 cc.		5 cc.			
	Titre.	20		5		3(zone)	20
B	Day.	1	4	7	11	18	26
	Ox serum injected.	20 cc.					
	Titre.	13	13	10	3(Zone)	5	20

The titre of complement on the various days is given as defined in the introduction and on all occasions where the titre is given as <5 there was a marked reduction in the amount of lysis by fractions of 1 M.H.D. Furthermore in both experiments a zonal phenomenon appeared indicating some inhibitory mechanism. This gives further proof that the absence of lysis /

lysis of cells sensitised by H. v S. I.B. by the serum of a rabbit previously injected with horse serum is due to complement fixation by a precipitin reaction.

Experiments with Sheep Serum.

Six rabbits were injected with sheep serum, 10 c.c. being given on each of two occasions with an interval of one week between. There was no reduction of haemolytic complement activity as estimated with cells sensitised with H.v S. I.B. This was an interesting result because in the test for complement there were mixed together

1. Sheep cells.		Sensitised cells.
2. H.v S. I.B. in 3. Horse serum	Crude H.v S. I.B.	
+		
4. Complement		Rabbit serum.
5. Antibodies to sheep serum		

Presumably therefore the suspension of sheep cells had been washed so free of sheep serum that there was no antigen:antibody reaction between this and the antibodies to sheep serum (5) because complement was not fixed. This would indicate that the sheep cells were easily washed free of sheep serum by washing three times in saline. In previous experiments a note was made of the difficulty of washing sensitised cells free of the non-specific proteins of the antibody containing antiserum. In the latter case the washing was /

was carried out much more rigorously because the centrifuged deposit of a 3 per cent suspension of cells was washed with a gross excess of saline whereas in the former the washing was effected using some three or four volumes of saline to one volume of deposited cells. Either the cells can be more readily washed free from their own serum than from a foreign serum or the specific sensitising antibody is still able to give the serological reactions of the serum of the species in which it was prepared. The latter suggestion cannot be discounted lightly when one compares the degree of the washing described above and also that the washing of sheep cells free from sheep serum means to wash them free of the medium in which they were previously suspended whereas to wash them free from the non-specific proteins of an H.v S. I.B. means washing them free of an original concentration of 1 per cent serum added for half an hour before washing. The interference with precipitin formation because of a gross antigen (sheep serum) excess has not of course been excluded.

DISCUSSION.

The serum of a rabbit previously injected with horse serum is unable to lyse a suspension of red cells sensitised by a haemolytic immune body prepared in a horse. This phenomenon has been shown to be essentially the same as Bordet's "anti-immune-body" effect which can be more simply interpreted in terms of complement fixation between the non-specific serum proteins of the sensitising immune body and their antibodies which are present in the serum being tested for complement. The evidence for the conclusion was varied. In the first place if the sensitised cells were thoroughly washed to remove the non-specific proteins and leave the specific antibody fixed to the cells the phenomenon frequently did not appear. Nevertheless, washing of the sensitised cells did not regularly render them lysable by a complement which contained antibodies to the serum of the haemolytic sensitising antibody serum and in this connection the exact relationship between a specific antibody and the globulin of the serum containing it is raised. A sensitised particulate antigen is said to behave in some respects as though coated with a layer of antibody serum globulin (Marrack, 1934) and it may be difficult to remove the non-specific globulin from an antigen:antibody combination. Washing of sensitised cells does not, of course, remove the specific immune body /

body molecules and a method of investigation into the exact nature of antibodies might be opened up from this viewpoint. Altmann (1912) injected washed sensitised cells into animals and obtained precipitins for the sensitising serum but since there is difficulty in washing away the non-specific proteins it would be instructive to repeat Altmann's experiments until free from these non-specific proteins. This point could be controlled by their ability to be lysed by a complement which also contained antibodies to the sensitising immune body.

Although the explanation of the absent lysis advanced here was quite a simple one there are a few interesting applications to be considered. It might be expected that the serum of an animal injected with horse serum would give an anticomplementary result in a complement fixation test if the haemolytic immune body used to sensitise the suspension of red cells were derived from a horse. This was investigated in 12 rabbits whose sera showed no fixation of complement with the Wassermann antigen. Samples of sera were collected before and after injections of horse serum and tested in a complement fixation test with Wassermann antigen. The tests which were all done at the same time were performed in duplicate using H.v S. I.B. and R.v S. I.B. In none of the tests did the serum appear as anticomplementary, i.e. the serum controls never fixed 2 M.H.D. of complement (the lowest amount used).

Table /

Table VI showed a result where 0.1 c.c. of serum from a test rabbit was able to fix more than 5 M.H.D. of a fresh complement and no doubt this serum would have given an anticomplementary result in a complement fixation test but this serum was admitted to be unusually active in this respect. In experiments similar to that quoted as Table VI, although multiple doses of complement were not inhibited, there was always the evidence obtained from the absence of lysis by fractions of 1 M.H.D. of complement as a whole range was tested.

Of perhaps greater interest is the fact that the phenomenon recovered before precipitins for horse serum had disappeared from the rabbit's serum. The relationship of complement fixation to precipitation has already been mentioned and the above result was not upsetting to the opinion advanced to explain the phenomenon of the absent lysis. The view has been advanced by Browning & Wilson (1911) that the precipitins which appear early and late following the injection of an antigen may differ qualitatively in their ability to fix complement when interacting with the antigen.

SUMMARY.

1. The serum of a rabbit previously injected with horse serum cannot lyse red cells sensitised by a haemolytic immune body from a horse.

2. This phenomenon is due to complement fixation between the horse serum of the sensitising immune body and the corresponding antibodies present in the rabbit's serum.

3. The phenomenon is essentially the same as Bordet's "anti-immune-body" effect which can be better interpreted in terms of complement fixation.

4. It is much more difficult to wash red cells free from the non-specific proteins of a sensitising immune body than from their own serum.

5. Conclusion 4 raises the question of the relationship of antibodies to the globulin of the antibody containing serum.

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THE EFFECT OF THERAPEUTIC INJECTIONS OF HORSE SERUM ON THE BACTERICIDAL AND ANTICOMPLEMENTARY ACTIVITY OF HUMAN SERUM.

It has been shown previously that, when a rabbit is injected subcutaneously with horse serum, the lysin content of its serum is depressed between th 7th and the 17th days after the injection. Further, the fresh serum of such a rabbit is unable to act as complement for a haemolytic serum prepared in the horse against sheep cells (H.v S. I.B.), though it can do so for a serum prepared in the rabbit (R.vS.I.B.) It contains antibodies which fix complement in the presence of horse serum and this activity may be great enough to abolish the action of added complement and so make it anticomplementary in a complement fixation test in which H.v S. I.B. is used as haemolysin.

Using the same technique as in the previous experiments the sera of patients treated with horse serum were examined to see if therapeutic doses of serum produce these effects in human beings.

METHODS.

1. Bactericidal tests.

As before.

2. Complement.

Complement was estimated by adding varying amounts of fresh rabbit serum to 0.5 c.c. of a 3 per cent /

cent suspension of well washed sheep red cells sensitised by the addition of 7 M.H.D. of haemolytic immune body. The range tested was 0.02, 0.04, 0.06, 0.08, 0.1, 0.15 and 0.2, and the activity was taken to be represented by the reciprocal of the smallest fraction of a c.c. producing complete lysis.

3. Wassermann reaction.

The Wassermann reaction was chosen as being typical of a complement fixation test. The technique adopted employed constant volumes of antigen and serum with varying amounts of guinea-pig serum as complement. The amounts of complement used were 2, 4 and 8 M.H.D., with two serum controls of 2 and 4 M.H.D. The tests were carried out in duplicate using cells sensitised with an immune body prepared in a horse and one prepared in a rabbit.

A haemolytic immune body prepared by injecting sheep cells into a horse is referred to as "horse versus sheep cells immune body" or abbreviated "H.v S. I.B." A similar haemolytic immune body prepared in a rabbit is referred to as "R.v S. I.B."

RESULTS.

Observations were made upon 15 patients in the Edinburgh City Fever Hospital who had been treated with diphtheria or scarlet fever antitoxin. The first specimen of blood was withdrawn on admission before the administration of therapeutic serum and the bactericidal activity of the patient's serum upon B. typhosus was estimated on the same day or, at the latest, after storing till the following day in the refrigerator (4°C.). One c.c. of the serum was heated to 55°C. for half an hour in a quill tube and stored in the refrigerator for the Wassermann test, which was performed upon it and upon the second specimen simultaneously. The second specimen was withdrawn on the 8th-10th day after the injection of serum. It will be noted that the bactericidal tests on the two specimens of serum from any given patient were performed at different times so that it was possible for slight differences in activity to be due to the conditions of the experiment. In the Wassermann tests, however, both specimens were examined at the same time and even slight differences would have to be regarded as significant. The Wassermann test was performed in duplicate on every specimen with H.v S. I.B. and R.v S. I.B. respectively. In this test evidence was sought /

sought of the fixation of complement in the actual test and of anticomplementary action in serum control when using H.v S. I.B. The results are shown in the table.

Ten other cases were examined on the tenth day only, that is, no test was done before the injection of therapeutic serum. In all of these the serum was actively bactericidal for B.typhosus and in none was there any "anticomplementary" effect in the Wassermann test with H.v S. I.B.

Although this investigation was not extensive it seems clear that the therapeutic injection of these amounts of serum does not depress the α lysin nor produce sufficient antibodies to show an anticomplementary effect in a complement fixation test. It must be remembered, however, that the amounts injected were relatively small as compared with those used in the experimental animals. The depression of α lysin was regularly produced by the injection of 10 c.c. of crude horse serum in a rabbit weighing 5 lb. Furthermore the therapeutic sera were refined and consisted of the globulin only, so that there would not be the same content of serum proteins to act as antigens. No observations have been possible on patients treated with larger doses of serum.

TABLE /

TABLE.

Activity of human serum before and after injections of therapeutic horse serum.

Case No.	Day.	Volume of serum injected(c.c)	Bactericidal activity towards B. typhosus.	Anticomplementary activity with H.v S. I.B.	Anticomplementary activity with R.v S. I.B.
1	1	15	4	0	0
	9		4	0	0
2	1	15	3	0	0
	9		4	0	0
3	1	15	3	0	0
	9		4	0	0
4	1	15	4	0	0
	8		5	0	0
5	1	15	4	0	0
	8		4	0	0
6	1	15	5	0	0
	11		4	0	0
7	1	15	6	0	0
	11		4	0	0
8	1	10	3	0	0
	10		3	0	0
9	1	10	4	0	0
	11		4	0	0
10	1	10	3	0	0
	12		4	0	0
11	1	10	4	0	0
	10		4	0	0
12	1	5	4	0	0
	7		4	0	0
13	1	5	4	0	0
	7		4	0	0
14	1	5	3	0	0
	8		3	0	0
15	1	5	4	0	0
	9		5	0	0

In the above Table day 1 is the day of admission before the patient received any serum.

SUMMARY /

SUMMARY.

1. No depression of the bactericidal activity of serum has been observed in human beings treated with 5-15 c.c. of therapeutic horse serum.

2. The serum of such patients does not cause an anticomplementary effect in a complement fixation test using a heamolytic immune body prepared in a horse.

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THE EFFECT OF RETICULO-ENDOTHELIAL BLOCKADE ON NATURAL ANTIBODIES AND NATURAL IMMUNITY REACTIONS.

INTRODUCTION.

The cells of the reticul-endothelial system are known to play an important part in resistance to infection. Thus, the phagocytic activity of these cells is an essential factor in clearing the blood stream of bacteria. Even virulent bacteria injected into the blood stream are rapidly removed and although the animal should die from such an inoculation there is always at least an initial rapid drop in the number of bacteria in the circulation. Wright (1927) has followed the course of events after an intravenous injection of virulent pneumococci in rabbits. This usually produces a fatal result, yet 90 per cent of the injected pneumococci are removed from the blood stream within an hour.

From time to time attempts have been made to measure the value of the reticulo-endothelial system as a body defence. The effect of splenectomy on resistance has been studied by Jungeblut (1927 who found a lowered resistance to infection by trypanosomes. Regendanz and Kikuth (1927) made similar observations but on the whole, the results of such experiments have never been very definite. Another method has been the blockade of the reticulo-endothelial system by injecting particulate matter into the blood stream. In this case the object has been to engage the activity of these phagocytic cells and thus depress their efficiency as

a /

a means of clearing the blood stream of infection. Jungeblut (loc.cit) showed that infection by Spironema recurrentis runs a more rapid course in mice following splenectomy and blockade of the reticulo-endothelial system. On the other hand Kilmer and Schamberg (1933) found that injections of Indian ink produced no alteration in the course of infection by Trypanosoma equiperdum in rats. Singer and Adler (1924) found that pneumococci did not disappear so rapidly from the blood stream of blockaded rabbits as of controls.

All of the above investigations have been concerned with the phagocytic activity of the reticulo-endothelial system but more recently this system has been studied from another aspect, viz. that these cells may be the origin of antibodies. This view would unify and simplify our conception of the mechanisms responsible for resistance and there is some evidence to support it. Carrel and Ingebrigsten (1912) found that antibodies were produced in tissue cultures of reticulo-endothelial cells if these cells were removed from an animal previously injected with antigen. Many publications have appeared to show that the production of antibodies may be interfered with if the activity of the reticulo-endothelial system is otherwise engaged, as for example, during blockade. Jungeblut and Berlot (1926) found that there was a

delay /

delay in the production of antitoxin in guinea-pigs following an injection of diphtheria toxin-antitoxin mixture if the animals were also injected with Indian ink. Roberts (1929) tested for antibody production in blockaded rabbits following injections of sheep's red cells, B.typhosus and human serum, and considered that there was some depression which was not sufficiently marked to justify any definite conclusion. Several workers have estimated the production of a haemolysin in blockaded rabbits following the injection of red cells. Gay and Clark (1924) reported a diminished output as also did Isaacs (1925) and Cannon et al. (1929). Ross (1926) found no difference as compared with controls and of these workers he was the only one to test his animals for the presence of a natural haemolysin at the onset of his experiments.

Some evidence might be drawn from such results which would point to the reticule-endothelial system as being the source of circulating antibodies and the following paper records the results of experiments designed to ascertain whether reticulo-endothelial blockade exerts any influence on the production of natural antibodies and other serum constituents concerned in natural immunity reactions.

METHODS.

Blockading Measures.

The substance used was Higgins Indian ink (white label) in 20 per cent dilution in normal saline. This was injected daily intravenously in 5 c.c. amounts with an additional intraperitoneal injection of 5 c.c. every alternate day. Control rabbits received injections of similar volumes of normal saline. Total volumes ranging from 70 c.c. to 125 c.c. of 20 per cent ink were injected over a period of two or three weeks, and certain natural antibodies were tested before and after this measure. The sample of blood for the retest was drawn off 24 hours after the final blockading dose of ink. For some examinations an intermediate sample of blood was withdrawn but here also the animal was bled 24 hours after the preceding injection of ink.

The total volumes of ink injected were greatly in excess of the amounts commonly used as a blockading measure.

Fully grown rabbits were used throughout and a record kept showed no loss of weight due to the treatment.

Technique of Tests.

The following natural immunity reactions of rabbits' sera were examined.

(a) /

(a) Haemolysis of sheep's red cells by heated serum (55°C for 30 minutes) along with guinea-pig complement.

(b) Property of fixing complement along with the lipoid antigen used in the Wassermann test.

(c) Bactericidal activity towards B.typhosus, V. cholerae, S. aureus and B. anthracoides.

(d) Agglutination of B. proteus and Friedlander's pneumobacillus.

(e) Complementing activity with a haemolytic system of sheep's red cells sensitised by the addition of a haemolytic immune body prepared in a horse.

(a) Haemolysis of Sheep Red Corpuscles.

Before the blockading measures were begun, blood was removed from the rabbit and 1 c.c. of separated serum was heated for half an hour at 55°C in a quill tube and stored in the refrigerator at 0°C - 4°C. A similar sample was withdrawn 24 hours after the last blockading dose of ink and likewise treated. In some cases an intermediate sample was withdrawn immediately before administering the dose of ink for that day, i.e. 24 hours after the preceding injection of ink. The natural haemolysin for sheep's red cells present in these heated sera does not deteriorate at 0°C - 4°C, and it was thus possible to titrate all the specimens from any given animal against the same specimen of sheep's /

sheep's corpuscles and the same complement. The haemolysin was titrated by testing a range of varying amounts of stored inactivated serum with constant amounts of sheep cells and complement. The range tested was

0.0025; 0.005; 0.01; 0.025; 0.05; 0.075; 0.1; 0.2; 0.5.

To each tube was added 0.5 c.c. of a 3 per cent suspension of well washed sheep cells and 0.1 c.c. of complement. Complement consisted of guinea-pig serum which had been absorbed with an equal volume of well washed deposited sheep's red cells in order to remove any natural haemolysin for sheep's red cells which might be present. The absorption was carried out at 0°C and this procedure absorbs immune body only, without fixing complement.

The "titre" of the haemolysin is conveniently given as the reciprocal of the smallest fraction of a c.c. of inactivated rabbit serum which produced complete lysis of the cells in 2 hours at 37°C.

(b) Property of Fixing Complement along with Lipoid Antigen of the Wassermann Test.

The ability of rabbit serum to fix complement along with Wassermann antigen was investigated, employing constant volumes of antigen and serum and varying doses of guinea-pig serum as complement. The test /

test consisted of a titration with 2, 4, 8 and 14 M.H.D. The strength of the reaction was recorded in terms of the number of M.H.D. of complement fixed and obviously this could only be given as lying between two successive amounts.

As in the case of the haemolysin, serum samples were withdrawn, heated in a quill tube for half an hour at 55°C and stored in the refrigerator so that all the samples from a given rabbit could be tested at the same time under the same conditions.

(c) Bactericidal Tests.

As described in section I.

(d) Agglutination.

Serial doubling dilutions of serum from 1/2 to 1/128 were made and an equal volume of bacterial suspension freshly prepared from an 18 hour old culture was added to each tube making the final range of serum concentrations 1/4 to 1/256. The "titre" for tabulation purposes was taken as the reciprocal of the highest dilution causing agglutination.

(e) Complement.

Complement was estimated with 0.5 c.c. of 3 per cent suspension of well washed sheep's red cells sensitised by the addition of 7 M.H.D. of immune body. The range tested was

0.01; /

0.01; 0.02; 0.03; 0.04; 0.05; 0.06; 0.075; 0.1; 0.2, and the activity was represented by the reciprocal of the smallest fraction of a c.c. producing complete lysis.

RESULTS.

(a) Haemolysis of Sheep's Red Cells.

As is well known a certain proportion of rabbits contain in their serum a natural haemolytic antibody for sheep's red cells. In many respects this natural haemolysin behaves similarly to the artificially produced haemolysin following injections of red blood corpuscles.

Table I shows the results obtained with 8 test animals and 3 controls.

TABLE /

TABLE I.

Rabbit No.	Day of Experiment.	Volume of 20% ink injected. (c.c.)	Titre.	
792	1	0	5	
	9	40	2	
	18	70	2	
793	1	0	20	
	9	40	<5	Depression
	18	70	<5	
273	1	0	10	
	14	90	13	
	20	125	20	
332	1	0	10	
	14	90	10	
	20	125	10	
442	1	0	10	
	14	90	10	
811	1	0	10	
	14	90	10	
812	1	0	20	
	14	90	<5	Depression
795	1	0	5	
	9	40	5	
	18	70	5	
		Volume of Saline injected.		
814	1	0	40	)
	10	70	20	)
328	1	0	13	)
	14	90	40	) Controls
	20	125	20	)
791	1	0	0	)
	9	110	0	)

In /

In two rabbits (793 and 812) marked depression of this natural antibody was produced and comment on this is reserved until the discussion at the end of the paper.

(b) Complement Fixation with the Lipoid Antigen of the Wassermann Reaction.

Heated serum (55°C for half an hour) from most rabbits is capable of fixing complement along with the lipoid "antigen" of the Wassermann reaction. As previously stated all the specimens from any given animal were preserved until the end of the experiment in order to test all the samples together under the same conditions.

Observations were made on 12 test and 2 control animals. No change of activity was detected in 5 test rabbits and Table II shows the results in the other 7 test and 2 control animals.

TABLE /

TABLE II.

Animal No.	Day of Experiment.	Volume of 20% ink injected. (c.c.)	Complement Fixed.	
338	1	0	4-8	
	14	90	8-12	
	20	125	8-12	
795	1	0	8-12	Slight
	19	80	>12	increase.
809	1	0	2-4	
	19	90	4-8	
332	1	0	8-12	
	20	125	4-8	
812	1	0	8-12	
	14	90	4-8	
793	1	0	8-12	Slight decrease.
	19	80	4-8	
794	1	0	8-12	
	19	80	4-8	
		Volume of Saline injected.		
Con:trols 786	1	0	8-12	
	18	130	8-12	
791	1	0	>12	Slight
	9	110	8-12	decrease.

In three rabbits a slight increase in activity was found while a decrease of the same order was found in four as well as in one of the control rabbits which was injected with normal saline. It is doubtful therefore if the alterations in activity observed in the /

the blockaded animals was of definite significance.

(c) and (d) Bactericidal Activity and Complement.

Bactericidal activity and complement are considered together because the latter is required in the bactericidal action of serum on certain bacteria (Mackie and Finkelstein, 1931). The bactericidal action of normal serum depends on two types of mechanism which have been designed α lysin (active along with complement) and β lysin (independent of complement). Of the organisms tested B. typhosus and V. cholerae are susceptible to α lysin while S. aureus and B. anthracoides are susceptible to the β lysin.

The results from 10 test rabbits and 2 controls are shown in Table III.

TABLE /

TABLE III.

Rabbit No.	Day of Experiment.	Volume of 20% ink injected (c.c.)	Bactericidal Activity. B. typhosus.	V. cholerae.	S. aureus	B. anthracoides	Complement.
329	1	0	4	3	1	1	10
	20	125	2	2	1	1	10
285	1	0	3	3	2	3	10
	20	125	2	3	1	0	13
447	1	0	3	4	1	1	10
	20	125	2	3	1	1	5
426	1	0	4	3	4	2	5
	20	125	2	3	1	2	5
425	1	0	3	2	2	1	5
	20	125	2	3	2	3	10
810	1	0	3	2	0	0	10
	14	85	2	2	2	1	13
809	1	0	4	2	1	2	10
	14	85	2	3	1	2	10
815	1	0	2	3	2	2	10
	14	85	2	4	2	2	10
812	1	0	2	3	2	2	10
	14	85	3	-	0	0	13
811	1	0	3	1	3	4	20
	14	85	2	4	3	2	20
		Volume of saline injected.					
Saline Controls: 280	1	0	4	2	2	2	5
	20	125	3	2	3	1	13
807	1	0	3	1	1	5	13
	14	85	2	2	2	4	10

The /

The bactericidal effects on the whole remained remarkably steady. The results with S. aureus and B. anthracoides were very constant with a few exceptions which, however, were not all changes towards either increase or decrease. For example, 285, 811 and 812 showed a marked fall in bactericidal activity towards B. anthracoides but 425 showed a definite increase. The measurements of α lytic action with B.typhosus and V. cholerae showed a remarkable constancy. There were slight but no marked changes and the fall in activity in 329, 426 and 809 towards B.typhosus could hardly be regarded as significant. It must be emphasised that the tests in the various rabbits were carried out before and after the blockading measures in contrast to the two previous tests where the samples were stored until the end of the experiment in order to allow of all the samples being titrated under the same conditions.

The complement titres remained fairly constant. A temporary fall in complement following injections of Indian ink has been described by Jungeblut and Berlot (1926) but the activity had returned within 24 hours.

(e) Agglutination.

Normal agglutinating antibodies may be present in rabbits although not with the same regularity as the antibodies already described. As has been shown by Gibson (1930) these are lacking in very young animals and /

and develop in the absence of any specific contact with the various bacteria. Gibson (loc. cit.) and Lovell (1932, 1934) both emphasize the specificity of these agglutinins. Results of agglutination tests against the pneumobacillus and B. proteus are recorded in Table IV.

TABLE IV.

Rabbit No.	Day of Experiment.	Volume of 2% ink injected (c.c.)	Agglutinin Titre. Pneumobacillus.	B.proteus.	
285	1	0	32	0	
	20	125	16	8	
426	1	0	256	0	
	20	125	256	0	
425	1	0	128	0	
	20	125	64	64	
810	1	0	32	0	
	14	85	128	0	
809	1	0	32	0	
	14	85	256	0	
811	1	0	64	0	
	14	85	16	0	
812	1	0	32	0	
	14	85	16	0	
		Volume of saline injected.			
280	1	0	128	4)	
	20	125	128	16)	Controls
807	1	0	16	0)	
	14	85	16	0)	

The /

The pneumobacillus was agglutinated regularly by rabbits' serum and in some cases (809 and 810) the activity underwent a marked increase. No animal showed any significant depression. The results obtained with B.proteus are interesting because two rabbits 285 and 425 showed the development of an antibody which had previously been found to be absent. The significance of this is difficult to assess.

EFFECT OF BLOCKADE ON ARTIFICIALLY PRODUCED ANTIBODIES.

At the conclusion of these experiments a number of rabbits received an injection of sheep's red cells and the blockading measures were continued to verify the conclusion of other workers who have investigated the effects of blockade on antibody production following the injection of an antigen. Cannon et al. (loc. cit.) have emphasised that small doses of blockading agents may stimulate the reticulo-endothelial system instead of depressing it and the injection of a large amount of antigen may stimulate antibody production to such an extent that there may be no difference between blockaded and control animals. Accordingly they recommend severe blockading measures followed by the injection of only a small amount of antigen. The rabbits used in the author's experiments had received even larger doses of ink than were used by Cannon and at /

at the conclusion of the observations on natural antibodies a series of animals received one intravenous injection of 2 c.c. of a 3 per cent suspension of sheep's cells. This dose is almost the same as that used by Cannon. The blockade was continued by injecting 5 c.c. of 20 per cent ink every alternate day thereafter, the controls receiving injections of saline. The technique for measuring haemolysin production was that already described except that the range was extended to include smaller amounts of rabbit serum and although differing from that employed by Cannon et al. the titres approximate to those given by them.

Results are recorded in Table V from 5 test and 2 control rabbits which were known to possess a very low titre of natural haemolysin.

TABLE /

TABLE V.

Number.	Day after injection of cells.	20% ink (c.c.)	Titre of Haemolysin.	
809	0	90	<5	
	7	115	20	
	13	125	5	
	18	125	<5	
	21	125	<5	
811	0	90	<5	
	7	110	50	
	13	120	100	
	18	120	50	
	21	120	50	
426	0	125	<10	
	7	140	10	
	10	150	400	
	14	150	400	
285	0	125	<10	
	7	140	<10	
	10	150	10	
	14	150	<10	
447	0	125	10	
	10	150	100	
		Saline.		
807	0	90	5	Controls
	7	110	40	
	13	125	100	
	18	135	40	
	21	135	40	
280	0	125	<10	
	7	140	<10	
	10	150	10	
	14	150	10	

Admittedly /

Admittedly the number of rabbits was small and the variation in individual response in antibody production is great. Nevertheless most of the workers already quoted who have tested for this particular antibody in blockade experiments have used as small a number, and with only one exception they did not test for the presence of a natural haemolysin in the rabbit at the beginning of the experiment. In addition they did not absorb any natural haemolysin for sheep's cells from the added guinea-pig complement. From the experiments above there was no indication that the blockade interfered with haemolysin production.

DISCUSSION.

Some evidence has been collected by previous workers which would point to the reticulo-endothelial system as being the source of circulating antibodies and there is also some evidence to show that injections of particulate matter which engages the activity of this system interfere with the production of antibodies following the injection of an antigen. This paper deals primarily with experiments to discover the effect on circulating antibodies of such blockading measures in the absence of the injection of an antigen, i.e. the effect of blockade on natural antibodies and natural immunity reactions.

In the first place there is some dispute whether natural antibodies are of the same biological nature as the immune bodies which result from the injection of an antigen; whether they are produced in the same way and whether they have the same specificity. Some of these questions can scarcely be answered but as regards specificity there is much evidence to indicate that the natural antibodies are specific. (Gibson, 1930; Mackie and Finkelstein, 1931; Lovell, 1932,1934). Whatever their site of origin in the animal body, natural antibodies would appear to be restored into the circulation very rapidly, as in the case of immune /

immune antibodies. Thus, excessive bleeding of rabbits seems to affect only slightly their concentration in the blood serum and the author has frequently removed 10 c.c. of blood from a rabbit daily for 8 days without disturbing bactericidal activity or complement. The removal of 40 c.c. of blood on two successive days followed by the removal of 10 c.c. on alternate days for a week did not alter the activity of bactericidins, complement or anti-sheep haemolysin.

The results of the experiments described here produced no evidence that blockade of the reticulo-endothelial system could depress the concentration of circulating antibodies. Cannon et. al. (loc.cit.) pointed out the difficulties of blockading the reticulo-endothelial system and stated that the result of too small a blockading dose might result in stimulation rather than depression. The amounts of ink used were greatly in excess of those used by former workers but the ink was always rapidly removed from the circulation. There was no evidence that the reticulo-endothelial system was becoming embarrassed by the blockade.

In all of the results there are only two which require special comment. Two rabbits, 793 and 812, showed a marked fall in haemolysin for sheep's red cells. In so far as all of the specimens collected from these rabbits were tested at the same time at the /

the conclusion of the experiment and since the haemolysin keeps well in the ice chest the results obtained in this examination might be regarded as true titres. In other observations, as for example bactericidal activity and complement, specimens from the same animal were tested at different times. Under the latter conditions deviations which are without significance are bound to occur, but this explanation was not applicable to the haemolysin. It is difficult to assess the significance of these two isolated experiments. There may have been some depression due to the injections of ink but since they were the only significant changes over the whole series of results the general conclusion was drawn that the blockading measures had no appreciable effect on natural antibodies.

In connection with the experiment to test the effect of blockade on haemolysin production following the injection of a small amount of sheep's cells the author used only those rabbits which had little or no natural haemolysin. The injection of this small amount of antigen did not regularly produce antibodies beyond concentrations which might exist normally and much of the previous work on this point would have to be reviewed.

CONCLUSIONS.

Blockade of the reticulo-endothelial system with injections of Indian ink does not depress the activity of natural antibodies or affect natural immunity reactions.

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BLOOD COMPLEMENT IN ACUTE GLOMERULONEPHRITIS

AND OTHER DISEASES.

The clinical material in connection with the investigation into the blood complement in acute glomerulo-nephritis and pregnancy conditions was supplied by W.M. Arnott, B.Sc., M.D., F.R.C.P., and G.D. Mathew, M.B., F.R.C.S.E., and the correlated study was published jointly with them.

BLOOD COMPLEMENT IN ACUTE GLOMERULONEPHRITIS AND OTHER DISEASES.

Many claims have been made that blood complement is reduced in amount in several diseases.

Paul and Pelyi (1925) described a slight fall in complement in allergic states, but the alteration was not great and was not found by Tilden (1934). More pronounced disturbances of complement activity have been described in yellow fever by da Costa Cruz (1929a and b), and this was not due to anticomplementary activity. Goldner (1929) concluded that complement was reduced in amount in all diseases involving extensive damage to the liver, and slight reductions have been described in eclampsia by Goldschmidt-Fürstner (1931). Slight alterations have been described in acute rheumatism by Veil (1932) and by Veil and Buchholz (1932) and in pneumonia by Dick (1912). In most of the above instances the disturbance was insignificant, and very few investigators made any examination of the range of complement activity in normal people.

A very pronounced disturbance in complement activity is found in acute nephritis (Gunn 1914, Kellet 1936, Kellet and Thomson 1939), and this fact may throw some light on its aetiology. Longcope (1929) /

(1929) and his co-workers have observed that the skin-reactivity of patients with glomerulonephritis to filtrates of cultures of haemolytic streptococci is very much greater than in other forms of streptococcal infection, such as uncomplicated tonsillitis. According to Friedemann and Deicher (1928) the blood of patients with postscarlatinal glomerulonephritis contains a greater concentration of antibodies than does the blood in non-nephritic cases of scarlatina at a comparable stage of convalescence. Further, acute glomerulonephritis has been produced in rabbits by a nephrotoxic serum (Masugi 1933, 1934, Arnott, Kellar, and Matthew 1936). The clinical, bacteriological, and experimental evidence, therefore, appears to indicate that diffuse glomerulonephritis is due either to a greatly exaggerated acquired sensitivity of the kidneys to the products of the streptococcus or to the effects of an exceptionally vigorous antibody-antigen reaction, such as might take place during the period of regression of the infection.

It has been suggested, apparently on comparatively little evidence, that pre-eclamptic toxaemia and eclampsia are also due to a similar type of reaction. Pregnancy toxaemias and acute nephritis have several points in common: both of them are associated with albuminuria, hypertension, and oedema, and both tend to /

to progress towards a hypertensive encephalopathy. There are also glomerular changes in eclampsia, which, however, are much less pronounced than in glomerulonephritis (Baird and Dunn 1933, Kellar, Arnott, and Matthew 1937). In the light of these known similarities between acute glomerulonephritis and eclampsia an attempt has been made to determine whether the similarity includes a reduction in blood complement. For this purpose many normal people were examined to establish the normal range of blood complement, which was then examined in cases of acute nephritis, healthy pregnancy, pre-eclamptic toxaemia and eclampsia.

TECHNIQUE.

Various amounts of serum were added to 0.5 cm. of a 3 per cent. suspension of sheep's red cells sensitised by the addition of 7 M.H.D. of haemolytic antibody. The range of serum examined was: 0.02, 0.04, 0.06, 0.08, 0.1, 0.15, 0.2, 0.4 c.cm., and the activity was taken to be represented by the reciprocal of the smallest amount of serum producing complete lysis of the cells after incubating the mixtures for two hours at 37°C.

So that the results of all the tests done over a period should be comparable, a preserved complement was titrated with every batch. The method used to preserve a standard guinea pig complement was that described by Ruffner (1929) and used extensively by Sonnenschein /

Sonnenschein (1930). The preservation is due to the presence of hypertonic solutions, and equal volumes of guinea pig serum and a solution containing 10 per cent sodium acetate and 4 per cent boric acid were mixed.

According as the activity of the preserved complement varied with the suspension of sensitised cells used adjustments, usually slight, were made in the activities of the complements under test.

RESULTS.

Normal Controls.

The complement content of the blood of 126 normally healthy patients in hospital for some minor gynaecological condition was observed. In this control series complete haemolysis of the test amount of cells was obtained with 0.2 c.cm. or less of serum in 122 patients, and in the remaining 4 patients 0.4 c.cm. of serum was necessary to produce complete lysis. In these 4 there was obvious partial lysis with 0.2 c.cm. and with 0.1 c.cm., although 0.4 c.cm. was the amount required to produce complete lysis.

Acute Nephritis.

Of 18 patients with acute glomerulonephritis 5 had their blood complement estimated once, 3 twice, and the remaining 10 three or more times. Of the 5 patients examined only once 1 showed a normal complement content, and of the 3 examined twice 1 showed a normal complement. In each of the remaining 16 a

reduction of complement was found. In many instances there was apparently no complement present at all, but there was no obvious correlation between complement content and any of the clinical features, such as albuminuria, hypertension, and fever. In 2 cases a complete absence of complement activity was found on the same day that the blood-urea was normal showing that the absence of complement was not due to an inhibition of its activity by retained nitrogenous substances. In another case the blood showed only the slightest trace of complement long after the patient had been discharged as cured.

Pregnancy control series.

Complement estimations were carried out on the blood of 157 women at all stages of pregnancy, and all showed a normal activity.

Pre-eclamptic toxaemia.

In 33 patients with typical pre-eclamptic toxaemia, characterised by hypertension, albuminuria, and oedema, who were examined on one or more occasions, no reduction in the complement was detected.

Eclampsia.

Repeated examinations were made on 8 cases of eclampsia during the convulsive phase, and no disturbance of blood complement was found

ACUTE RHEUMATISM.

Fifty cases of acute rheumatism were examined and a total of 174 tests were made so that most patients were examined repeatedly. No evidence was found that the complement was in any way disturbed.

OTHER DISEASES.

Specimens of blood for complement were examined from cases suffering from the following diseases but no alterations were found: -

Pneumonia (Lobar)	10 cases.
Pneumonia (Bronchial)	7 cases.
Scarlet Fever	20 cases.
Whooping Cough	8 cases.
Syphilis	40 cases.

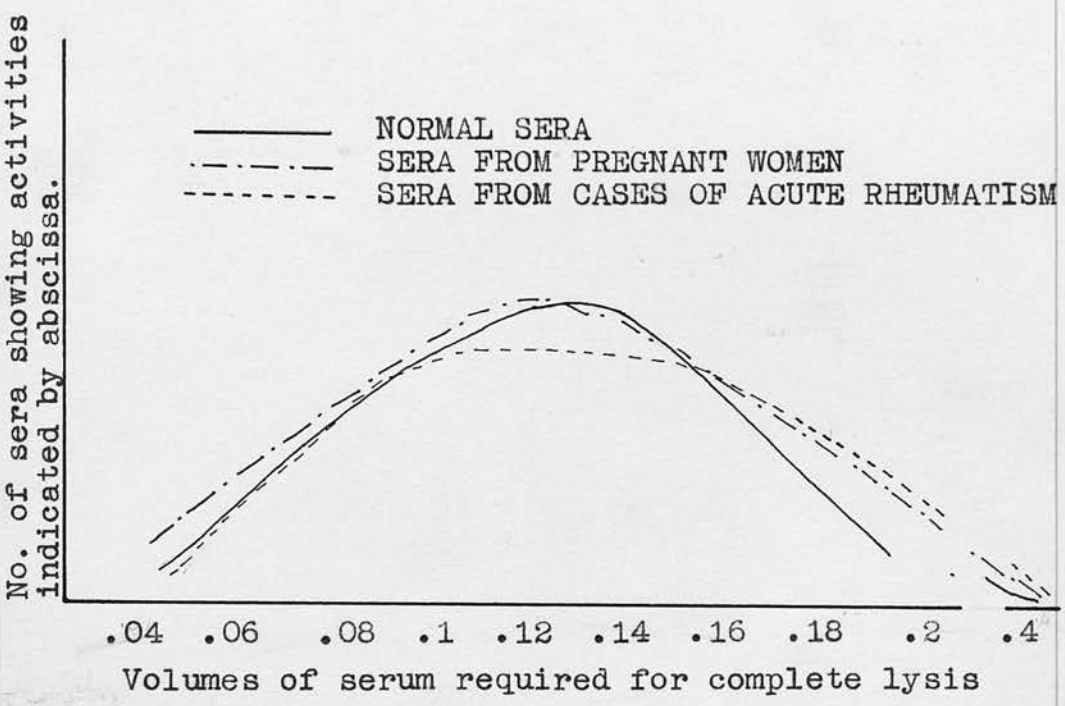
DISCUSSION.

A regular reduction in blood complement was found in acute nephritis and in no other disease, including pre-eclamptic toxaemia and eclampsia. The similarity between acute glomerulonephritis and eclampsia does not therefore include this suggestion of an immunity reaction. It appears likely that the similarity is due to the fact that they both share a widespread arteriolar spasm which is productive of hypertension, albuminuria, encephalopathy and oedema.

It /

It would appear that many of the claims that there is a disturbance of blood complement in many diseases, cannot be easily confirmed. It would appear also that not enough attention has been paid to the variation in activity found in normal bloods.

In the above series for example the three large groups showed activities of blood complement with the following distributions:-



SUMMARY.

Blood complement is regularly reduced in acute nephritis and no such disturbance was found in any other disease examined including toxaemia of pregnancy.

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IMMUNITY TO DIPHTHERIA.

IMMUNITY TO DIPHTHERIA.

In sections 1 and 2 of this thesis are described the results of experiments designed to examine the properties of natural antibodies. The exact relationship of these natural antibodies to the antibodies which arise as the result of specific contact with a bacterium is not clearly defined.

It is possible that in the blood there are present in small amounts a large number of antibodies (not necessarily antibacterial) which are the result of stimulation by an antigen. The antigen may be a chemical substance absorbed unchanged through the alimentary canal as it is now accepted that this can occur.

It is known, however, that certain natural antibodies are inherited according to Mendelian law, as for example the isohaemagglutinins and these cannot be due to antigenic stimulation.

It would be impossible, however, to classify the natural antibodies into those which appear in the blood in the absence of antigenic stimulation and those which conceivably might be due to such stimulation. Reference has been made in the general introduction to the fact that in the interplay between a host species and /

and a bacterial parasite there appear all grades of severity of reaction. The occurrence of infection without apparent damage to the host is a common occurrence and such contact produces some degree of resistance in the host with the appearance of antibodies. It would indeed be interesting if it were possible to separate such antibodies from those antibodies which might more correctly be designated "natural".

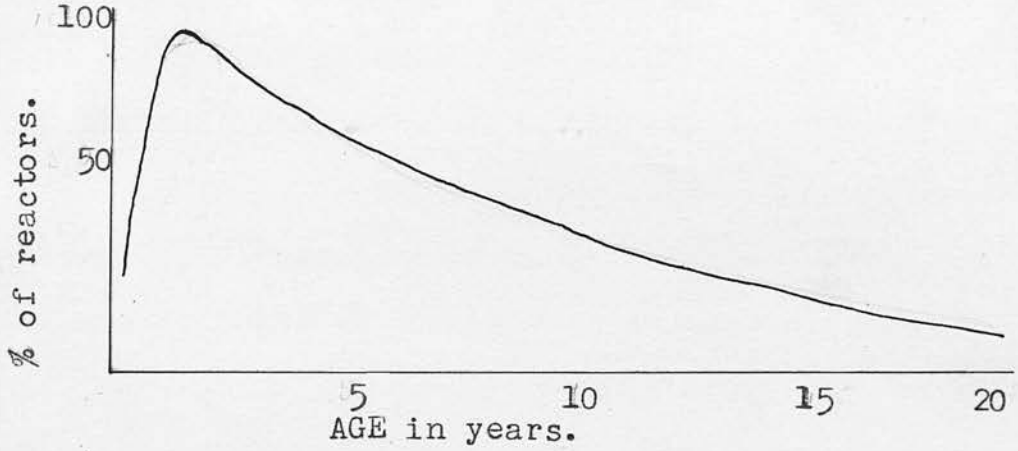
It is of interest to know that the writer developed a Widal reaction positive to a titre of 1:360 to B.typhosus as a result of the experiments described in section 1. This was discovered accidentally by a colleague who was anxious to collect a number of normal sera which would not react with the enteric group. The writer suffered from no untoward effects and needless to say the Widal reaction was negative to B. para A. and B. para B.

Immunity to diphtheria makes an interesting study because many authorities are of the opinion that the Schick negative state (indicating immunity) which develops with age is not due to specific contact with the diphtheria bacillus but due to a normal physiological process. This would mean that an antibody capable of specific neutralisation of diphtheria toxin appears /

appears normally in the blood and its presence there is as inexplicable as the presence of the natural antibodies.

The Schick reaction, however, is of value not only in determining susceptibility or immunity in the individual but also in ascertaining the rate at which antitoxin appears in the blood if large numbers of people of all ages are examined. The simple Schick test, of course, merely determines whether the antitoxin concentration in the blood is above or below a certain amount.

In white races, the number of reactors to the Schick test shows the following distribution



The immunity to diphtheria (as evidenced by the Schick test) in the first year of life is due to a congenital passive immunity which rapidly disappears. The largest group of Schick positives occurs in the age group 2 - 5 years and this corresponds with the age /

age group showing the heaviest incidence of the disease.

The immunity which appears and is responsible for the decreasing number of reactors from the age of 2 onwards is an active immunity.

Two views have been advanced to explain the rising "herd" immunity. One view is that the immunity is due to some physiological maturation process and develops in the absence of any specific contact with the diphtheria bacillus. The other view is that the rising immunity is due to specific contact with the infecting agents with resulting subclinical or latent infections. The evidence on the subject is confusing.

A correlation between blood groups, which are inherited according to Mendelian law, and Schick reactions in young children has been described by Hirszfeld, Hirszfeld & Brokman (1924) and the correlation is advanced as evidence to support the view that the reaction to the Schick test is determined by a normal physiological process. This view has been challenged by Rosling (1928).

Much of the evidence in favour of the view that immune persons (Schick negative) acquire this state in the absence of specific contact has been obtained by investigating the reactions in races in whom diphtheria is rare. Thus, Schick tests have been carried out amongst /

amongst the Eskimos by Heinbecker & Irvine-Jones (1928) and Asbelew & Margo (1932); in East Africa by Kleine & Kroo (1930); in Southern Africa by Grasset (1933) and Grasset & Perret-Gentil (1933); in Annam by Souchard & Tournier (1937); in Katanga by Serra (1936); in Indo-China by Vaucel, Joyeux & Hoang-tich-Try (1936) and in Nigeria by Cauchi & Smith (1934). All of these workers consider that the Schick negative state develops with age in persons of these races though diphtheria is rare or even unknown, at approximately the same rate as it does amongst Europeans. Asbelew & Margo (1932), in addition to determining the Schick reaction, took throat swabs from 200 Eskimos, but failed to isolate C. diphtheriae. They claimed, therefore, that not only was diphtheria unknown in the community, but that the diphtheria bacillus was absent. For the latter conclusion, however, their survey was rather small.

The results of Schick tests in countries where there is more than one race are of special interest. In Formosa, Sugie, Honda & Kawai (1937) performed over 30,000 Schick tests in children up to 15 years of age and found 85% of positive reactors in Japanese children and 44% positive in the Chinese children. They found also, however, that the Schick-positive rate /

rate in Japanese children born in Formosa was lower than that found in children born in Japan.

In New Zealand, Turbott (1931) found 80% of white children Schick-positive as against 10% of pure Maori children. In children who were half-breeds he found 23% positive. This work was done in a somewhat isolated district where diphtheria is very rare amongst the Maoris.

On the other hand, there is no doubt that latent infections can play a very definite part in immunizing against diphtheria. Dudley (1923) found that the number of reactors to the Schick test in a semi-closed community decreased considerably following an outbreak of diphtheria. Dungal (1932) in Iceland, where diphtheria is rare, recorded the effects on the Schick test of an epidemic of diphtheria which had occurred eleven years previously. He found a very slow rise in the percentage of non-reactors between the ages of 5 and 12 years. In children aged 12, however, there was a sharp increase in the number of non-reactors, presumably because they were just old enough to have experienced the epidemic eleven years previously. Three years later, Dungal & Sigurjonsson (1935) did not find this sharp rise in incidence of non-reactors in that age group.

Of /

Of interest too, are the marked differences, in results obtained by testing different social classes of the same race. Zingher (1923) determined the Schick reactions of school children and found many more reactors amongst the more fortunate classes than amongst the less fortunate.

Pulley & Fleisher (1938) found that 40% of students in St. Louis University were still Schick-positive and that there was some correlation between the reactions and the size of town in which the student had lived.

It is of obvious interest to know if the rate of immunisation (ratio of Schick negatives to Schick positives at various ages) is the same in all social classes. If the view is correct that the rising immunity (production of antitoxin) is a normal physiological process then one would expect that there would be no difference in the ratio non reactors : reactors, between different social classes in the same race or community. If, however, the correct view is that the rising immunity is due to specific contact with the C.diphtheriae in the absence of overt disease then one would expect that the immunity would develop more quickly in the less fortunate social classes due to their conditions of living. At any given age children from any social class will show more /

more reactors than children from a less fortunate social class.

To examine this point Schick tests were carried out on two groups of youths, viz. medical students aged 20 - 22 and militiamen, aged 20.

The militia-men were chosen for the test not only because they were the same age as the students but also because they represent a "cross section" through the community. Youths from all social classes would be present in the proportions in which they occur in the population at large.

The medical students were examined in their third year of University life and the militia-men were examined about three weeks after the commencement of their training.

RESULTS.

In the following table are given the results of tests on those who gave no history of diphtheria and no history of immunisation.

Class	Total	Schick +	Schick –	Percentage positive.	P.
Students	243	171	72	70	$p_1 = .7$
Militia-men	103	37	66	36	$p_2 = .36$

The /

The standard deviation of $p_1 - p_2$

$$\text{is} \quad \sqrt{\frac{.7 \times .3}{243} + \frac{.36 \times .64}{103}}$$

$$= \sqrt{.003102}$$

$$= .056 \quad (2)$$

$$p_1 - p_2 = 0.34$$

$$= 6\,2$$

The odds against the above difference being due to the interplay of chance are in the region of 1,000,000 to 1.

The high incidence of reactors among the students as compared with that amongst the militiamen can be interpreted in terms of living conditions for the students on the whole were drawn from more privileged social classes and had not been as exposed to infections as had been the average member of the community.

The incidence of 36% amongst the militia-men was higher than expected from previous experience of the test in the general population but it must be noted that Edinburgh is a residential city and has a higher proportion of more fortunate social classes than would be found in other cities and the militia-men were drawn from Edinburgh and district.

DISCUSSION /

DISCUSSION.

Immunity to diphtheria can be correlated directly with the presence of antitoxin in the blood. Even in the absence of clinically recognizable attacks of diphtheria the majority of people are immune before reaching adult life. One view put in explanation of this fact is that the immunity develops as a normal physiological property of growth, the other is that the immunity, which increases with age, is due to contact with the respective infecting agents. The evidence quoted appears to show that specific contact undoubtedly plays some part. Whether or not it plays the whole part is still undecided.

The observations recorded here are of particular value because the tests were made on young adults at an age when the majority of people do not react to Schick toxin. If antitoxin can be produced by physiological processes it should be present in the great majority of persons who have reached the age of 20. Moreover, although the physiological production of antitoxins might conceivably differ in various races, there ought not to be marked differences between different classes of the same race. On the other hand, if it is admitted that specific contact with the infecting agent can produce a rise in immunity /

immunity among people who are more than usually exposed, we should expect to find fewer reactors.

If the view is correct that there is a rising immunity due to a physiological process then the students must be accepted as a population approaching normal and their degree of resistance would be due to physiological activity plus some minor contribution from specific contact. The militia-men would owe their degree of resistance to the same physiological process plus a larger contribution from specific contact. The fact that 70% of the students at age 20 were Schick positive suggests that physiological processes play no part in determining the immune state.

If specific contact alone determines the grade of immunity, then the militia-men seem to represent an average population and the medical students would be considered a biassed group in that they had been less exposed to infections in their upbringing than had the average individual of their age.

The latter interpretation would appear to be the rational one.

SUMMARY.

(1) Great differences in the number of young adult reactors to the Schick test were found in different /

different social classes.

(2) In white races the immunity to diphtheria (as evidenced by the Schick test) in the absence of previous overt disease seems to be caused wholly by contact with the C. diphtheriae.

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