On some views of the relation of humoral action to phagocytosis together with some remarks and observations on phagocytosis in vitro.

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PART I

In an investigation of the mechanism of immunity against the Streptococcus Pyogenes, Denys and Leclef (1) concluded that, in vitro, normal rabbit serum was not bactericidal for that organism nor did it impede its growth. But the addition of pleural-exudation cells produced retardation of growth or even a destruction of the microbes (as judged by the plate method). "The more we add leucocytes to the serum, the more development is hindered. If the quantity of leucocytes is sufficient we can even detect a transient diminution of the number of microbes." Denys and Leclef lay stress on the fact that retardation of growth or destruction of the streptococci in such case depends on the actual presence and vitality of the leucocytes (i.e. is not due to a leucocytic secretion). The increase in the number of streptococci does not follow the death of the leucocytes, but "generally the multiplication of the organisms begins when the leucocytes are still in full possession of their motility as demonstrated by examination on the warm stage."

Yet one fails to gather from this account by what means this retardation of growth, or it may be destruction /

(1) La Cellule XI p.175 (1895 Sur le Mécanisme de l'Immunité chez le Lapin Vacciné contre le Streptocoque Pyogène.

is brought about; there is no bactericidal action of the serum, by itself, and phagocytosis in such serum is very feeble (both in vitro and in corpore). In contrasting the behaviour of leucocytes in normal and in immune rabbit serum they say, "to appreciate the influence, which results from change of serum, let us first consider what takes place between microbes and corpuscles of an untreated animal, in the serum of an untreated animal, when a medium quantity of organisms is added. As regards the streptococci, one sees an arrest of development but generally this arrest only lasts some hours and gives place to a very marked increase. As regards the leucocytes, there is found an absence of phagocytosis or a phagocytosis of no great vigour; yet these cells have not ceased to show signs of life (amoeboid changes), and it is not uncommon to find them all living, with no sign of feebleness or degeneration, when the fluid has become a veritable culture. But if the leucocytes of the normal rabbit are suspended in the serum of the vaccinated animal, the result is extremely different. As regards the streptococci, increase is delayed as long as the leucocytes remain alive, or at least as long as their vitality is not impaired. Indeed, multiplication of the organisms is not only delayed but the diminution of the number of colonies, on the plates, points to an active destruction of the streptococci. As to the leucocytes, one finds an /

an active phagocytosis and generally a distinct prolongation of life, in comparison with the leucocytes of the same animal immersed in their own serum". With regard to phagocytosis in vitro they conclude that "the leucocyte of the normal animal bathed in the immune serum gains, in presence of the streptococcus, a potency altogether new". Again, "the normal white corpuscle acquires, in the immune serum, all the potency of the leucocyte of the vaccinated animal; the leucocytes of immunised animals possess their potency in virtue of the properties of the serum". In normal serum the leucocyte of the immunised animal behaves just as the "normal" leucocyte in normal serum. This has been affirmed in quite another quarter (1) and has been termed "the indifference of the leucocyte". Neufeld and Rimpau (as also Bulloch and Atkin loc.cit.) extended this "indifference" to various species of leucocytes in one and the same serum (immune serum, N. & R.: normal human serum B. & A.).

With the further findings of Denys and Leclef one is not here specially concerned; these are concerned with the mechanism and immunity as investigated in corpore. These are contradictory, phagocytosis being apparently absent in the pleura of rabbits both immunised /

(1) Proc. Roy. Soc. vol. 74 (1905) Bulloch and Atkin.

immunised and normal, when streptococci were injected there, whereas in the subcutaneous tissue of the ear in the immunised rabbits plagocytosis is prompt and vigorous; this Metchnikoff (1) comments upon; he seems to fall into error, however, in saying, that the pleural surface "should have been scraped in order to ascertain whether the phagocytic reaction was localised in this region". Denys and Leclef state (p.214) that they did this carefully and did not find any more organisms on the parietal pleura than in the exudate. Another experiment of Denys and Leclef is interesting; they state that the afflux of leucocytes occurs equally in normal and in immunised animals but that phagocytosis occurs only in the immunised. A rabbit is killed one or two days after an intra-pleural injection, when the symptoms of a fatal issue are evident: its pleura is opened and the exudate obtained; "very often this contains motile leucocytes alongside the streptococcus culture. This exudate is divided into two portions; to the first is added a little blood or serum from a normal animal; to the other a little blood or serum from an immunised animal, and the two portions are placed in the incubator. After half-an-hour or more one can see that the leucocytes of the tube made up with blood from the immunised rabbit are crammed with streptococci, while those of the control tube /

(1) "Immunity in Infective Diseases." Eng. trans. 1905, p.246.

tube still show for the streptococci all their former indifference".

Finally, one would note that Denys and Leclef reject the theory that the immune serum acts as a "stimulin" and maintain that, "the immunised animal resists the streptococcus (i) by a direct action of the serum, (ii) by its leucocytes. The latter always owe their activity to the serum".

The following is part of Metchnikoff's criticism (1) of the foregoing investigation; it might be applied, one does not know whether justly or not, to much that is referred to below. "Researches extending over a long period have shown us that the study of phagocytosis in vitro can give only a very inexact and imperfect idea of the course of the phenomena in the living animal. Usually the leucocytes taken from the exudations, although amoeboid, no longer fulfil their phagocytic functions at a time when in the animal they would ingest micro organisms with the greatest rapidity. As a general rule existence outside the living body weakens them very considerably. But in some cases, rare it is true, the leucocytes, although inactive in the animal, exhibit intense phagocytosis when introduced into a hanging drop of fluid from an exudation or even of urine. In any case it /

(1) "Immunity in Infective Diseases". Eng. trans.1905, p. 284.

it is very hazardous to infer from phenomena which appear under these artificial conditions what takes place in the living animal". Metchnikoff's objection that clumping of the streptococci in the immune serum might simulate partial destruction is probably correct: Neufeld and Rimpau accept it. It seems now to have been established that immune serum exerts no bactericidal effect upon the Streptococcus Pyogenes.

Metchnikoff's view of the order of things in acquired immunity against micro-organisms is expressed as follows:- (1) "In connection with my work on immunity against the micro-organism of swine pneumo-enteritis I was able to demonstrate that the serum of vaccinated rabbits, incapable of preventing the multiplication of the specific cocco-bacillus, is also powerless to deprive it of its virulence: it is without the power of causing its agglutination or of neutralising its toxin. In short, this serum appears to exercise no direct action on the micro-organism, yet, in spite of that, it prevents its pathogenic action. With these results before me, I was led to assume a certain stimulating action of the serum on the defensive elements of the animal organism and especially on the phagocytic system. The discovery of the fixative property of serums would lead us to believe /

(1) Immunity p. 270 & 284.

believe that this stimulation was entirely useless and that the permeation of micro-organisms by the fixative was amply sufficient to bring about their destruction and removal from the animal. A living micro-organism in its normal form, endowed with full virulence and provided with its fighting weapon, the toxin, but at the same time permeated by the fixative substance, might behave in the animal in some special way. It might excite a strong positive chemiotaxis of the leucocytes and be ingested and destroyed by these cells with greater facility. A priori, there would be nothing to object to in this view, but certain facts are opposed to it. Thus, in the case of micro-organisms just cited, (swine erysipelas: Mesnil. anthrax: Sawtchenko), we see bacteria permeated not only with the fixative but also with cytases, capable of producing a fatal infection. We are thus compelled to accept the theory of an influence of protective serums not only on the micro-organisms but also on the organism of the animal into which they are introduced. As this influence manifests itself in the form of a strong phagocytosis, it is only natural that we should attribute it to the existence of a stimulating action of the serums of vaccinated animals on the phagocytes of the normal animals. Also, an adaptation of the leucocytes is invoked /

invoked (p 107 & 558). Bacteria treated with a specific serum and thereafter freed of that medium retain all their original virulence (Gentilly coccibacillus and the Pneumococcus: Issaeff. Gamaleia's Vibrio: Samarelli.:Streptococcus: Bordet. Swine-erysipelas: Mesnil.) This view - based on in vivo tests - is opposed to the "opsonic" hypothesis developed by Wright and Douglas and corroborated and extended by Bulloch and Atkin (v. infra). On the other hand, in natural immunity "the phagocytes act, so to speak, motu proprio (p.201): ..... they enter into a struggle against the micro-organisms and rid the animal organism of them without requiring any previous help on the part of the body fluids", (p.206): i.e. without antitoxins agglutinins alexins or fixatives. Dean (in loc. cit. infra.) remarks that this statement with regard to natural immunity "that the leucocytes free the organism of bacteria without aid from the humours" is apparently largely based on Bordet and Gengou's results obtained by their method of testing for the presence of "fixateur". The results obtained by the use of that method are not above criticism. That the amount of fixateur present in normal serum compared with immune serum is small, is true, but, as suggested by Savtchenko, the amount necessary to prepare the organism for phagocytosis may be /

be small compared with that necessary for bacteriolysis".

According to Metchinkoff, the non-occurrence of phagocytosis, where leucocytes and micro-organisms are brought together, is due to a reaction between the microbe and the phagocyte, - "a negative chemiotaxis" -.

Recently, the idea that "bacterio-tropic" substances in the body fluids are necessary for the occurrence of phagocytosis, has been maintained in several quarters. Here we have various attempts to link together the humoral and the phagocytic theories of immunity. This is definitely the stand-point of Neufeld and Rimpau (1): "there is very often a third kind of specific serum action (i.e. besides antitoxic and bactericidal)..... this in principle, according to our view, is nearly related to the bactericidal type but requires, as contrasted with this, a direct cellular co-operation and our decisive experiments are linked to older researches which originated entirely under the influence of Metchnikoff's phagocyte theory". Neufeld and Rimpau repeated some of the interchanged-cells-and-sera experiments of Denys and Leclef /

(1) Deutsche Medizinische Wochenschrift nr. 40: 1904 Ueber die Antikörper des Streptokokken-und-Pneumokokken-Immunserums (for this view cf. also Gruber & Futaki: Wright etc.)

Leclef: they also carried out absorption experiments and found that leucocytes, treated with immune serum for 20 minutes at 37°C and thereafter washed and placed in normal serum, were unable to take up the virulent streptococcus whereas, the streptococcus, so treated and the serum removed by washing with saline sulution, was actively ingested by leucocytes in normal serum. "The specific serum then acts, not as a stimulin for the leucocytes but directly on the bacteria which now secondarily are taken up by the cells." The serum was "inactivated" by heating for 30 minutes at 59° C and phagocytosis went on as before. Accordingly, "the bacteria take no complement from the serum but exclusively a relatively heat-stable substance, which we ought to place in analogy with the immune bodies of Pfeiffer". (This result was not due to killing of the streptococci, for killed streptococci behaved just as the living under the given conditions, i.e. phagocytosis did not occur in normal serum nor in saline solution and first appeared when specific serum was added.) But it may be noted, (and in this is an objection to these cell-exudation experiments viz.- the cells are often less active than human leucocytes got by the method of Wright and Douglas,) that, even with a little-virulent race of streptococcus, Neufeld and Rimpau did not find phagocytosis in normal serum.

Hektoen /

Hektoen and Ruediger [1] employed mixtures of blood and exudation-cells from a number of animals: leucocytes from certain rodents (= blood cells + exudation cells) were not nearly so potent as human leucocytes. The latter, doubtless, are obtained by a more efficient method. As a digression, one would like to quote here a table given by Hektoen and Ruediger: their experiments are somewhat inexact in method but a salient fact like the following is of interest.

	Human Leucocytes	Guinea-pig leucocytes	Rabbit leucocytes	Dog leucocytes	Goat leucocytes	White Rat leucocytes	Horse leucocytes
Strept. 300	24	50	20	50	30	40	50
Strept. 381P	23	0	0·5	25	...	5	...
Strept. 104	20	2	0	13	0	0	4

Savtchenko [2] states that if a quantity of a "saprophytic" race of any septicaemic organism be introduced /

(1) Journal of Infectious Diseases 1905 vol. 2 p 128.
(2) Ann. de l'Inst. Past. 1902- p 108.

introduced into the abdominal cavity of an animal, at first it is ingested, later this is not so. Gruber and Futaki[1] in examining the phagocytic activity of guinea-pig leucocytes in normal guinea-pig serum (in vitro) found that many different kinds of organisms even virulent strains, e.g. of typhoid, were freely ingested in the active serum but that, in most cases, in the inactivated serum phagocytosis was greatly diminished or almost nil. In the case of two virulent races employed (Fowl cholera and Asiatic cholera) phagocytosis took place neither in active nor inactivated serum. They emphasise the thermolability of the elements in normal serum which are concerned in phagocytosis. On the other hand Dean in his experiments used two races of Staphylococcus "one highly virulent, recently isolated from the human body, the other an old laboratory culture. No marked differences were observed in the counts obtained from these". To return, Neufeld and Rimpau consider that their type of immunity may be extended to the "septicaemic" infections in general. One may remark that they suggest that the "amboceptor-laden" bacteria are complemented within the leucocytes. In their second communication Neufeld and Rimpau[2] describe the /

(1) Munch. Med. Wochenschr Feb. 1906 "Seroaktivität und Phagozytose"
(2) Zeitschr. f Hyg. u Infectionskranktn. Sep. 1905.

the sera which they have examined as "bacteriotropic" sera, in opposition to the bacteriolytic sera: "the action is not bactericidal nor strictly antitoxic: the bacteriotropic substances act on the receptors of the bacterial cell and on these the virulence of the organism depends".

Mennes (1) used the method of Denys and Leclef in his investigation of the immunity of rabbits against the Pneumococcus, with the result, that retardation of growth or decrease in the number of pneumococci was noticeable, not in normal serum nor normal serum with leucocytes nor even in immune serum, but only when white blood corpuscles (pleural exudate) were added to immune serum, no matter whether the leucocytes came from a normal or from an immunised animal. "The primary immunising element is the serum and not the leucocytes." On the warm stage he found that there was no phagocytosis in the serum of a normal rabbit, but in immune serum "the leucocytes fall almost with animosity, (fast mit Erbitterung), on the pneumococcus.

(1) Zeitschr. f. Hyg. u. Infectionskrankhtn. 1897 p 426 "Das Antipneumokokken-Serum und der Mechanismus der Immunität des Kaninchens gegen den Pneumococcus"

Denys and Marchand [1] found that the serum of the horse immunised against the streptococcus was in no way bactericidal: in this they considered it differed from that of the immunised rabbit. Such immune horse serum "contains a substance which renders the leucocytes of the immunised (passively) animal extremely active". So also Bordet [2] found that such preventive horse serum was not at all bactericidal: it was only feebly, if at all, agglutinative. He found, in opposition to Denys and Leclef, that phagocytosis in vitro went on in normal rabbit serum apparently as well as in mixtures containing preventive serum. In both kinds of mixtures growth of the organism ultimately took place.

Further, phagocytosis of the streptococcus (Marmorek's streptococcus: highly virulent) went on fairly actively in the peritoneal cavity of the normal unprotected rabbit, in which a leucocytic exudation had been previously caused: at least this was so if the dose of organisms was small (p.201). Bordet states that the liquid portion (sérosité) of such a cellular exudate is bactericidal, "as Denys and Leclef found" (?). Even if a copious inoculation were made from a young /

(1) Bull. Acad. roy. de méd. de Belg. 1896.

(2) Ann. de l'Inst. Past. 1897 "Contribution à l'étude du sérum antistreptococcique".

young culture on such "serosite" next day the liquid was sterile. This action does not take place in vivo.

Organisms grown (24 hr.) on a mixture of preventive serum and broth were found after filtering and washing to be highly virulent, apparently as virulent as those grown on broth and normal horse serum. On this experiment Metchnikoff lays great stress: but Bordet remarks that, from it one is not justified in concluding that in vivo the serum can exercise no attenuating action. It is not stated that the animal employed for the test was previously "prepared" by intraperitoneal broth injection and even in the animal protected by previous inoculation of preventive serum, injection of the streptococcus into the peritoneal cavity is said to be much more dangerous if there be no recent cellular exudate (p.203). This experiment is somewhat discounted by another in which the organisms were mixed with the preventive serum and allowed to remain so mixed for some hours before injection: as compared with a rabbit (A) which received, separately, first preventive serum and later the organisms, the first rabbit (B) survived for a definitely longer period: both rabbits had received preliminary intraperitoneal broth injections and rabbit (B) was injected with /

with normal horse serum at the time when the other (A) received preventive serum. This experiment was repeated several times with the same result. Phagocytosis in the first rabbit (B) is more complete and more prompt: it seems to take place more easily than in the other rabbit (Bordet).

However although the preventive serum in vitro "causes no profound change in the streptococcus", in the peritoneal fluid of the protected animal morphological changes are often seen. In the struggle a smaller race of streptococcus appears and at the period when the "phagocytic crisis" sets in it is chiefly those which are ingested. If the case is to terminate fatally, normal forms (?25–35 hours after onset of infection) are still found free: these are surrounded by a stainable aureole (v.figs. 4 and 5 p 212 Ann. l'Inst. Past. 1897). In the earlier part of his communication Bordet emphasises the co-relation of the "aureole" with negative chemiotaxis.

Metchnikoff (1) cites the work of Bordet and of Salimbeni on the mechanism of immunity against the streptococcus: "in presence of these precise results, the researches of Denys and Leclef are deprived of their importance". §Phagocytosis in this particular case would seem to play a cardinal rôle.

In /

(1) Immunity p. 246 (Eng. trans.).

In his research on the bactericidal action of serum, in particular on the immunity of guinea-pigs against the Cholera Vibrio, Bordet (1) found that these organisms injected into the jugular vein of immunised guinea-pigs were ingested by the phagocytes with extraordinary rapidity and that Pfeiffer's phenomenon in such case was insignificant in the general blood-stream. The presence of leucocytes was closely related to the bactericidal power of serum and exudates. Levadidi (2) in a similar research, but at a date when the phenomena of haemolysis were known, besides repeating this experiment of Bordet's found, among other things, that "sensitised" organisms injected into the blood stream of a normal animal, phagolysis having been averted by a previous broth injection, were ingested in similar fashion without the occurrence of extracellular lysis: also, that the bacteriolytic power of normal sera was due to the intervention of the complement and a normal "sensibilisatrice". Metchnikoff considers that in acquired immunity the blood plasma contains fixatives: these aid the action of the cytases which are intracellular in /

(1) Ann. de l'Inst. Past. 1895 "Les leucocytes et les Propriétés actives du Sérum chez les Vaccinés.

(2) Ann. de l'Inst. Past. 1901. "Sur l'état de la cytase dans le plasma des animaux normaux et des organismes vaccinés contre le vibrion cholérique".

in the living animal and only exceptionally and abnormally free in the blood plasma or in exudates (p.556 et seq.)

On the other hand there is no detectable fixative in normal serum and in view of this Metchnikoff suggests three possible conditions: (1) that the cytases can act without the help of any fixative. This he seems to dismiss on the ground of a certain observation of Bordet and Gengou's: B Proteus Vulgaris withdrew no cytase from normal guinea-pig serum yet was greedily phagocyted in the peritoneal cavity of a guinea-pig: "since fixative is absent from the serum and since, nevertheless, it must exist for the needs of digestion, it must clearly be concluded that (2) it is found inside the phagocytes." (3) "Its quantity is perhaps so small that when it has passed into the serum its action is entirely lost or nearly so. Fresh researches are necessary to elucidate this delicate point" (p.202). (The enumeration (1), (2), (3) is not in Metchnikoff but is inserted here for convenience).

Savtchenko,[1] in a study of the phagocytosis of guinea-pig red blood-cells under the influence of the serum of rabbits treated with guinea-pig erythrocytes, is led to the conclusion that "the most probable hypothesis is that the immunising fixative substances are stimulins for the phagocytes in that, on the one hand, they show a special affinity for their specific object, for example the microbe, on the other hand, for the protoplasm of the phagocytes and in particular for the cytase contained in these. From this point of view immunising substances serve in the phenomenon of phagocytosis as intermediaries between the object phagocyted and the phagocyte. For this reason they quite deserve the name of "intermediary bodies" (Zwischen Körper) which Ehrlich, led by consideration of their humoral action in immunity, has given them."

Wright[2] objects to the terminology here introduced (i.e. "le fixateur" and "la substance sensibilisatrice" "(1) as suggesting that the phenomena of phagocytosis are analogous to those of haemolysis: (2) that like amboceptor the "incitor" substance (v. infra) exerts its specific effect only in the case where it is reinforced by complement; (3) that the mechanical /

(1) Ann. de l'Inst. Past. 1902" Du Role des Immunismes (Fixateurs) dans la Phagocytose.

(2) Proc. Roy. Soc. vol. 77 Jan. 1906.

mechanical movements of the phagocyte in ingestion of particulate matter are analogous to the chemical action of the complement." The point of this last objection is perhaps not quite apparent.

In a previous publication (1) Savtchenko had concluded from experiments in vitro and in vivo that, in the case of guinea-pigs immunised (mostly passively) against the spirillum of relapsing fever, extracellular destruction of the organism in peritoneal exudate could be averted by the injection of 3 c.c. of bouillon 12 hours or so before the inoculation with organisms. In such exudate spirilla (added in serum) show no Pfeiffer's phenomenon even if normal guinea-pig serum be simultaneously added; the spirilla are ingested by the leucocytes from the outset.

"The immunising substance (la "philocytase", le fixateur") in the free state does not exist in the peritoneal cavity in presence of leucocytes or at least it exists in too small quantity to show its action on the microbes:.....it is taken up by the leucocytes and for this reason one finds no Pfeiffer's phenomenon in the peritoneal cavity" (or in exudate in vitro). The same intermediary action is stated as in the later communication.

Phagocytosis /

(1) Ann. de l'Inst. Past. 1901; Sartchenko & Melkich "Etude sur l'Immunite dans la Fievre recurrente".

Phagocytosis of the spirilla by guinea-pig leucocytes cannot be elicited in vitro and only slowly in vivo in the case of normal guinea-pig exudation: on the other hand organisms in the fluid of the immunised animal under the given conditions show no sign of lysis and are readily phagocyted. Unfortunately for purposes of comparison the guinea-pig possesses competent natural resistance to the organism.

The research on the phagocytosis of red blood cells is of great interest; Savtchenko found on examining the phagocytic action of the leucocytes in guinea-pig peritoneal exudation resulting from injestion 24 hours previously of a mixture of bouillon and haemolytic serum that such leucocytes washed free of exudation-fluid ingested in vitro normal red blood corpuscles of the guinea-pig. That the leucocytes had really absorbed "fixateur" was indicated by the fact that normal guinea-pig blood allowed to clot after admixture with such washed leucocytes yielded a serum which had a slight but definite haemolytic property. Again, leucocytes from a normal guinea-pig ingested very few of the red blood corpuscles of the guinea-pig which had been treated with the haemolytic serum Savtchenko sums up: "fixateur can determine phagocytosis of elements which are not phagocyted in the normal state and that in two fashions": that is to say /

say as stated above by modification of the specific element, microbe or red blood corpuscle, and secondly (the condition which obtains in the first stages of poisoning with haemotoxins and which results in phagocytosis of red cells without symptoms of haemolysis) by modification of the leucocyte. One should note also that Savtchenko was able to demonstrate both in corpore and in vitro that washed red corpuscles of the guinea-pig treated with the specific serum (which had been heated for half-an-hour at 55° C.) and thereafter well washed with saline solution were taken up by normal guinea-pig leucocytes: in the in vitro experiment the leucocytes were the washed cells of peritoneal exudate: in the other case (in vivo) lysis was in evidence or not according as the guinea-pig had not or had been prepared by a preliminary bouillon injection. Savtchenko suggests also that the amount of "fixateur" necessary to prepare the special element for phagocytosis may be small compared with that necessary for lysis. However, as the conditions are not those of active immunity Savtchenko's conclusion that "fixateur" may act on the leucocyte does not directly confute the view expressed by others that the leucocyte is indifferent in acquired immunity (active).

Barratt /

Barratt (1) expresses the view based on an experiment in which he demonstrated in vitro phagocytosis of hen's erythrocytes in dove anti-serum (which however was not haemolytic although the dove some time previously had received a series of injections of hen's erythrocytes: the serum might be called "sub-haemolytic") that amboceptor was not necessary for phagocytosis.

One could object to this conclusion for the reason just mentioned. Further, the serum should have been "inactivated" by heating to 58° C for 30 minutes as was done with the normal sera in his other experiments. More weight might be attached to the experiments in which normal rabbit serum - haemolytic for goat and guinea-pig erythrocytes - was found after inactivation not to give rise to phagocytosis in such mixtures as rabbit leucocytes + goat erythrocytes + inactivated rabbit serum. However it is conceivable that in this case the action of complement preponderates very greatly and that the amount of amboceptor is really quite small, as small indeed as the quantity of erythrocytic opsonin which Barratt states is present in certain normal sera and which can only be detected by a special manoeuvre. In that case the facts would be in analogy with Dean's results for ~~fresh~~ normal? and immune serum /

(1) Proc. Roy. Soc. vol. 76 1905. "The Phagocytosis of Red Blood-Cells".

serum with regard to staphylococcus....."The loss (by heating) in the case of sera such as normal sera which contain only a small quantity of the substance which influences phagocytosis is so great that a method where very small quantities are used makes the demonstration and estimation of the substance impossible". In other anti-erythrocyte sera Barratt showed that digestion with the specific red cells removed the substance which caused phagocytosis. After such digestion different kinds of leucocytes could be used to demonstrate phagocytosis of the red cells. Leucocytes withdrew the substance much less than specific red cells if indeed they did so at all. But the treated leucocytes were not tested ; only the serum in which the leucocytes had been incubated. This therefore is no direct refutation of Savtchenko's conclusion: bulk for bulk serum conceivably could "sensitise" more leucocytes - perhaps an almost indefinite amount - than red cells. Further Savtchenko's was an "in vivo" test as regards the leucocytes this is "in vitro".

Barratt refers this substance to the "group of opsonins first described by Wright and Douglas". Barratt states that erythrocytic "opsonins" are present in normal sera in relatively small amount: this was demonstrated by the employment of a large quantity of serum (inactivated) as compared with the bulk of erythrocytes. /

erythrocytes. It may be noted that the substance seems to withstand, partially at least,- e.g. 4 sera out of 6 resisted heating to 69°C for 30 minutes - higher temperatures than the bacterial "opsonins".

A new phase of immunity has arisen with the description by Wright and Douglas (1) of a new mode of action of the body fluids on bacteria. The main arguments brought forward by Wright and Douglas were corroborated and extended by Bulloch and Atkin (2). Having found that human serum, even after anti-staphylococcus inoculations exerts no bactericidal effect on Staphylococcus Pyogenes; Wright in co-operation with Douglas proceeded by a very ingenious method (v.infra) to examine the respective rôles of phagocytes and blood fluids in phagocytosis. The standard was not immune serum but normal serum. In contrast with much that has been alleged by those who have used the exudation cells of animals,it has been found that in normal human sera (and indeed it has been /

(1) Proc. Roy. Soc. Vol. 72 (1903) "An Experimental Investigation of the Rôle of the Blood Fluids in connection with Phagocytosis": Proc. Roy. Soc. vol. 73 (1904) "Further Observations on the Rôle of the Blood Fluids in connection with Phagocytosis. Also Proc. Roy. Soc. vol. 74 (1904) "On the Action exerted by the Human Blood Fluids on the Tubercle Bacillus etc." and "On the Action exerted by the Human Blood Fluids on the Staphylococcus Pyogenes etc."

(2) Proc. Roy. Soc. vol. 74 (1905) "Experiments on the Nature of the Opsonic Action of the Blood Serum".

been found for other cells and sera) white blood corpuscles as obtained by and employed in this method commonly show phagocytic activity with regard to many organisms. Definite elements in normal blood serum (and other body fluids) were postulated for the production of this phagocytosis and to these the name "opsonins" was given (from "opsono" I cater for: the blood fluids are considered to modify the bacteria in such a way that they become available food for the phagocytes: in using the term with respect to immune sera Gruber and Futaki refer to this action thus,-... ..."Opsonine welche die Bakterien zu einer den Phagocyten schmackhaften Speise machen"). The data for this hypothesis are broadly as follows:- leucocytes in serum which had been heated 10-15 minutes to 60°-65° C and then cooled, showed for staphylococcus pyogenes a greatly diminished phagocytic activity or indeed none at all. The thermolabile element was held to act not on the leucocyte but on the bacteria because inactivation of the serum after mixture with bacteria had no effect in preventing ingestion of these bacteria in the heated serum (mixtures containing bacteria heated in previously inactivated serum showed only traces of phagocytosis). However in their communication (1) which deals with the phagocytosis of tubercle bacilli Wright and Douglas /

(1) Proc. Roy. Soc. vol. 74.

Douglas found in the three experiments they record a distinct fall in the phagocytic count where bacilli were heated after incubation with unheated serum: this they say "is at present without explanation"; also, in their experiments the leucocyte does not show itself altogether indifferent.

The existence of opsonic elements in the blood plasma was not considered to necessarily exclude the existence, in addition, in the serum of bodies directly stimulating the phagocytes. Digestion of fresh normal serum with typhoid bacilli led to a diminished opsonic power of that serum, "an anti-opsonic" effect (Wright and Douglas Proc. Roy. Soc. vol. 72 p 368). Wright's general view of this "opsonic" action was stated as follows:- "It would seem probable that the bacteriolytic, bactericidal and bacterio-opsonic effects exerted by the blood fluids are each in their degree manifestations of a digestive power exerted by the blood fluids on bacteria brought into contact with them".

These observations were extended (1) to a number of micro-organisms besides the Staphylococcus Pyogenes (Bacillus Typhosus, Vibrio Cholerae Asiaticae, B.Coli, B. Dysenteriae, B. Pestis, Micrococcus Melitensis, Diplococcus Pneumoniae). The Bacillus Diphtheriae and /

(1) Proc. Roy. Soc. vol. 73.

and Bacillus Xerosis were considered to be insensible to the opsonic action of the blood fluids, phagocytosis of these organisms being quite as free in the heated as in the unheated serum (or even greater in the heated serum). Phagocytosis of the cholera vibrio and of the typhoid bacillus was very marked in heated serum.

Bulloch and Atkin remark in the preface to their investigation that "contrary to general opinion Douglas and Wright found that the leucocytes were capable of engulfing microbes only when the latter had been attacked by the serum or plasma.....Wright and Douglas found that the opsonic substance was more or less thermolabile being destroyed in 10 to 15 minutes at a temperature of 60° to 65°C." The opsonic theory was very generally considered to imply that phagocytosis could not be elicited apart from serum action.

Lohlein(1) demonstrated presumably as a refutation of the opsonic theory that phagocytosis in vitro could take place in a medium of saline solution (the leucocytes having been well washed); Lohlein holds that "apart from all intervention of the body humours normal guinea-pig leucocytes ingest and digest, in vitro, anthrax bacilli and cholera vibrios of relatively high virulence. These leucocytes can also ingest harmless /

(1) Ann. de l'Inst. Pasteur 19 p.647 (Oct.1905) "Sur la Phagocytose in vitro de microbes pathogenes" (premier memoire).

harmless races of Staphylococci and Streptococci: virulent races of Streptococci escape phagocytosis. As regards Bacillus Coli we have found definite differences in the intensity of the phagocytic process according as different microbial races were examined." Lohlein checked these results by experiments in which phagocytosis of the given organism was tested in the peritoneal cavity of the living animal: the results were found to correspond. It was also found that human leucocytes obtained and prepared in Wright's method were phagocytic for virulent anthrax in a medium of saline solution: guinea-pig leucocytes (washed) in these conditions ingested virulent cholera organisms and transformed them into granules in a medium deprived of "sensibilisatrices": this is held sufficient "to definitely refute the assertion of Wright and Douglas" that the spherulation of the intra-cellular ingested micro-organisms, which has often been ascribed to the agency of the leucocytes in in reality due to the action of the blood fluids." Lohlein maintains that phagocytosis is a "cellular act" which can go on without the intervention of the active principles contained in the body humours.

In a very detailed research Bulloch and Atkin (1) defined /

(1) Proc. Roy. Soc. vol. 74 p.380 (Jan.1905)
"Experiments on the Nature of the Opsonic Action of the Blood Serum".

defined opsonic action: they showed that opsonin could be completely removed from normal serum by bacteria and this at 0°C or at 37°C., the micro-organisms so treated (and washed free of serum?) being phagocyted vigorously on the addition of washed leucocytes. "The action of heat is to destroy opsonin and not merely to convert it into a non-opsonisable (?) modification". Opsonin is here stated to be eminently thermolabile, 10 minutes at 60°C reducing the opsonic power to nil and lesser pemperatures even 50°C for 10 minutes reducing it very greatly. Wright had found a similar but not so great, progressive destruction according as the serum was heated at temperatures between 50° and 60°C: recently (1) Wright has pronounced differently on this point with regard to immune serum. His view as to normal serum was expressed thus:- "The opsonic power of the blood fluids is but little impaired by the action of heat until these have been exposed to temperatures above 50°C. The following are the results of a typical experiment:- Phagocytic power obtained with the serum before exposure to heat 12.7: with the same serum heated for 10 minutes to 45°C., 13.1: with the same serum heated for 10 minutes to 50°C 10.2: with the same serum heated for 10 minutes to 55°C., 5.7."

Bulloch and Atkin consider that the opsonin is not /

(1) Proc. Roy. Soc. vol. 77.

not identical with any of the antibodies hitherto discovered in the serum: the opsonin is of relatively simple constitution."

The opsonic theory from the outset has been applied clinically, at first as a control for inoculation with vaccines the so-called "opsonic index" (i.e. healthy serum and patient's serum compared as regards phagocytic reaction with the same washed blood corpuscles and suspension of bacteria and the result expressed as a ratio) being taken as the guide whether or not the period for further inoculation had arrived. Very often but not invariably the opsonic power of the patient's serum falls after inoculation with a vaccine and this "negative phase" lasts for an indefinite period: further inoculation during the "negative phase" superinduces a more pronounced "negative phase" and such cumulation is associated with aggravation of the general and local clinical condition of the patient. In the early stages of thetheory a persistently low index was the datum exploited for diagnosis: at least this was inferentially so (for tubercle especially; also for staphylococcus): or, this low index might, in the absence of symptoms, simply mean a predisposition to infection. Wright gives in one of his earlier communications a case of tubercular peritonitis where the index was above normal (Proc. Roy. Soc. vol. 74). The demarcation however /

however between health and disease was arbitrary and the index in disease showed considerable range in the series of cases examined by Wright and Bulloch: this criticism is applicable to Bulloch's [1] classification of lupus cases, viz.- that those with a very low opsonic index do not improve with X-ray or Finsen light treatment whereas those in the "normal" limit are likely to improve with that treatment. Wright states with regard to staphylococcal infection, "in view of these observations (a list of cases and indices) and of the fact that we have not come across any instance of the association of a normal phagocytic power with a staphylococcus infection, the conclusion would seem justified that a low phagocytic power and staphylococcus infection are related to one another by some fact of causation." But cases of acute tubercular infection have been found to show oscillating indices frequently well above normal [2]. Wright's [3] view of this is as follows:- "In cases of strictly localised tubercular infection the index is low and this condition of low /

(1) Trans. Path. Soc. Lond. vol. 56 pt July 1905.

(2) Urwick: Brit. Med. Journ. July 22 1905: also Wright Proc. Roy. Soc. Jan. 1906.

(3) Proc. Roy. Soc. Jan. 1906. "On the Possibility of Determining the Presence or Absence of Tubercular Infection by the Examination of a Patient's Blood and Tissue Fluids."

low opsonic power has preceded the infection. No auto-immunisation is going on: inoculation with tubercle vaccine can call the machinery of immunisation into play. The constant fluctuation in the opsonic power of the blood in cases of active pulmonary tuberculosis and other active forms of tubercular infection furnishes, as we can hardly doubt, evidence of a periodic conveyance of tubercular elements into the blood: and of a response to such stimulation on the part of the machinery for immunisation. The low opsonic indices registered in connection with active tuberculosis would in other words be "negative phases" such as supervene - as one of us has shown - upon the inoculation of all vaccines: the high opsonic indices would be positive phases such as normally succeed upon the negative phases just mentioned: and the normal opsonic indices would correspond to periods of transition between negative and positive phases or, as the case may be, to periods in which the blood is returning after a positive phase to the condition quo ante." But the variations are often considerable from day to day and even while constitutional disturbance is present the opsonic index may oscillate abruptly from sub-normal to very considerably above normal and vice versa or remain persistently, although irregular, above normal in precisely that class of case (v.Wright's cases /

cases in loc. cit.) These patients are not improving: auto-inoculation in such cases ought according to the theory to result in cumulative negative phases: also, inoculation with a tubercle vaccine is not likely to benefit this class of patient.

In the earlier investigations exudation fluids (which had been in process of accumulation or had been accumulated for sometime) blister fluid, fluid from abscesses, were generally found to give a very low index as compared with the blood serum. But exudates have been found upon occasion to give higher readings than the blood serum (e.g. in case of tubercle v. Wright Proc. Roy. Soc. Jan. 1906: pleural exudate, opsonic index = 1: peritoneal exudate 0.1: blood serum 0.7.; the peritoneal effusion was the chief clinical feature in the case and from these observations Wright pronounced the case tubercular and not malignant: the peritoneal exudate is supposed to have formed when the blood opsonic index was low, the pleural exudate when it was high.)

Lastly Wright considers any appreciable phagocytosis of tubercle bacilli in a patient's serum, after heating, to be an evidence of infection; i.e. there is present in the heated serum of those who are subjects of a systemic infection, as distinguished from a strictly localised infection, or who have been subjected to /

to inoculations of a tubercle vaccine an element which incites Phagocytosis." But systemic infection here includes a considerable diversity of cases and one is almost led to think that the case is "systemic" or not according as the opsonic index is at, or above, normal or below it (in which case, i.e. the fact it would be "strictly localised").

With regard to this point of diagnosis the figures given by Wright strike one as arbitrary and variable, so much so that, for the number of observations given - and one must remember that these observations cannot claim to be beyond the range of experimental error - one is not inclined to regard this datum more favourably than the former means of diagnosis, i.e., alleged persistently low index in those who are subjects of infection.

To complete this chain of reasoning records dealing with the heated serum of patients who have persistently low indices and who are suffering from chronic lesions, should have been shown. If their heated serum corresponds to the heated serum of healthy individuals, well and good; if it gives higher counts the hypothesis as to auto-immunisation etc. as formulated above falls to the ground.

Wright's rules for guidance in diagnosis (tubercular lesions) in their present form must be pronounced /

pronounced unlikely in a great proportion of cases to give a clue where the clinical features are obscure.

The present writer found in a case of genito-urinary infection with B. Coli, (that organism being present in the urine in almost pure culture: it was identified as B. Coli) that the patient's index ascertained on five occasions was distinctly above normal and variable: a 20-hour agar growth of the particular bacterial race causing the infection was employed in the test. The patient had rigors (not severe) from time to time with slight rises of temperature.

The specificity of opsonins has been alleged and the taking of differential opsonic indices (i.e. phagocytosis of different organisms in the same fluids) has been used for diagnosis. But the organisms are not "standardised" e.g. there cannot be, strictly, a comparison of the phagocytosis of dead tubercle bacilli in an exudate with phagocytosis of living staphylococci in that fluid even though the same standard serum is used as a control for each.

The relative position and importance of "opsonins" in immunity, in general, is not yet defined. Some, who emphasise the thermolabile element, incline to identify opsonin with complement (Crofton. Journ. Hyg. Oct. /

Oct. 1905: and so, also, in a sense Dean associates complement with phagocytosis): others (v.supra Neufeld and Rimpau, Savtchenko etc. also infra, Dean) laying stress on thermostability consider that the substance is identical with immune body. Gruber and Futaki seem to consider (?) that opsonin in immune serum and alexin in normal serum function in the same way as regards phagocytosis. Leishman (v.infra) has used the term "Stimulin" even in view of the findings of Wright and Douglas.

At least one effort has been made to bring the opsonic hypothesis into line with previous knowledge. Dean (1) found thermostability of the "substance which influences phagocytosis" in staphylococcus immune serum and, also, in certain cases and under certain conditions, in normal sera. He takes up the position that "opsonin" is identical with immune-body. "The fact that this specific substance is present in small amount in normal serum is in accordance with the numerous observations of the occurrence of immune substance in normal sera. One need only refer to the normal antitoxin (e.g. of diphtheria) anti-ferments etc. and to the fact that the bacteriolytic and haemolytic actions of normal serum are due to the presence /

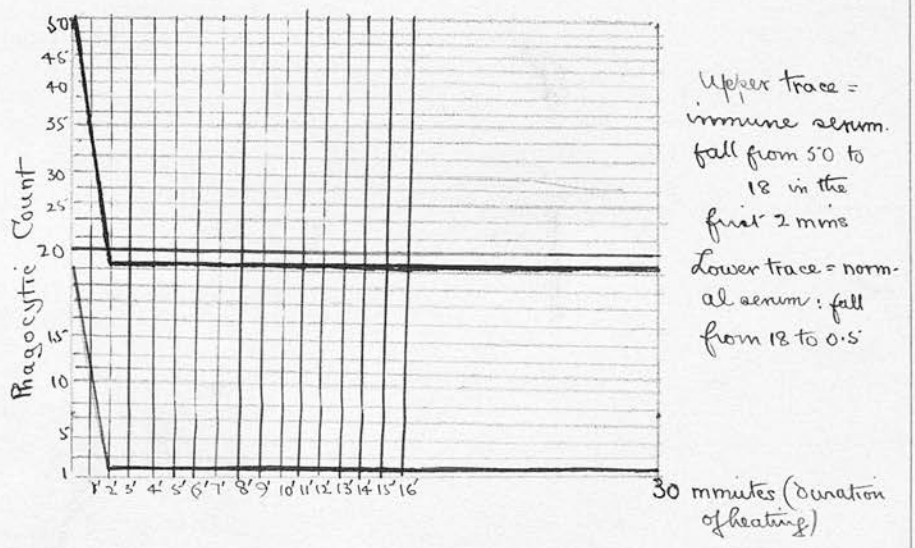
(1) Proc. Roy. Soc. vol. 76 B (1905) p 506 "On the Nature of the Substance in Serum which influences Phagocytosis".

presence in the serum of an immune substance plus a complement, as has been firmly established by the work of Pfeiffer, Bordet, Moxter, Ehrlich and Morgenroth and others. In giving the name "opsonin" to the substance which becomes attached to the micro-organisms and prepares them for phagocytosis Douglas and Wright have, therefore, named a property of serum which had already been recognised by a number of different workers..... It may be convenient to adopt Wright and Douglas' term of "opsonin" for the particular property in question (i.e. preparation of the microbe for phagocytosis). The only danger attached to such a course is, that one might be led to regard the opsonin as actually a different substance (i.e. from that known as "fixateur" and "substance sensibilisatrice) and not merely a property of immune serum."

One would refer to two of Dean's experiments. That opsonin was present in normal human serum after heating to 60°C for 20 minutes was shown by the fact that staphylococci treated with a relatively large quantity of such serum (cf. Barratt, supra) that is to say having been incubated in that serum for 15 minutes at 37°C and thereafter freed of serum were actively phagocyted in saline solution on the addition of leucocytes. The contrast is between 0 per leucocyte for untreated cocci in heated serum and 60 per leucocyte /

leucocyte for the treated cocci in saline solution, the test being performed in capillary tubes with small quantities of serum etc. in the method of Wright.

Again Dean shows as a curve the rate of destruction of the "substance" in normal and in immune serum respectively, by phagocytic counts from mixtures in which the portions of serum had been heated to 60°C for increasing periods up to half-an-hour.



The initial fall is extremely marked: in fact the first two minutes seem to have completed the destruction of the agents in the two sera which are concerned in phagocytosis so far apparently as these can be destroyed at such a temperature within a reasonable period. Dean is scarcely accurate in referring to this fall as a "fractional destruction" of the "substance". According to his own theory "the substance" /

substance" resists heat and some other factor has been destroyed. Indeed, Dean recognises that complement may have some function, although not an essential one, in regard to phagocytosis. With regard to heated sera he says:- "One must remember that Wright and Douglas' method probably demonstrates the presence of "opsonin" over only a very short range. The brief time during which the substance is allowed to act on the cocci probably admits of only fairly high concentrations being indicated." But the supposed immune body is just as concentrated in the unheated as in the heated normal serum, and for that reason it may be doubted whether it is the essential agent in the act of phagocytosis. (as far as normal serum is concerned).

Dean also records some experiments indicating that bacteria treated with heated immune serum washed and thereafter centrifugalised in normal serum leave the latter comparatively little impoverished as regards "the substance" when it is tested with fresh cocci and so also vice versa.

Besredka in the Bulletin de l'Institut Pasteur criticises Dean's position very favourably.

Leishman (1) has also emphasised the thermostability /

(1) Trans. Path. Soc. Lond. vol. 56 part III p. 344.

thermostability of "the substance" in some cases (he calls it "Stimulin") viz.- in sera from animals immunised against Micrococcus Melitensis and B. Typhosus and in sera from patients convalescent from infections with these organisms. Very small quantities of such sera added to phagocytic mixtures increased the count very greatly. Heating to 60°C for 10 minutes did not diminish this "stimulin" effect.

With a view to determining whether this increased phagocytosis was due to stimulation of the leucocytes or action on the bacteria the following experiment was done. All the sera employed were heated to 60°C. A suspension of typhoid bacilli was divided into three equal portions "A", "B" and "C". "B" was mixed with 0.5 c.c. of heated typhoid serum derived from an immunised rabbit and the mixture was allowed to digest for 1 hour at 37°C. "At the end of this time it was centrifuged until the supernatant fluid was glass-clear: this was pipetted off and replaced by normal saline in which the germs were once more shaken up and the centrifuge again employed. After the washing to remove the remains of the serum the supernatant salt solution was removed and a fresh quantity added to bring the suspension up to its original volume. In this way the germs had been given the opportunity of absorbing /

absorbing or "fixing" the substance contained in the heated immune serum, if they were capable of doing so. The third portion - suspension "C" - was treated in a manner precisely similar to "B" with the exception that heated serum from a normal rabbit was employed in place of the typhoid rabbit's serum. Three experiments were then done with each of these three suspensions, normal human leucocytes and normal heated human serum being employed in each case.

Experiment 10.

(a)

		Susp. A. (untreated)	B. (digested with typhoid serum)	C (digested with normal serum)
F.G's corpuscles 3 vols " heated serum 3 vols	Phag Ind.	38	23	35

(b)

F.G's corpuscles 3 vols. " heated serum 2 vols. Typhoid rabbit's heated serum (diluted 1 in 2) 1 vol.		76	49	70

(c)

F.G's corpuscles 3 vols. " heated serum 2 vols. Normal rabbit's heated serum (diluted 1 in 2) 1 vol.		43	28	34

..... With the untreated suspension "A" a two-

fold /

two-fold increase of phagocytosis results from the addition of the typhoid serum while a precisely similar effect is obtained with suspensions "B" and "C" which had been treated with typhoid serum and normal serum respectively. Had the substance contained in the typhoid serum been fixed by the bacteria of suspension "B", and had the mechanism of phagocytosis been similar to what Wright and Douglas have shown to be the case in opsonic action, I should have expected the experiment conducted with this emulsion to have shown throughout higher phagocytic indices, than in the case of those performed with suspension "A" and "C", and, further that the degree of stimulin effect, as compared with the two controls would have been less. In place of this, phagocytosis with "B" suspension is less than with the others while the stimulin effect of the addition of typhoid serum is precisely the same. Without wishing to insist too strongly upon the results of this experiment it appears to me to suggest that the effects recorded above are due to a real stimulation of the leucocytes by the immune serum and are not the results of a sensitising action of the serum upon the bacteria similar to the action of opsonins."

Wright has replied (1) on this question of thermostability /

(1) Proc. Roy. Soc. vol. 77 B 516 1906. "On Spontaneous Phagocytosis and on the Phagocytosis which is obtained with the Heated Serum of Patients who have responded to Tubercular Infection or as the case may be to the inoculation of a Tubercle Vaccine"

thermostability: in view of his previous work the recognition of the fact that "spontaneous phagocytosis" can occur constitutes a considerable change from the interpretation generally put upon the "opsonic" hypothesis. Wright finds that phagocytosis of tubercle bacilli can take place in a medium that is practically entirely saline solution and that the phagocytosis can be increased (even to a higher count than in a 2-fold dilution of normal serum: the count with undiluted serum is not given) or diminished, within limits, according as the dissolved salt content of the mixture is less or more. To eliminate phagocytosis due to other causes than the serum present the salt content in phagocytic mixtures is arranged to be over 1% NaCl. That phagocytosis can go on in an immune serum (that of a patient whose opsonic index had been raised from 0.17 to 1.8 by repeated inoculations of new tuberculin) is incidentally admitted, the salt content of the mixture being above 1% NaCl.

Digestion for half-an-hour at 37°C with a suspension of tubercle bacilli can remove the "incitor element" from heated immune serum; this incitor element is therefore an opsonin: this is spoken of provisionally /

provisionally as the opsonin found in the heated immune serum. As to thermostability Wright recalls that in his earlier observations there was a residual phagocytosis in heated serum. Is this due to a residuum of the thermolabile opsonin or to a thermostable body?

This matter of normal serum is shelved and the question discussed is, whether the opsonin in heated serum, where phagocytosis is marked, is really a residue of the thermolabile substance or a really thermostable substance. There is no attempt to show that the opsonin in heated serum can be destroyed by heating, if that is sufficiently prolonged, which one feels is the plain question at issue (v.the curve depicted by Dean for staphylococcus immune serum). An experiment is done in which an immune serum is heated before dilution in one series and after dilution in another and the phagocytic reaction tested in the progressively increasing dilutions: the phagocytosis ceases first in the serum which was diluted before heating and the conclusion arrived at, is, "that the opsonin found in heated serum is destroyed by heat when the serum is sufficiently diluted." Four such results are given: when one scrutinises them one observes a marked rise in three in the initial dilutions of the serum which was /

was first diluted and then heated.

	Phagocytic count in serum heated before dilution.			Heated undiluted serum			Phagocytic count in serum diluted before heating		
	I	II	III	I	II	III	I	II	III
				2·4	-	1·4			
2 fold	1·9						3·3*	2·7*	1·5*
4 fold	2·7	1·5	·85				1·7	1·9	1·5*
8 fold	1·1	1·4	0·7				0·6	1·2	1·6*
16 fold	1·0	1·6	0·7				0·45	0·3	0·2
32 fold	·97	1·5	0·25				0·2	0·05	0·0
64 fold	·75	0·9	0·0				0·08		

1. = pooled serum of 6 patients who had been inoculated with a tubercle vaccine.

2. = serum of a patient who had been inoculated with a tubercle vaccine.

3. = serum of a patient who had been inoculated with a tubercle vaccine.

* these counts do not bear out the foregoing assertion as to dilution, the count in the second series ought to fall from the outset. Wright refers to Dean's conclusion that the opsonins found respectively in unheated and heated immune sera were identical. "The plain teaching of our experiments is that the opsonin which is found in the heated immune serum of a patient who /

who has responded to tubercular infection, or as the case may be to the inoculation of a tubercle vaccine, does not differ with respect to its resistance to heat and sunlight from the opsonin which is found in unheated normal serum....."

"We have only to remark in conclusion that if we prefer to speak of the opsonin as a thermolabile element, and Dean prefers to speak of it as a thermostable element, there is nothing at issue between us except the question as to whether it is in harmony with usage and with the genius of the English language as employed in scientific discourse, to characterise as "thermostable" an element of which at best residual traces remain in the case of the normal serum where this has been heated to 60°C and in the case of the immune serum where this has after adequate dilution been heated to the same temperature."

Still, Dean has some claim to distinguish - he does so but not sufficiently explicitly - the thermolabile from the thermostable element, if the latter resist heating to 60°C for periods up to half-an-hour without showing progressive destruction: and again, if normal horse serum after 4 hours at 60°C can exercise an opsonic effect.

It is a conception requiring some elaboration that /

that the "substance" heated in dilution is more completely destroyed than when heated in concentration, the same bulk being heated in each case, say for 15 to 20 minutes at 60°C. In Wright's experiment there is no standard for "adequate" dilution.

To meet Dean's argument as to the limitation of Wright's method a healthy (normal) serum should have been contrasted with a diluted immune serum at that dilution which (unheated) gave a count equal to that of the normal serum, both sera – the normal undiluted and the diluted immune serum – being tested before and after heating. The immune serum after heating might be more potent than the heated normal serum in which case one would infer the presence of a really heat-stable substance. Both might show a similar count after heating in which case it might be allowable to conclude that the substance is one and the same in each serum. But if such an immune serum show a lower or a higher count according as it is heated after or before dilution then one is at a loss what to infer, that is to say on the basis of Dean's hypothesis.

PART II

Wright's "opsonic" theory assumes that phagocytosis and even phagocytosis "in vitro" is, as such, an index of the efficiency of the mechanism of defence against micro-organisms.

This is doubtful. As to "phagocytic counts", by means of which Wright considers that the theory can be employed as an accurate quantitative measurement of this defence, one is inclined to think that the value of such enumeration is limited: besides the possibility of error due to the mechanical procedure there are two "personal equations" in the matter, the observer and the individual leucocyte (cf. Wright Proc. Roy. Soc. vol. 77; he remarks that, in heated serum, not every leucocyte but only one here and there is phagocytic). Where the phagocytic difference between two mixtures is gross, enumeration is superfluous: on the other hand one can, with care, discriminate fairly well between mixtures which, as regards phagocytosis, might to a general scrutiny appear very similar.

Dean places no great confidence in phagocytic counts: Wright points out that Dean has used very dense suspensions and has obtained counts so high that quantitative estimation is out of the question. Dean's argument, however, does not depend on "refinements of enumeration". Wright's counts in some places are much /

much too high (30 per leucocyte and upwards). An average of from 6 to 10 per leucocyte is perhaps as high as one would, with any confidence, care to count. For any degree of accuracy it is essential that the suspension of bacteria be homogeneous, i.e., that there be no considerable clumps of organisms. One has found that, for this reason, some organisms, e.g. B.Diphtheriae and B. Pyocyaneus, are very unsuitable: in such case only very gross differences could be considered of significance. Some organisms form very scanty growths in 24 hours and it is difficult to get a suspension of proper density unless one employ several cultures or very little saline solution. The Staphylococcus Pyogenes Aureus is convenient; Bulloch and Atkin used Staphylococcus Albus. The observations given below were made with living cultures (20-24 hours old) mostly grown on agar. The staphylococcus chiefly employed was, as one had reason to know, a (laboratory) culture of very low virulence: another race which had been comparatively recently isolated from the throat of a patient was also used (Staph. 2). Both behaved similarly. Suspensions of these cultures were made in 0.8 % Na Cl solution: the suspension was centrifugalised to free it of clumps, the upper portions being then pipetted off: this /

this last was used as suspension for the phagocytic tests.

As to technique, an adaptation of Wright's procedure was used, in that comparatively large quantities (15-20 c.c.) of human blood were withdrawn from a forearm vein into a glass-barrelled syringe, well cleaned, sterile and washed out with citrate solution just before use. Such a method would seem to have advantages (better preservation of the blood obtained in such quantity and by this means) Blood from the same individual (healthy male) has been used throughout. The blood was gently poured - the needle having been removed from the syringe - into an equal volume of 1% solution of citrate of soda in 0.8% solution of sodium chloride, this solution having been slightly warmed just previous to the addition of the blood: care was taken in emptying the syringe not to wet the sides of the centrifuge tube with blood. All the solutions, tubes etc. had been sterilised: care was taken that the centrifuge tubes and incubation tubes etc. were clean. Another portion of blood (for serum) was poured into another centrifuge tube and allowed to clot. Both tubes were centrifugalised. From the first tube the supernatant "citrated plasma" was removed and the blood corpuscles centrifugalised /

centrifugalised three times in large quantities of 0.8% Na Cl solution ("washed corpuscles"). Less concentrated citrate may be used (½% Urwick): but one has once seen a pale clot form in the citrated plasma obtained as above: also one knows that others in taking opsonic indices have had trouble with blood clotting in capillary tubes (? due to insufficient citration) even where 1% citrate had been used. Wright has used various concentrations of citrate even 10% (1 part citrate solution to 9 parts blood Proc. Roy. Soc. vol. 73 p 139) but in this case the first portion of blood entering the citrate solution receives rather harsh treatment because 3% citrate is sufficient to inhibit phagocytic activity though that faculty can be restored by washing the corpuscles in saline solution.

Wright's technique was a modification of Leishman's procedure for the investigation of phagocytic activity; Leishman measured off equal quantities of blood and bacterial suspension in a capillary pipette and incubated a drop of the mixture on a slide under a cover-glass: at the end of fifteen minutes the cover-glass was slid off and the film stained with Leishman's stain. Wright introduced the citration of the blood, washing of the blood corpuscles.

Wright /

Wright receives the blood in a special capsule with a limb recurved so that it could be hung in the hand-centrifugalisation machine. This capsule was made from glass tubing in the Bunsen flame. Citrate solution was filled in through the capillary recurved limb up to a mark on the capsule and then blood from the finger up to the same level. This capsule was centrifugalised and the corpuscles washed in it just as described above. Capillary pipettes were used for measuring, mixing and incubating the quantities of serum corpuscles and suspension of bacteria. As to the saline solution: Wright (also Bulloch) used 0.85% and recently Wright has used stronger salt solution for the reason given already. In the following experiments 0.8% was used because one concluded (rightly or wrongly) that the blood cells were better preserved in 0.8% NaCl than in 0.85 %. Only recently has the matter of the salt content been discussed. As to the citration: if the citrated plasma be well centrifuged a white deposit consisting of blood-plates is seen lying on the surface of the layer of corpuscles. These platelets - one sees them afterward in the smears - are often large: very commonly one finds them grouped round white cells, especially in smears from heated serum mixtures: this grouping is also well seen on /

on the warm stage. Occasionally these platelets are apparently inside the white cell (polymorph or monomuclear). On two occasions one has found the organisms distinctly clumped and in such clumps one could generally find platelets (once Staphylococcus and once Micrococcus Melitensis). Levaditi found that cholera vibrios in the blood stream adhered to the blood-plates (Ann. de l'Inst. Past. 1901 p 922). The upper layers of the centrifugalised deposit are said to be specially rich in white corpuscles (Wright) One's custom was to gently mix the deposit to ensure uniformity. The mixtures were generally made and incubated in specimen tubes (i.e. small corked tubes about an inch long, with ½-¼in. diam.) which had been well cleaned and sterilised: the corks were boiled and dried. 1 c.c. pipettes graduated to tenths and hundredths were used for measuring the constituents of the mixtures. The proportions generally used were 0.3 c.c. blood corpuscles, 0.3 c.c. serum or other medium, 0.1 c.c. susp. of bacteria. These are Wright's proportions 3. 3.1. Incubation = 20 minutes at 37°C. Smears are then made of the mixtures after the tubes have been emptied into watch-glasses. The smears are perhaps best when made rather thick and with a free edge as recommended by Bulloch: along this edge one finds good leucocytes as a rule. Wright /

Wright and Bulloch used slides that had not been specially cleaned. This is not advisable (the film washes off or is "streaky"). Wright used sand-paper on the middle of the slide to ensure that many leucocytes will be found in one place. One has not found this an advantage. As to staining, Leishman's stain is perhaps the best: Jenner's is also very good. eosin and methylene blue tends to leave a deposit in the free edge, the particular place one wishes to be distinct. For counting, one's impression is, that it is advisable to have the cells faintly stained yet moderately well-defined. If the nucleus be dark (e.g. if one stain with Leishman's stain and use distilled water too freely afterward) one cannot see through it nor see bacteria lying on it. One has found heated serum mixtures, for some reason, hard to stain: the nucleus tends to be very dark and if one "clear" the slide with fresh Leishman the protoplasm of the leucocytes becomes invisible. However with practice one can determine whether cocci are "in" or "out" even when the protoplasm is very pale. Of course it is a question whether, in slides made from mixtures in this way, the bacteria have really been ingested or are merely lying <u>on</u> the cells. However that does not matter /

matter much as they ought to lie "on" the cells as much in one slide as in another if the number of cells and bacteria is the same in each.

Some difficulties in counting may be mentioned:-

1. Poor definition of the white cells. These mixtures are not so easily stained as blood. It is difficult to get the white cell suitably stained and to have plenty such cells on the slide. There are often thin processes projecting from the leucocytes and these are apt to be missed: also, the formation of large Vacuoles is misleading for one may think that the inner edge of the Vacuole is the outer limit of the cell and miss the thin external rim of the Vacuole.

2. Small outlying portions of nucleus are often very like a coccus.

3. Digestion of the micro-organisms spoils the counting. Staphylococci show only very slight changes after 20 minutes in the incubator. Other organisms are less resistant (? even Streptococci). One finds in the case of "digested" Staphylococci a paler blue and occasionally pink staining. Cell granules may be confused with the last more particularly if the stain has brought out the granules of the cells.

4. /

Incubation = 20' at 37°C

UNHEATED SERUM 0.15cc Washed Corpuscles 0.15cc Susp Staphylococcus 0.05cc	552/100	I	
{UNHEATED SERUM 0.15cc Washed Corpuscles 0.15cc} 0.3cc these incubated 20' at 37°C after which is added Susp Staph 0.05cc	542/100	II	
UNHEATED SERUM } 0.15 there were Susp. Staphylococci } 0.05 incubated 20' at 37°C & then Washed Corpuscles added: 0.15cc	427/100	III	
HEATED SERUM 0.15cc (30' at 60°C in hot air chamber) Washed Corpuscles 0.15cc Susp. Staph. 0.05	138/100	IV	
HEATED SERUM 0.15cc } These (as above) Washed Corpuscles 0.15cc. } were incubated 20' at 37°C + then was added Susp. Staph. 0.05cc.	166/100	V	
HEATED SERUM 0.15cc Susp Staphylococci 0.05cc} these incubated 20' at 37°C & then added Washed Corpuscles 0.15cc	80/100.	VI	

Experiment which would seem to show that preliminary incubation of serum and corpuscles or serum and staphylococci does not reinforce the subsequent phagocytic effect when the 3rd factor is added & incubation repeated. No agglutination of the organisms was noticed in III & VI: such a condition would have accounted for the difference in these two cases.

4. Grouping of the organisms round the white cells as sometimes happens.

The process of counting is laborious: 15 or 20 white cells are too few in most cases: 50 give a fair indication: it is better to count 50 twice, i.e., on two different slides.

As to the kind of cells in which phagocytosis is counted, polymorphs only are examined. This is perhaps too arbitrary. The mononuclears are phagocytic in vitro. Bordet and Salimbeni found this to be very marked in the living animal. Indeed with regard to opsonic indices in tubercle one wonders why the mononuclears were not chosen for the counts. The eosinophile cells are also phagocytic.

In general, one must consider that although the examination of the cellular activity (and humoral activity) in this way is interesting, even instructive, the conditions are unnatural and one might call them morbid: it is more than probable that many leucocytes are destroyed in the washings. One sees a variable but generally quite appreciable number of broken down leucocytes in the films.

In examining phagocytosis with larger (measured) quantities of corpuscles serum etc. than were used in Wright's /

I

	A	B	C	
Unheated Serum 0·3cc Washed Corpuscles 0·3 Susp. Staph. 0·1	421	216	126	I
Unheated Serum 0·15cc Saline Solution (0·8%) 0·15cc Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	314	190	44	II
Heated Serum 0·3cc. Washed Corpuscles 0·3cc Susp. Staph 0·1cc.	171	170	21	III
Heated Serum 0·15cc Saline Solution 0·15cc Washed Corpuscles 0·3cc Susp. Staph. 0·1cc	143	342	61	IV
"Citrated Plasma" 0·3cc Washed Corpuscles 0·3cc. Susp. Staph 0·1cc.	222	107	53	V
Heated "Citd Plasma" 0·3cc Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	229	364	75	VI
Saline Solution (0·8%) 0·3cc. Washed Corpuscles 0·3cc Susp. Staph 0·1cc	92	45	4	VII
Saline Solution (0·85%) 0·3cc. Washed Corpuscles 0·3cc. Susp Staph 0·1cc.	7/50			VIII

Wright's method and with relatively more dilute suspensions of staphylococcus one noticed that in serum which, as one thought had been heated at 60° for a long enough period phagocytosis though diminished was still present; also that heating citrated plasma did not seem to affect it in the same way. One thought it worth while to make some further observations on heated serum and heated citrated plasma: with the following results (p 59.)

Serum corpuscles and cultures prepared on 3 separate occasions for A B and C. Suspension of bacteria more dilute in B than A: in C than B. Serum and cit. plasma heated in hot air chamber at 60°C for 25-30 minutes. Incubation in "specimen tubes" = 20 minutes at 37°C Figures in table = cocci in 100 polymorpho-nuclear leucocytes.

TABLE I

II

	Specimen tube	Quill tubing	
Citrated PLASMA 0·3cc. Washed Corpuscles 0·3cc. Susp. Staphylococcus 0·1cc	131/50 158/50 126/50	223/50 199/50	I
HEATED Citrated PLASMA 0·3cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	359/50	251/50 223/50	II
HEATED Citrated SERUM 0·3cc Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	373/50	274/50	III
UNHEATED SERUM 0·3cc Washed Corpuscles 0·3cc Susp Staph. 0·1cc	400/50	410/50	
HEATED SERUM 0·3cc Washed Corpuscles 0·3cc. Susp Staph. 0·1cc.	330/50	340/50	

Heating in hot air chamber: this accounts for the high figures in the case of the heated serum (imperfect heating)

Conclusions:-

1. Heating the serum has decreased the phagocytosis.

2. "Citrated plasma" seems to differ in this respect from serum: there is marked increase after heating in B.

3. Dilution of heated serum has less effect in decreasing phagocytosis than dilution of unheated serum: there is marked increase in B.

4. Phagocytosis goes on to some extent in 0.8% NaCl solution.

Another experiment was done with regard to citrated plasma: two sets were incubated one in quill tubing, the other in "specimen tubes": serum heated with citrate solution was also examined. Heating incubation etc. as before. The figures indicate counts in 50 leucocytes: e.g. three different slides were used in the three counts in 1 first column and so on.

vide TABLE II

Conclusion:-

That heating "citrated plasma" does not always decrease its phagocytic effect: it would seem sometimes to have the opposite effect. One has never found similar results for blood serum in a test conducted /

UNHEATED SERUM	Specimen Tube UNHEATED SERUM 0·3cc Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc	378/30	403/50	213/50
	Quill Tubing UNHEATED SERUM 0·3cc. Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	387/30	414/50	250/50
	Capillary Tube UNHEATED SERUM 3 parts Washed Corpuscles 3 parts Susp. Staph 1 part	296/30	335/50	75/50
HEATED SERUM	Specimen Tube HEATED SERUM 0·3cc Washed Corpuscles 0·3cc Susp Staph 0·1cc.	120/30	320/50	107/50
	Quill Tubing HEATED SERUM 0·3cc Washed Corpuscles 0·3cc Susp Staph. 0·1cc.	315/30	344/50	146/50
	Capillary Tube HEATED SERUM 3 parts Washed Corpuscles 3 parts Susp Staph 1 part	335/30	507/50	67/50
		A	B	C

conducted in this way (ie. in the larger bulk). Heating seems almost always to reduce the "phagocytic property" in serum (v.infra: there is one exception to this in Table III col. "B") at least with regard to staphylococcus and a good many other organisms. Wright and Douglas found "citrated plasma" almost exactly equivalent to citrated serum in similar dilution.

As one had found on one occasion that digestion of similar mixtures in quill tubing alongside specimen tubes gave no difference where the unheated serum was concerned but a higher count for the quill-tubing-heated-serum mixture as compared with the corresponding specimen tube ($^{66}/_{100}$ as cpd. with $^{21}/_{100}$) one made the following observations to test the "specimen tube" method.

Specimen tubes: quill tubing into which the total mixture was drawn out of a specimen tube: and capillary tubes according to Wright's method were used. It may be noted that Wright obtained different results if the bore of the pipettes was varied but the results were not constantly in favour either of the wide or the narrow calibre. Three suspensions of the same growth of Staphylococcus were used being as regards density as 1: 1 in 2: 1 in 4. Heating incubation etc. as before.

vide TABLE III.

IV

	'Specimen' Tube	Quill Tubing	Capillary Tube
UNHEATED SERUM Washed Corpuscles Susp Staph.		395/100	296/50
SERUM heated on water-bath 30' at 60°C Washed Corpuscles Susp Staph	103/100	107/100	
SERUM heated in hot air chamber 30' at 60°C. Washed Corpuscles Susp Staph	107/100	162/100	152/100
SERUM heated in capillary tube in hot air chamber 30' at 60°C Washed Corpuscles Susp Staph			104/100

Conclusion:-

That where heated serum is concerned phagocytosis is brisker in quill tubing and capillary tubes than in the specimen tubes: in the case of the denser suspensions "A" and "B" the effect of heating is less obvious than in "C".

Another question was forced on one here, viz.- Will another method of heating give different results from those obtained as above? (i.e. hot air chamber).

An experiment was done with serum heated on the water bath 30 minutes at 60°and another portion in the hot-air chamber 30 minutes at 60°C, also a small quantity was heated in the hot-air chamber for 30 minutes at 60°C, with this result

vide TABLE IV.

Conclusion:-

That destruction of the "phagocytic" property of serum is more thorough if it be heated on the water bath. Even in serum heated on the water bath for 30 minutes at 60°C phagocytosis is not abolished.

Does citrated serum, when heated on the water bath behave as blood serum so heated? Also, what is the effect of the citrate solution with regard to phagocytosis?

		Staph. '2'	Staphylococcus '1'	Staph. '2'
I	Unheated Serum 0·3cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	317/20 375/20 692/40	324/50	367/50
II	Heated Serum 0·3cc. Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	205/50 234/50	110/50 43/25	87/50
III	Citrated Plasma 0·3cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	300/50 282/50	162/50	126/50
IV	Heated Citrated Plasma 0·3cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	196/50 223/50 237/50	93/50 108/50	54/50
V	Citrate of Soda solution (1% in 0·8% NaCl) 0·15cc. Unheated Serum 0·15cc. Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	453/50 439/50		125/50 108/50
VI	Citrate of Soda solution 0·2cc Unheated Serum 0·1cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.		170/50 175/50	114/50
VII	Serum / Citrate of Soda solution } of each 0·15cc. heated together 0·3cc. Washed Corpuscles 0·3cc. Susp. Staph 0·1cc.	239/50 284/50	102/50	
VIII	Heated Serum 0·15cc. Citrate of Soda solution 0·15cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	209/50 227/50	81/50	
IX	Citrate of Soda solution 0·3cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	34/50 10/20		

Heating = 15' at 60°C on water-bath.

TABLE V.

Conclusions:-

The mixtures containing heated serum + citrate solution and heated citrated plasma tend to fall off to the heated serum count. There is no rise in these counts. Citrate solution neither by itself nor along with serum has any stimulating effect on the phagocytosis. There is no reason to consider citrated plasma (ττ) more or less potent than citrated serum (∇) and (∇τ). (As one will see below, Table dilutions of serum differing only in this degree are not readily distinguishable by this method.)

Two attempts were made to show some difference in the "opsonic" content of "plasma" and serum. "Citrated plasma" obtained as above described is obviously not equivalent to a similar volume of equal parts blood serum and citrate solution. Wright mixed citrate solution with blood which had clotted, the amount of citrate being equal to the volume of the newly drawn blood: the blood clot was broken up with the citrate and the mixture centrifugalised. The "citrated serum" and "citrated plasma" were found to give identical results. Will any difference be evident if the blood is received not into an equal volume of citrate solution but into a quantity = 56% of its volume, i.e. allowing that the blood corpuscles are /

VI

	Staphylococcus ①	Staphylococcus ②
Citrated Plasma 0·3cc. Washed Corpuscles 0·3cc. Susp Staph 0·1cc.	225/50	193/50
Citrated Serum 0·3cc. Washed Corpuscles 0·3cc Susp Staph 0·1cc.	117/50 124/50	207/50 154/50

VII

	Staphylococcus ①	Staphylococcus ②
"Plasma" 0·3cc Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	163/50 165/50	245/50 270/50
Serum 0·3cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc	162/50 201/50	291/50 253/50
Heated "Plasma" 0·3cc. Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	56/50	104/50
Heated Serum 0·3cc. Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	47/50	126/50

are equivalent to 44% of the volume of the blood? 5 c.c. of blood was drawn from a vein into the syringe which contained 2.8 c.c. of citrate solution, the syringe being slightly warmed. The syringe was emptied into a paraffined tube, this device being used to prevent any tendency to clotting. No clotting occurred. The citrated plasma thus obtained was contrasted with citrated serum (i.e. 0.15 c.c. serum 0.15 c.c. citrate solution) in the usual way: suspensions of two different staphylococci were used. v. TABLE VI.

Conclusion:-

That citrated plasma so obtained seems to have a phagocytic effect about equal to that of the citrated serum.

Gengou (1) in a research on the source of alexine in normal serum made use of paraffined tubes for withdrawal of blood and paraffined tubes for centrifugalisation of the blood. Clotting of the blood was, he found, by this means much delayed and he obtained a fluid which was much less bactericidal than blood serum (Metchnikoff). With this result in view an attempt was made to get a fluid which would be, perhaps, more akin to circulating plasma than ordinary blood serum, and which would be free from admixture of citrate /

(1) Metchnikoff: Immunity p. 190.

VIII

			Ser. htd. 15'	Ser. htd. 30'
I	Unheated Serum	0·3cc	222/50	
	Washed Corpuscles	0·3cc	238/50	374/50
	Susp. Staph.	0·1cc		
II	Serum Mixture, 2 in 3 (i.e. 2 parts unheated with 1 part heated serum)	0·3cc.	339/50	300/50
	Washed Corpuscles	0·3cc		
	Susp Staph	0·1cc.		
III	Serum Mixture, 1 in 2	0·3cc.		287/50
	Corpuscles	0·3cc.		
	Susp. St.	0·1cc.		
IV	Serum Mixture, 1 in 3	0·3cc.	368/50	243/50
	Corpuscles	0·3cc.		
	Susp St.	0·1cc.		
V	Serum Mixture 1 in 6	0·3cc.		228/50
	Corpuscles	0·3cc.		194/50
	Susp. St.	0·1cc.		
VI	Serum Mixture 1 in 10	0·3cc.		188/50
	Corpuscles	0·3cc.		203/50
	Susp. St.	0·1cc.		
VII	Serum Mixture, 1 in 20	0·3cc.		205/50
	Corpuscles	0·3cc.		227/50
	Susp. St.	0·1cc.		
VIII	Serum Mixture 1 in 40	0·3cc.		141/50 193/50
	Corpuscles	0·3cc.		
	Susp. St.	0·1cc.		
IX	Serum Mixture 1 in 80	0·3cc		178/50
	Corpuscles	0·3cc.		177/50
	Susp. St.	0·1cc.		
X	Heated Serum	0·3cc.	76/50	83/50
	Corpuscles	0·3cc.		107/50
	Susp. St.	0·1cc.		

See also back of Table XVIII

citrate solution.

The syringe was coated thinly but completely with sterile vaseline. After withdrawal, the blood (10cc.) was gently poured into a well paraffined tube which had been kept warmed at 34-35 C°. This tube was at once centrifugalised. The supernatant fluid separated much more quickly than blood serum usually does. Clotting took place some time after removal of the "plasma" from the deposit: the clot was small and had a white cap. This plasma was compared with serum from blood withdrawn at the same time and allowed to clot in the usual way. Also, the two fluids were heated 15 minutes at 60°C on the water bath for further comparison. Suspensions of two races of staphylococcus were used. The result was as follows:- (v. Table VII)

Conclusion:-

This experiment shows nothing analogous to Gengou's results. The two fluids seem to be equivalent.

What is the result if heated serum be mixed with unheated?

Two experiments have been done. One with serum heated 15 minutes at 60°C, the other with serum heated 30 minutes at 60°C: the sera were heated on the water bath: the experiments were done with serum, corpuscles etc. obtained and prepared on two occasions. (v.Table VIII).

The /

The result in the first column is remarkable. (It would seem that this effect can be abolished by heating the serum for a longer period.(?)) As to the second column it will be noticed that the counts fall off definitely though irregularly: the thermolabile element is in evidence down to a dilution of $\frac{1}{80}$ i.e. there is a distinct fall from IX to X. This gives rise to the reflection that this method of comparing unheated sera may discriminate only between gross differences of "opsonic" content. The thermolabile element is much less in VII than in IV, in VIII than in V yet on a count of 50 corpuscles these mixtures might show little difference.

Blood corpuscles do not remain suspended in serum that has been heated on the water bath at 60°C. They fall out in less than 20 minutes in the mixtures, e.g. in the mixtures used in the experiment resulting in the figures in table VIII (second row) the corpuscles had fallen out in V, VI, VII, VIII, IX and X, so also in III in the other row. This sedimentation shows quite quickly in capillary tubes. Brisk phagocytosis may go on although the corpuscles have fallen to the bottom of the tube, e.g. III (a) table VIII. In heated serum on the warm stage the leucocytes are quite active. The "falling out" is due to an excessive "rouleaux" /

* An expt was done with 3 portions of serum
(i) 0·9 cc htd. 15′ at 55°C
(ii) 0·9 cc " 5′ at 60°C
(iii) 0·9 cc " 30′ at 60°C
} The serum was used as ~~in the~~ above v. back of Table XVIII
Result :- counts analogous to those of col. 2, table VIII

"rouleaux" formation of the reds: this shows well in hanging drop preparations and also in the films. Films made of the heated serum mixtures have, for this reason, a granular appearance to the naked eye (invariably for the human serum used)

One has not noticed this sedimentation in any other medium, salt solution, citrate solution nor in heated citrated plasma. The leucocytes tend to clump more in heated serum than in unheated: however they often clump in unheated serum: the bacteria seem to have something to do with this, for one has noticed it especially in some cases, e.g. in films made from mixtures containing B. Typhosus and Cholera Vibrio: but in general the groups of leucocytes are bigger in heated serum, forming sometimes quite enormous masses. Dean (and others) remarks this clumping of the leucocytes: Neufeld and Rimpau say it only takes place when bacteria are present.*

One has not found, as Hektoen and Ruediger state to be the case, that heating bacteria (staphylococci) to 60°C, after "opsonisation" i.e. incubation of serum and bacteria at 37°C for 20 minutes, abolished phagocytosis if the mixture be subsequently tested by the addition of corpuscles: there was marked phagocytosis. Staphylococci grown 4 days on human blood serum - which was removed by centrifugalisation and washing - were /

* There is not any constant change in the appearance of serum as a result of heating to 60°C: one has seen it clouded but not generally. Citrated plasma becomes (? always) opalescent

IX

Unheated Serum Washed Corpuscles Susp. B. Coli (treated with unheated serum)	0·3cc 0·3cc 0·1cc.	258/50	I
Heated Serum Washed Corpuscles Susp. B. Coli (treated with unheated serum)	0·3cc. 0·3cc 0·1cc.	21/50 30/50 46/50	II
Saline Solution (0·8%) Washed Corpuscles Susp. B. Coli (treated with unheated serum)	0·3cc 0·3cc 0·1cc	14/50 20/50	III
Serum (after treatment with 3 loopfuls B. Coli) Washed Corpuscles Susp. "normal" B. Coli	0·3cc 0·3cc 0·1cc.	5/50 22/50	IV
Unheated Serum Washed Corpuscles Susp. "normal" B. Coli		157/50	V
Heated Serum Washed Corpuscles Susp. "normal" B. Coli		1/50	VI
Saline Solution Washed Corpuscles Susp. "normal" B. Coli		4/50	VII

The suspension of "normal" B. Coli was less dense than that of the "treated" B. Coli (both suspensions in 0·8% NaCl solution) This is the reason for the difference of the counts in I & V. Same culture used for each suspension.

Serum heated 15' at 60 °C on water bath. (Heated Serum)

Incubation of mixtures = 20' at 37 °C

were freely ingested by the leucocytes (in a mixture containing fresh serum). One has treated serum with a relatively large quantity of small pieces of fine silk thread which had previously been boiled in distilled water and dried: this portion of serum was centrifugalised free of thread and examined as usual. No diminution of phagocytic effect followed: the count rather rose ($^{32}/_{50} \rightarrow {}^{40}/_{50}$). This is in contrast to what occurs when one treats serum with bacteria in the same way. 1 c.c. of serum was treated with three loopfuls of a 24 hour agar culture of B. Coli (i.e. incubation at 37°C for 15 minutes): this mixture was then centrifugalised, the deposit washed and filtered. The serum so treated with bacteria showed great diminution of phagocytic effect when tested with a suspension of "untreated" bacilli: on the other hand the treated bacilli do not behave very differently from the untreated. V. Table IX. This at variance with Bulloch's results for the Staphylococcus.

Serum which has been heated (on the water bath) at 60°C for 15 minutes does not seem to modify this organism so that it cannot be taken up in unheated serum.

1 c.c. of heated serum was treated with three loopfuls of agar culture (24 hours) of B. Coli i.e. incubated /

X

UNHEATED SERUM 0·3cc. Washed Corpuscles 0·3cc. Susp. B. Coli (treated with heated serum as described) 0·1cc.	180/40	I	
HEATED SERUM 0·3cc. Washed Corpuscles 0·3cc Susp. B. Coli (treated with heated serum as described) 0·1cc.	1/40	II	
UNHEATED SERUM 0·3cc Washed Corpuscles 0·3cc Susp. "normal" B. Coli. 0·1cc.	69/30	III	
HEATED SERUM 0·3cc Washed Corpuscles 0·3cc Susp. "normal" B Coli 0·1cc.	0/30	IV	

Suspension of "normal" B. Coli less dense than that of "treated" B. Coli. Same culture used for each.

There is however no sign that phagocytosis in I is less brisk than usual, i.e. the count in I does not approximate to that in III.

Serum heated 15' at 60°C on water bath (HEATED SERUM)

Incubation 20' at 37°C.

incubated together at 37°C. The organisms were centrifugalised, washed and re-suspended. When compared with a less dense control suspension (untreated with serum but centrifugalised etc. as the other) of the same culture the treated bacilli seem to be ingested in the same way, (this corresponds to Bulloch's result for staphylococcus). v. Table X.

One has not found that variation of the salt content (i.e. slight variation) of the mixtures - in the absence of serum - has any marked effect on phagocytosis.

In place of serum, salt solutions as in Table XI were examined. In an experiment given in Proc. Roy. Soc. Jan. 1906 Wright shows as a curve how phagocytosis (of tubercle bacilli) increased as the salt content of a serum mixture diminished from 0.8 to 0.7 % Na Cl.: in the greater dilutions he attributes the phagocytosis entirely to the 0.6 % Na Cl solution used for dilution: on the other hand the count fell steadily to 0 as the Na Cl content rose from 0.92% to 1% (that is, one and the same serum was diluted in the first case with 0.6% saline, in the other with 1.3% saline). There is nothing analogous here. (XI) One considers however that phagocytic counts in smears from mixtures in which serum is replaced largely or entirely /

~~XII~~

Saline Solution (0·6%)	0·3cc.	16/25 (?)	I
Washed Corpuscles	0·3c.c.		
Susp. Cholera Vib.	0·1cc.		
Saline Solution (1·2%)	0·3cc.	20/50 (?)	II
Washed Corpuscles	0·3cc		
Susp. Cholera Vib	0·1cc.		
Saline Solution (0·8%)	0·3cc.	22/25 (?)	III
Washed Corpuscles	0·3cc.		
Susp Cholera Vib.	0·1cc.		
Saline Solution (0·6%)	0·3cc.	0/30	IV
Washed Corpuscles	0·3cc.		
Susp B. Proteus	0·1cc.		
Saline Solution (1·2%)	0·3cc.	3/30	V
Washed Corpuscles	0·3cc.		
Susp. B. Proteus	0·1cc.		
Saline Solution (0·8%)	0·3cc.	3/30	VI
Washed Corpuscles	0·3cc		
Susp. B. Proteus	0·1cc.		

→ Count in serum = 172/50

Unheated Serum	0·3cc.	337/50	I
Washed Corpuscles	0·3cc.		
Susp. Staph. Ry. Aur.	0·1cc.		
Urine (boiled: filtered)	0·3cc.	21/50	II
Washed Corpuscles	0·3cc.		
Susp. Staph. Ry Aur.	0·1cc.		
Saliva	0·3cc.	208/50 * (circa)	III
Washed corpuscles	0·3cc		
Susp. Staph. Ry Aur.	0·1cc.		

X

* The staphylococci were somewhat agglutinated.

entirely by salt solution are of doubtful value. In such case one has found that disrupted leucocytes are extremely numerous; incubation with saline solution seems to cause this: sometimes one has to search diligently for entire or approximately entire polymorphs. These broken-down leucocytes are often mere faint reticula: sometimes they are recognisable as leucocytes of a certain kind: in the earlier stages the nucleus is swollen and projects out of the cell. Broken-down leucocytes occasionally show ingested cocci. The smears from saline mixtures after staining show almost invariably much deposit (Leishman's stain being used).

In general, phagocytosis in salt solution is small. Two other media - saliva and urine - were examined. v. Table XII

A number of organisms were examined with regard to phagocytosis in unheated serum and in serum heated at 60°C on the water bath. B. Subtilis, B. Proteus, B. Coli, Micrococcus Melitensis, a Torula, Streptococcus Pyogenes, examined in tubes in the usual way showed decrease sometimes more, sometimes less but always marked, in the heated serum. B. Coli showed this more than any other organism examined, and for this reason was used in the experiment mentioned above (B. Coli /

Phagocytosis of Micrococcus Melitensis in various media.

Incubation = 20' at 37°C

UNHEATED SERUM	0·3cc.	219/20	
WASHED CORPUSCLES	0·3cc.	281/20	
Susp. Micrococcus Melitensis	0·1cc	(circa)	I
HEATED SERUM (10' at 60°C on w. bath)	0·3cc.	28/20	
Washed Corpuscles	0·3cc.	30/20	II
Susp. Mic. Melit.	0·1cc.		
HEATED SERUM (20' at 60°C on water bath)	0·3cc.	12/20	
Washed Corpuscles	0·3cc.		III
Susp. Mic. Melit.	0·1cc.		
SALINE SOLUTION (0·8% NaCl)	0·3cc.	9/20	
Washed Corpuscles	0·3cc.		IV
Susp. Mic. Melit.	0·1cc		
CITRATED PLASMA.	0·3cc.		
Washed Corpuscles	0·3cc.	62/20	V
Susp. Mic. Melit	0·1cc.	74/20	
HEATED CITRATED PLASMA (20' on the water bath)	0·3cc.	2/20	
Washed Corpuscles	0·3cc		VI
Susp. Mic. Melit.	0·1cc.	3/20	

The difference between I and V seems rather remarkable.

Coli treated with heated serum). B. Pyocyaneus showed undoubted decrease but because of its tendency to agglutinate and because many leucocytes were phagocytic in the heated serum one could not estimate comparative phagocytosis except in the roughest fashion. For this particular organism there seemed to be less phagocytosis in citrated plasma than in heated serum: in the citrated plasma the bacilli were gathered in enormous masses and probably the majority of the leucocytes were never in a position to ingest them. Wright states that B. Diphtheriae is ingested as freely in heated as in unheated serum. One examined this organism twice (two races): the organism is clumped in the mixtures and some phagocytosis goes on in heated serum. 24-hour blood serum cultures were used: count in unheated serum $^{106}/_{27}$ (= 4 approx), in heated serum $^{81}/_{100}$ (0.81). The result was similar on the other occasion.

With regard to the Pneumococcus, Wright found that it behaved as did the majority of the organisms examined. Hektoen and Ruediger stated that none of the leucocytes mentioned on p. || ingested the pneumococcus, except those of the horse, and those only slightly. Mennes found that it was not ingested in normal serum (v. supra). Huber confirmed the results of Mennes for human leucocytes but also "made the interesting /

XIV

Suspensions of 24-hr. blood agar growths used in each case

		Pneumococcus ex sput. thro' 2 rabbits	Pneumococcus on blood agar 1 month		
Two tubes a & b.					
UNHEATED SERUM	0·3cc.				(a) & (b) = separate tubes
Washed Corpuscles	0·3cc.	48/50 (a)	108/50		
Susp. Pneumococcus	0·1cc.	41/50 (b)		I	
Done in capillary tubes; 15' incubation.					
UNHEATED SERUM	3 vols.				
Washed Corpuscles	3 vols.	31/50	?	II	
Susp. Pneumococcus	1 vol.				
SERUM HEATED 15' on water bath at 60°C	0·3cc.				
Washed Corpuscles	0·3cc.	6/50	32/50	III	
Susp. Pneumococcus	0·1cc.				
SALINE SOLUTION (0·6%)	0·3cc				
Washed Corpuscles	0·3cc	0/50		IV	
Susp. Pneumococcus	0·1cc				
UNHEATED SERUM	0·3cc.				
Washed Corpuscles	0·3cc	27/100	31/50	I	
Susp Pneum.	0·1cc.				
SERUM HEATED 10' at 60°C on water bath	0·3cc.				
Washed Corpuscles	0·3cc.	2/100	9/50	II	
Susp. Pneum.	0·1cc.				
SERUM HEATED 20' at 60°C on wat. bath	0·3cc.				
Washed Corpuscles	0·3cc.	5/100		III	
Susp. Pneum.	0·1cc.				
SALINE SOLUTION (0·8%)	0·3cc				
Washed Corpuscles	0·3cc	2/100		IV	
Susp. Pneum.	0·1cc.				
CITRATED PLASMA.	0·3cc				
Washed Corpuscles	0·3cc.	0/50	0/30	V	
Susp. Pneum.	0·1cc.				
CITRATED PLASMA (heated 15' at 60°C on water bath)	0·3cc				
Washed Corpuscles	0·3cc.	2/50	10/50	VI	
Susp Pneum.	0·1cc				

interesting observation that these could ingest a-virulent pneumococci" (Neufeld and Rimpau). Gruber and Futaki found that guinea-pig leucocytes took up the pneumococcus in "active" (normal) serum. Macdonald (1), using glycerine agar culture of the pneumococcus, has examined the "opsonic curve" in a long series of cases of acute pneumonia.

One examined phagocytosis of the pneumococcus on four occasions: on the first two one found that the organism was ingested in normal serum to some extent and very little in heated serum. It is difficult to obtain sufficiently copious growths of it in 20 hours. However by using several tubes of blood agar one obtained fairly dense and fairly uniform suspensions of two strains: one had been grown on blood-agar for a month by frequent subculture, the other had very recently been obtained from pneumonic sputum and had been passed through two rabbits. These suspensions were examined with this result (v. Table XIV). The suspension of the recently isolated pneumococcus was the more dense: even in the other, however, phagocytosis was much less marked than it would have been in the case of a staphylococcus suspension (little virulent) of similar density. With regard to the Cholera Vibrio and Typhoid Bacillus, phagocytosis in serum which has /

(1) Trans. Path. Soc. Lond. vol. 57 pt. 1.

has been heated to 60°C is very marked (cf Wright, Proc. Roy. Soc. vol. 73: Leishman supra: recently Harrison, Trans' Path. Soc. Lond.) In his description Wright (loc. cit.) seems to identify, in this case, opsonic, more or less, with bacteriolytic effect. He states that, as regards cholera, the ingested organisms, in unheated serum are mostly spherulated before ingestion: those ingested which have a normal shape were ingested before the serum had time to act on them. There is no vacuolation to be seen in the white cells of unheated-serum mixtures. On the other hand in heated serum the organisms intracellular and extracellular show no spherulation: vacuolation round ingested vibrios is marked (loc. cit. p 138 and fig. p 141). As to typhoid bacilli the conditions are the same only no vacuoles are figured either for the heated-serum or unheated-serum cells: in heated serum the typhoid bacilli are "morphologically unaltered and have preserved their staining properties unimpaired". Metchnikoff (Immunity p. 198) states that typhoid bacilli undergo little or no change in serum.

One has found that phagocytosis of the Cholera Vibrio and Typhoid Bacillus is very marked in heated serum. One has seen (for cholera vib.) however, very marked vacuolation in a smear made from an unheated serum /

serum mixture. On the other hand one has found in the case of heated-serum ingested cholera organisms which showed "digestive" changes (swelling and pink staining): one did not see such marked vacuolation as Wright figures. The cholera organisms used was a laboratory grown race. The typhoid bacillus keeps its proper shape within and without the cells much better in heated serum than in unheated: in examining cells (heated serum) in two or three slides one found here and there colour changes and imperfect staining of the ingested bacilli: one has even seen what one took to be intracellular spherulation. In unheated serum the extracellular typhoid bacilli were few (so also cholera): these bacilli were rounded and stained a deep blue: in the cells there was much pink granulation also many blue spherules and a number of unchanged bacilli. Vacuolation was fairly evident in heated and unheated-serum cells (in contrast to Wright's figures). In the unheated serum mixtures one did not see nearly so much extracellular spherulation as Wright figures. The Finkler-Prior organism was examined: in heated serum phagocytosis was marked (rather less than in the case of Cholera Vib.) and on the whole ingested organisms showed little change but there sometimes seemed to be swelling of the /

the ingested organisms and sometimes one noticed pink granules in the cells not unlike bacterial débris. On the other hand vacuolation in the polymorphs in unheated serum was extremely marked, sometimes quite enormous.

Wright suggested that this method might be applied as a test for the efficiency of therapeutic sera (antimicrobic): their efficacy to be gauged by their phagocytic effect. Crofton (1) carried this out with several specimens of antistreptococcic and antistaphylococcic serum. He found that the sera, unless quite fresh, gave rise to no phagocytosis when examined with his own corpuscles: even when less than a month old, such a serum gave a count of 0. On "reactivation" with his own unheated serum Crofton obtained a higher count with Antistrept. serum than with his own serum diluted with saline to a corresponding volume. He considers from his experiments that the phagocytic effect (even in normal serum) is due to the interaction of a thermolabile with a thermostable substance. The Antistaphylococcic serum could not be "reactivated" in this way.

The following experiments were done with some phials /

(1) Journ. Hyg. vol. 5 No. 4.

phials of anti staphylococcic and antistreptococcic serum. The antistaphylococcic serum - which was five months old - gave on two occasions a relatively much bigger increase when "reactivated" than Crofton found for his antistreptococcic serum. On a third occasion this was not so. The antistreptococcic serum ($2\frac{1}{2}$ months old) showed no such increase (incubation 30 minutes and 15 minutes respectively). The streptococcus is not a good organism for phagocytic counts (at least the strain employed was not). A separate phial of anti-serum was used for each set of counts.

Dean obtained marked "opsonic" effects from horse serum four years old. The sera used were obtained from Dr. Dean.
(see Tables XV - XVIII)

XV

I	Unheated Serum 0·3cc. Washed Corpuscles 0·3cc. Susp. Staphylococcus 0·1cc.	134/50	138/50
II	Serum heated on waterbath at 60°C for 30' 0·3cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	85/50	63/50
III	Unheated Serum 0·3cc. Anti-Staphylococcic Serum 0·01 c.c. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	117/50	127/50
IV	Unheated Serum 0·25cc. Anti-Staph. Serum 0·05cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	302/50	287/50 *
V	Unheated Serum 0·2cc Anti-Staph. Serum 0·1cc. Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	222/50	234/50 *
VI	Unheated Serum 0·1cc Anti-Staph. Serum 0·2cc. Washed Corpuscles 0·3cc. Susp. Staph 0·1cc.	121/50	95/50
VII	Heated Serum (as above) 0·25cc. Anti-Staph. Serum 0·05cc Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	38/50	29/50
VIII	Heated Serum 0·2cc. Anti-Staph. Serum 0·1cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	13/50	5/50

Unheated Serum 0·5cc. Washed Corpuscles 0·5cc. Susp. Staphylococcus 0·1cc.	268/100	I	
Unheated Serum 0·5cc. Anti-Staph Serum 0·1cc. Washed Corpuscles 0·5cc. Susp. Staph. 0·1cc.	521/100 *	II	
Heated Serum 0·5cc. (60°C. on waterbath) Anti-Staph. Serum 0·1cc. Washed Corpuscles 0·5cc. Susp. Staph. 0·1cc.	96/100	III	
Saline Solution 0·5cc. (·8%) Anti Staph Serum 0·1cc. Washed Corpuscles 0·5cc. Susp Staph 0·1cc	8/100	IV	

Unheated Serum 0·3cc. Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	100/50	I	
Heated Serum 0·3cc. (30' at 60°C on wat bth) Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	29/50	II	
Unheated Serum 0·25cc. Anti-Staph. Serum 0·05cc Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc.	91/50	III	
Unheated Serum 0·15cc. Anti-Staph. Serum 0·15cc. Washed Corpuscles 0·3cc Susp. Staph. 0·1cc.	47/50	IV	
Heated Serum (as above) 0·15cc. Anti-Staph. Serum 0·15cc. Washed Corpuscles 0·3cc. Susp. Staph. 0·1cc	2/50	V	

Incubation of mixtures = 30' at 37°C

UNHEATED SERUM 0·3cc. Washed Corpuscles 0·3cc. Susp. Streptococcus 0·1cc.	445/100	I
HEATED SERUM (at 60°C 30' on nat. bth) 0·3cc. Washed Corpuscles 0·3cc. Susp. Streptococcus 0·1cc.	169/150	II
UNHEATED SERUM 0·3cc. ANTI-STREPTOCOCCIC SERUM 0·01cc. Washed Corpuscles 0·3cc Susp. Streptococcus 0·1cc	388/100	III
UNHEATED SERUM 0·25cc ANTI-STREPT. SERUM 0·05cc. Washed Corpuscles 0·3cc. Susp. Streptococcus 0·1cc.	256/100	IV
UNHEATED SERUM 0·15cc ANTI-STREPT. SERUM 0·15cc. Washed Corpuscles 0·3cc Susp. Streptococcus 0·1cc.	183/100	V
UNHEATED SERUM 0·1cc. ANTISTREPT. SERUM 0·2cc Washed Corpuscles 0·3cc. Susp. Streptococcus 0·1cc.	254/100	VI
HEATED SERUM 0·3cc. ANTI-STREPT. SERUM 0·01cc. Washed Corpuscles 0·3cc. Susp Streptococcus 0·1cc.	97/100	VII
HEATED SERUM 0·25cc ANTI-STREPT. SERUM 0·05cc Washed Corpuscles 0·3cc Susp Streptococcus 0·1cc.	76/100	VIII
HEATED SERUM 0·15cc ANTI-STREPT. SERUM 0·15cc. Washed Corpuscles 0·3cc. Susp. Streptococcus 0·1cc.	24/100	IX
HEATED SERUM 0·1cc ANTI-STREPT. SERUM 0·2cc. Washed Corpuscles 0·3cc Susp. Streptococcus 0·1cc.	69/100	X

XVIII

Incubation for 15' at 37°C

Unheated Serum 0.3cc Washed Corpuscles 0.3cc Susp Streptococcus 0.1cc.	95/50 90/50	I	
Serum Heated 0.3cc (10' at 60° on w.b.) Washed Corpuscles 0.3cc Susp Streptococcus 0.1cc	23/50	II	
Serum Heated 0.3cc. (20' at 60°C on w.b.) Washed Corpuscles 0.3cc. Susp. Streptococcus 0.1cc.	29/50	III	
Unheated Serum 0.3cc. Anti-Strept Ser. 0.01cc. Washed Corpuscles 0.3cc. Susp. Streptococcus. 0.1cc.	91/50 89/50	IV	
Unheated Serum 0.25cc. Anti-Strept. Ser. 0.05cc Washed Corpuscles 0.03cc. Susp. Streptococcus. 0.01cc	142/50 102/50	V	
Unheated Serum 0.2cc Anti-Strept. Ser. 0.1cc Washed Corpuscles 0.3cc Susp. Streptococcus 0.1cc	81/50	VI	
Serum Heated 0.25cc (10' at 60°C on w.b.) 0.05cc. Anti-Strept. Ser Washed Corpuscles. 0.3cc. Susp. Streptococcus 0.1cc	1/100	VII	

V shows a doubtful increase.

Mixt	Ser. htd. mal. bth. 15′ at 55°C	Ser htd 5′ at 60°C	Ser htd 30′ at 60°C.
Ser (unheated) Bacps. Sump	353/50	511/50	368/50
Ser. 2 htd. Ser. 1 Bacps Sump	293/50	500/50	341/50
Ser. 1 htd Ser. 2 Bacps Sump.	288/50	532/50	418/50
htd Ser. Bacps Sump.	64/50	103/50	77/50
Cit Plasm. Bacps Sump		224/50	
htd Cit Pl. Bacps Sump.		142/50	

The higher counts in the centre column are due to inadvertence in that the series was given about 24′ instead of 20′ in the incubator.