

In contrast with the observations incorporated in Table II in the body of the paper these observations were conducted in phagocytic mixtures containing 0·85 per cent. instead of 1·1 per cent. of NaCl. It is shown in the next following communication that spontaneous phagocytosis is absolutely abolished only in the case when the salt content of the phagocytic mixture exceeds 1 per cent.

The source of fallacy to which attention is here called falls, no doubt, for all practical purposes, entirely out of account.

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.

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(Communicated by Sir John Burdon-Sanderson, Bart., F.R.S. Received October 21,—Read November 23, 1905.)

It has been indicated in the foregoing paper than an *incitor element** is to be found in the blood of those who have made an immunising response to tubercular infection, or, as the case may be, to an inoculation of a tubercle vaccine. This fact does not stand by itself.

Recital of Previous Observations on the same Subject.

The observations of *Metchnikoff*, following in sequence upon the classical researches of R. Pfeiffer on the intraperitoneal destruction of bacteria by the aid of immune sera, first drew attention to the fact that very active phagocytosis comes under observation when bacterial cultures, or as the case may be spermatozoa, are introduced into the peritoneal cavity of normal animals in association with heated serum derived from immunised animals.

* The term "incitor-element" (Latin—*incito*: I hasten, I urge forward, I bring into rapid movement) is here employed to denote the element in the heated serum which promotes phagocytosis. By employing this term, pending the elucidation of the nature and mode of action of the element in question, we secure the advantage of leaving these issues unprejudged.

*Savtschenko** obtained in experiments conducted *in vitro* with the heated sera of animals which had been subjected to injections of red blood corpuscles, phagocytosis of these formed elements.

Neufeld and Rimpau,† working with heated sera derived from animals which had been immunised against streptococcus and pneumococcus, and conducting their experiments *in vitro*, have described these immune sera as possessing a power of inciting phagocytosis. This power was, be it remarked, not numerically measured.

Leishman,‡ employing the numerical method for the measurement of phagocytosis which was devised by him with the modifications introduced by one of us in conjunction with Douglas, ascertained that the sera derived from Malta fever convalescents, or as the case may be from men who had undergone anti-typhoid inoculation, retain, after heating, elements which promote phagocytosis.

Dean, working with the same methods, without however conforming to the easily realised conditions§ which are essential to the accuracy of the enumeration, has described incitor elements in the heated serum derived from animals which had been immunised against staphylococcus.

Lastly, *Douglas*, employing again the same methods, has obtained evidence of the presence of an incitor element in the heated serum derived from himself and others after inoculation with a sterilised culture of the plague bacillus.

Views of the Observers above mentioned on the Nature of the Incitor Element contained in the Heated Serum.

Influenced by the theoretical conception that the increased resistance to bacterial invasion which is obtained by bacterial inoculation is in every case referable to a modification of the phagocytes,|| *Metchnikoff* originally spoke of the incitor element as a *stimulin*.

* 'Annales de l'Institut Pasteur,' 1902.

† Neufeld and Rimpau's paper was published in the 'Deutsche Med. Wochenschrift' in September, 1904, 12 months after the first description of the opsonins in these 'Proceedings.'

‡ 'Path. Soc. Trans.,' 1905, vol. 56.

§ "I should not feel disposed," remarks this author ('Roy. Soc. Proc.,' Series B, vol. 76, p. 511), "to place quite the same reliance as Wright and Douglas on the numerical accuracy of the results which can be derived from their method. Where the leucocytes are very full, *i.e.*, where the counts are high—it is impossible to differentiate results by the method of enumeration." In spite of the perfectly self-evident experimental limitation of our method, which Dean here recognises, this worker employs in practically all his published experiments bacterial suspensions which give him an *average* phagocytic count often of 50 and more bacteria in the leucocyte. Such a count is altogether incompatible with accurate quantitative work.

|| The correctness of the view that artificial immunity depends upon a modification of

This appellation may, we think, be characterised as unfortunate, *first*, because the mode of action of the incitor element was prejudged; *secondly*, because the appellation suggests (in contravention to everything which has come to light with respect to immunisation) that there are elaborated in the animal organism in response to inoculations, not *vaccinotropic* elements (elements which have a chemical affinity for the vaccine) but *leucocytropic* elements (substances which have a chemical action on leucocytes).

At a later date the terms "sensitiser" and "fixing substance" (*la substance sensibilisatrice* and *le fixateur*) were applied by Metchnikoff to the incitor element. This nomenclature is, it seems to us, almost equally infelicitous—infelicitous because it imposes upon the mind the following ideas:—(a) that the phenomena of phagocytosis are analogous to those of hæmolysis; (b) that the incitor substance, like the "amboceptor" of Ehrlich, exerts its specific effect only in the case where it is reinforced by a complement; and (c) that the mechanical movements of the phagocyte in the ingestion of particulate matter are analogous to the chemical action of the complement in the case where red blood corpuscles are dissolved by a hæmolytic serum.

With the exception of Leishman,* who, with a view to conforming to the original nomenclature of Metchnikoff, and also because his own experiments incline him to adopt the same point of view, speaks of the incitor substances as *stimulins*, all the other observers† take the view that the

the leucocytes was first inquired into by Denys and Leclef ('La Cellule,' 1895, vol. 11), in connection with their experiments conducted on rabbits with streptococcus. The doubt with regard to the correctness of Metchnikoff's view which found expression in the paper of these authors was further justified by the experiments of Mennes ('Zeitsch. f. Hygiene,' 1897, vol. 25), conducted with the blood of animals immunised against the pneumococcus. Finally, the incorrectness of the view that immunisation depends on a modification of the leucocytes was for the first time unambiguously established by one of us working in conjunction with Douglas ('Roy. Soc. Proc.,' vol. 72, p. 369, and vol. 73 p. 129). Our results were afterwards confirmed by Bulloch ('Roy. Soc. Proc.,' vol. 75).

* *Loc. cit.* and 'Journ. of Hygiene,' 1895.

† It may be remarked in this connection that Neufeld and Rimpau, while satisfied that the incitor substances in the serum exert an opsonic action on the bacteria, suggest that the term *opsonins* should be here rejected and that the substances here in question should be called *bacteriotropins*. Pending the discussion of the questions of the mode of action of the incitor elements in the heated serum, and of their identity or non-identity with the opsonins found in normal blood, it will suffice here to remark with respect to the proposed nomenclature of Neufeld the following:—

(a) The term *bacteriotropins* (since it connotes nothing more than the property of entering into chemical combination with bacteria) is more appropriate as a generic term for the whole class of substances which combine chemically with bacteria, than as a specific designation for the substances which prepare the bacteria for phagocytosis.

(b) All considerations of the comparative merits of Neufeld's terminology and my terminology apart—there must, I apprehend, remain to me as the author of the term

incitor element in the immune serum exerts an opsonic action upon the bacteria, preparing them for phagocytosis.

Sources of Fallacy which must be Eliminated before the Question as to the Nature of the Incitor Element in the Heated Serum can be Properly Investigated.

Before an inquiry into the nature of the incitor constituent of heated "immune serum" can be properly taken in hand, the sources of fallacy which are incident to such an inquiry must be realised. A *first* source of fallacy is associated with the occurrence of *spontaneous phagocytosis*. A *second* source of fallacy arises, as we shall see in a subsequent section, in connection with the fact that the incitor power of the heated immune serum is influenced in a remarkable and, for the present, quite inexplicable manner by the duration of the exposure to heat and by the temperature employed.

Fallacy of Spontaneous Phagocytosis.

It will enforce itself upon the mind on considering the protocols of the original experiments published by one of us in conjunction with Douglas in these 'Proceedings'* that the phagocytosis is not completely

bacteriotropic substances ('Lancet,' December 23, 1899) as against Neufeld the right of assigning to this term its technical signification.

Dean likewise, while championing the view that the incitor element is an opsonin, and while dissatisfied with the ambiguity of the terms "fixateur" and "substance sensibilatrice," and while conceding that "it may be convenient to adopt the term *opsonin*," employs instead the periphrasis "*the substance which prepares the micro-organisms for phagocytosis*," denying himself the convenience of the term opsonins "in order to mark the danger that one might be led to regard the opsonin as actually a different substance, and not merely a property of immune serum." My fellow-worker, Douglas, and I have not claimed for ourselves anything more than this: that we have, by the aid of an accurate quantitative method, adapted from Leishman, placed in a clear light the rôle of the blood fluids in relation to phagocytosis, a rôle which was practically everywhere ignored or misconceived, and which had at best been "glimpsed" by one or two observers whose work, undertaken with very defective and fallacious technical methods, was, as Dean's own analysis shows, of a very unconvincing character. We submit that the clarification of the rôle of the blood fluids which was effected by us would have remained incomplete and ineffective if we had not alighted on the terms "opsonic power" and "opsonins," or some other apposite and equally convenient nomenclature to denote, as the case may be, the power or "*the substance in the serum which prepares the micro-organisms for phagocytosis*."

We would also submit that the ultimate—and we hold for the present unapproachable question—as to whether the opsonic effect we have described is only one of a series of diverse effects exerted by a single *antitropic* substance, or whether it is the result of the specific activity of an independent chemical unit in the serum, is not prejudged by the employment of the term *opsonin*.

* Vols. 72, 73, and 74.

abolished by the heating of even a normal serum. The residual phagocytosis registered in the protocols must, as reflection will show, be either *spontaneous phagocytosis*, meaning by this phagocytosis occurring apart from any co-operation of the serum, or phagocytosis dependent upon the chemical activity of an element which has resisted the destructive action of heat.*

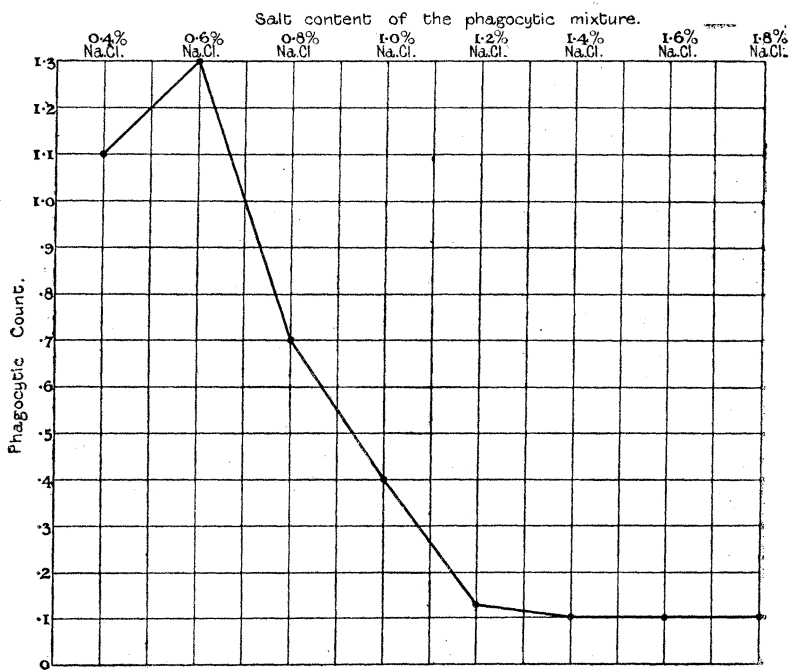
When face to face with the consideration that the elimination of all spontaneous phagocytosis must be a necessary preliminary to the proper investigation of every question which has reference to the presence of an incitor element in heated serum, a suggestion from our fellow worker Captain Stewart R. Douglas, I.M.S., led us to inquire whether the phagocytic activity of the leucocyte might not be affected in a conspicuous manner by the salt content of its fluid environment. Captain Douglas's suggestion was a happy one. For, as will appear in the next section, we found that in certain concentrations of salt the leucocytes display considerable spontaneous phagocytosis with respect to the tubercle bacillus, while again in other salt concentrations spontaneous phagocytosis with respect to these micro-organisms is entirely suppressed.

Investigation of the Influence of the Salt Content of the Fluid Environment of the Leucocyte upon Spontaneous Phagocytosis.

The general results of our experiments conducted with tubercle bacilli will be best submitted in the form of the subjoined graphic curves.

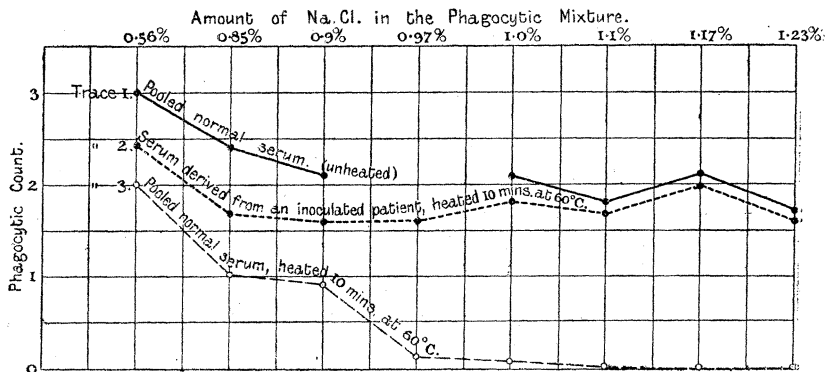
In *Curve 1* we show the phagocytic counts obtained in an experiment conducted without any admixture of serum. In these experiments one volume of washed blood corpuscles, suspended in 0.85 per cent. NaCl solution, was mixed in each case with an equal volume of suspension of tubercle bacilli in distilled water, and with one volume of a graduated solution of salt. It will be observed that the spontaneous phagocytosis which is here in question is greatest where the phagocytic mixture contains 0.6 per cent. of NaCl, and that the count falls off in a gradual manner, and finally reaches a figure which does not differ sensibly from zero when a concentration of 1.2 per cent. NaCl is arrived at.

* In favour of the former of these two alternative explanations of the residual phagocytosis is, *first*, the difficulty of conceiving in connection with the experiments conducted with carmine and Indian ink particles that these were chemically acted upon by the serum; *secondly*, the difficulty of explaining, otherwise than as a result of individual differences in phagocytic activity as between leucocyte and leucocyte, the fact that in preparations made with heated normal serum and tubercle bacilli suspended in physiological salt solution, the phagocytosis is generally restricted to a very small percentage of the leucocytes instead of coming into evidence, as in the case of experiments conducted with unheated and active serum, in association with practically all the mature leucocytes.



CURVE 1.

In *Curve 2* we show the phagocytic counts obtained in films prepared from phagocytic mixtures containing a *double* volume of undiluted serum, a *double* volume of washed corpuscles suspended in 1 per cent. NaCl, and a *single* volume of a suspension of tubercle bacilli made in the same menstruum, supplemented in each case by a *single* volume of a solution of sodium chloride of progressively increasing strength.



CURVE 2.

Three different sera were here subjected to experiment—

1. The pooled unheated serum derived from eight normal men ;
2. The same serum after it had been exposed to a temperature of 60° C. for 10 minutes ; and
3. Serum from a patient who had been subjected to therapeutic inoculations of tubercle vaccine. This serum, like the last, had been exposed to a temperature of 60° C. for 10 minutes.

It will be seen that, as in Curve 1, where no serum was employed, the highest phagocytic counts were with each serum obtained where the concentration of the sodium chloride was least.

In the case of Trace 3 (obtained with the heated normal serum) the phagocytosis must be interpreted throughout as purely spontaneous phagocytosis.

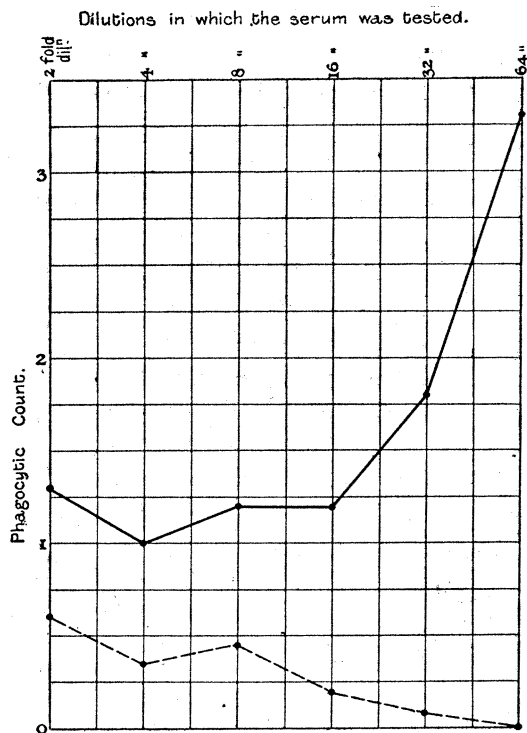
In Trace 1 and Trace 2 it must, in the case where low concentrations of NaCl are in question, be interpreted as spontaneous phagocytosis supplemented—to an extent corresponding with the differences between the counts in these traces and those in Trace 3—by phagocytosis dependent upon the chemical action of the serum. Lastly, in these two first traces the phagocytosis registered where high concentrations of NaCl were employed must be entirely dependent upon the chemical action of the serum.

In Curve 3 we show the effect of making progressive dilutions of one and the same normal unheated serum with, in the one case a 0·6 per cent. NaCl solution, and in the other case a 1·3 per cent. NaCl solution, using, as in the experiments above, in each case one and the same suspension of tubercle bacilli and one and the same washed blood cream.*

It will be seen that while in the lower trace the phagocytic count sank away in an almost regular manner to zero as the opsonins of the serum were more and more diluted, in the upper trace the phagocytic count increased as the serum was diluted by a less concentrated salt solution.

We do not see room to doubt that in the case of the lower trace spontaneous phagocytosis was completely suppressed, and that such phagocytosis as was obtained was due exclusively to the action of the opsonins, and that in the case of the upper trace the phagocytosis obtained in the outset was due to spontaneous phagocytosis supplemented by the action of the opsonins, while the increased phagocytosis in the latter part of the trace was entirely due to spontaneous phagocytosis.

* By this procedure there were obtained in the first case phagocytic mixtures whose salt content diminished from 0·8 to 0·7 NaCl, and in the second case phagocytic mixtures in which the salt content increased from 0·92 to 1 per cent.



CURVE 3.

Fallacy which may be introduced by the exposure of the Serum for a different period to different degrees of Temperature.

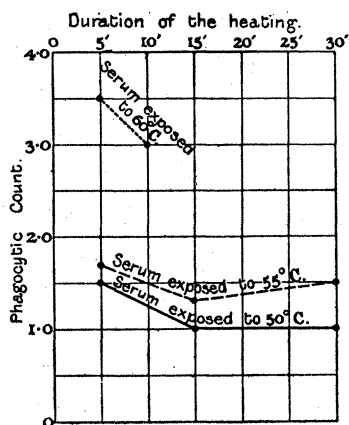
In view of the research of Dreyer,* which brought out the fact that the agglutinating power of a serum may, as progressively higher temperatures, or as the case may be progressively longer exposures are employed, be first lost and afterwards recovered, it suggested itself that an analogous effect might possibly be exerted upon the incitor element of an immune serum when exposed for different periods to different temperatures.

The results of a typical experiment carried out with such a serum are embodied in the *Curve 4* below.

It will be seen that while the incitor power of the immune serum was in each case preserved, a very different phagocytic count was obtained according as the serum was exposed to temperatures of 50°, 55° and 60° for a shorter or longer time.

It will be manifest, in view of these results, that where experiments are conducted with heated sera care must be taken to see that in every case the same conditions are observed in the matter of the heating of the serum.

* 'Brit. Med. Journ.,' September 10, 1904.



CURVE 4.

Investigation of the Question as to whether the Incitor Substance which is found in the Heated Serum of Persons who have responded to Tubercular Infection, or as the Case may be to an Inoculation of Tubercle Vaccine, is a Leucocytotropic Element—to which the Appellation “Stimulin” would Apply—or Bacteriotropic Element, to which the Term “Opsonin” would Apply.

The absorption method of Ehrlich, which has already been employed by Neufeld and Rimpau in connection with the investigation of the nature of the incitor element contained in the serum of animals immunised against streptococcus and pneumococcus, was obviously the method indicated for employment in connection with the problem here before us. It was also manifest, in view of the facts detailed in the previous section, that the comparative experiments instituted with heated immune serum before, and after digestion with tubercle bacilli and subsequent centrifugalisation, would yield unfallacious results only on adhering rigidly to the same conditions in the matter of the heating of the serum, and on arranging the experimental conditions in such a manner as to achieve in the phagocytic mixtures employed in each case a salt content of over 1 per cent. NaCl.

A series of experiments conducted with these precautions showed in an uniform manner that the incitor element can be completely extracted from heated immune serum by digestion for half an hour at 37° C. with a suspension of tubercle bacilli.*

It is thus clear that the incitor element which is found in heated serum of

* This result is in conformity with the results obtained by Neufeld and Rimpau in connection with streptococcal and pneumococcal immune serum, and by Dean in connection with staphylococcal immune serum.

persons who have responded to tubercular infection, or as the case may be to the inoculation of a tubercle vaccine, is an opsonin. We may, pending the discussion in the next section of its identity and non-identity with the opsonin of the unheated normal serum, speak of this opsonin in a provisional manner as the *opsonin found in the heated immune serum*.

Question as to whether the Opsonin found in Heated Immune Serum is or is not Identical with that found in the Unheated Normal Serum.

Leishman, who has spoken of the incitor element in the heated immune serum as a *stimulin*, in common with Neufeld, working in conjunction with Rimpau, and Dean, who have shown that this incitor element functions as an *opsonin*, have laid emphasis on the thermostability of the incitor element. Both Leishman and Neufeld urge that the character thermostability differentiates the incitor elements they have in view from the thermolabile opsonins described by one of us in conjunction with Douglas. Neufeld goes further, and contends that the particular opsonins which have been described by him as thermostable alone possess any significance in connection with the protection of the organism against bacterial disease. In support of this contention Neufeld adverts to the fact that man, although he is, according to experiments recorded by one of us in conjunction with Douglas, the possessor of thermolabile opsonins against the plague bacillus, is none the less not protected against this micro-organism.

Before investigating the question of fact as to the identity or non-identity of the opsonins of the normal and immune organism, which are discriminated from each other by Neufeld, we may be allowed to comment on the standpoint which he takes up. We submit that he proceeds upon an entirely erroneous conception when he assumes that the non-immunised human organism does not offer a resistance to such bacterial infections as plague. We submit, further, that it is erroneous to conceive of the normal organism as differing from the immunised organism in a qualitative manner. Rather, does not the theory of Ehrlich brilliantly teach that in immunisation we are never building upwards from a level of absolute non-resistance, but always building upon a foundation which is already laid—calling into existence in increased quantity and conveying into the blood only such chemical agents as exist already preformed in the body?

Reverting from this digression, we may address ourselves to the investigation of the facts, and may inquire whether they plead against or in favour of the identity of the opsonins which are found in the unheated normal blood with the opsonins which are found in the heated immune blood.

In the investigation of the facts we have built upon the following postulates:—

- (a) If the so-called thermostable opsonins are in reality thermostable, it will make no difference to the result whether the serum is heated in a diluted or in an undiluted condition. If on the other hand the thermostable opsonins represent nothing other than a residuum of thermolabile opsonins which has escaped destruction by heat, it may quite well happen that the serum will be completely inactivated if, before the heat is applied, the serum is adequately diluted.
- (b) Again, if the serum as derived from an immunised organism contains in its native condition a mixture of opsonins, which are respectively thermolabile and thermostable, we may, in conformity with the all-round greater chemical stability of thermostable substances, expect that the thermolabile opsonins will be destroyed when exposed to sunlight, and that the thermostable opsonins will remain unaffected.
- (c) Lastly, if the reputedly thermostable opsonins constitute an altogether new and distinct category of opsonins produced in the course of immunisation, we may expect, at any rate in cases where the immunisation has been carried very far, to find the thermostable opsonins greatly in excess of the thermolabile opsonins. In such a case it would be reasonable to expect the heated serum to bear almost as much dilution as the unheated serum before the point is in each case reached where the opsonic power is lost. On the contrary, if the so-called thermostable opsonins represent only an undestroyed residuum of the ordinary thermolabile opsonins, we may expect the heated serum to forfeit its opsonic power by dilution sooner than the unheated serum.

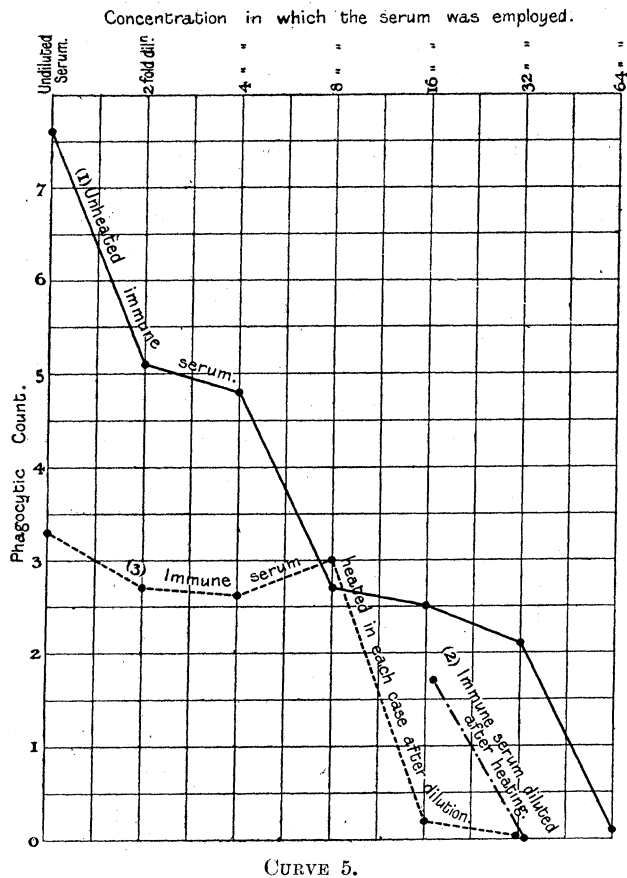
The graphic curves which are subjoined will serve to bring before the eye the results of, in each case, a typical experiment instituted with a view to the resolution of the questions suggested above.

Curve 5 furnishes an answer to the questions suggested in (a) and (c); *Curve 6* an answer to the question suggested in (b).

Explanation of Curve 5.—The experiment, whose results are here graphically set forth, had a double object in view. Its first object was to determine whether the tuberculo-opsonic power of the serum derived from an inoculated patient would be only partially abolished in the case where heat is applied to the undiluted serum, and would be completely abolished when heat is applied to the diluted serum. Its secondary object was to determine how far one and the same serum could be diluted before and after heating before its tuberculo-opsonic power was extinguished.

The serum which was employed for the purposes of this experiment was obtained from a patient whose opsonic index had been raised from 0·17 to

1:8 by repeated inoculations of new tuberculin, and who had under the influence of these inoculations completely recovered from tuberculous ulcers of the leg, which had laid bare the tendons, and which had for a period of 13 years previously to the commencement of the inoculation treatment defied all treatment.



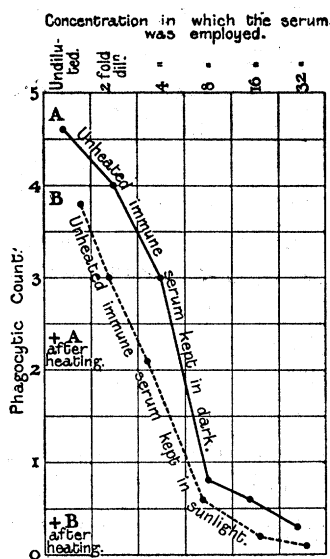
So far as the quantity of serum which was available allowed of this being done, these questions were investigated, the precautions explained above being in each case rigidly observed.

It will be seen on reference to the graphic curve, and on comparison of the phagocytic counts registered in the case of the 16-fold dilutions, that while the serum which had been first heated and then diluted (Trace 2) gave a phagocytic count of 1.7, the serum which had been first diluted and then heated (Trace 3) gave a phagocytic count of practically zero. It will further appear on referring to the three traces that, while the opsonic power of the

unheated serum was maintained till a 64-fold dilution was arrived at, the opsonic power of two samples of serum which were heated respectively before and after dilution was extinguished when in the former case a 32-fold, and in the latter case a 16-fold dilution was arrived at.

Comment.—The experiment shows that the opsonin found in heated serum is destroyed by heat when the serum is sufficiently diluted.*

Explanation of Curve No. 6.—In the experiment here in question we employed a serum derived from a patient with tubercular peritonitis, who had responded to infection in a characteristic manner.



CURVE 6.

Dividing it into two portions, we exposed one portion to direct sunlight for a period of six to eight hours, keeping the other portion in the dark in an incubator at 22° C. for the same time.

We now measured the opsonic power of each portion of serum both in the unheated condition and after exposure to 60° C. for 10 minutes. In the case of the unheated samples we tested in each case not only the undiluted serum but also in each case a series of progressive dilutions. In the case of the samples which were heated we tested only the undiluted sera.

It will be seen on comparing the phagocytic counts obtained with the insulated and non-insulated sera respectively, that while in the case of the unheated samples the serum which had been exposed to sunlight gave

* Further experiments bearing on this question will be found in the Appendix.

throughout almost as high a phagocytic count as the serum which had been kept in the dark, in the case of the heated samples the serum which had been exposed to sunlight gave a zero result, while the specimen which had been kept in the dark gave a count of 2·3 bacilli to each leucocyte.

Comment.—The experiment shows that the reputedly thermostable opsonin is—in contradiction with what is known to hold of other thermostable elements—eminently heliolabile.

Conclusions with respect to the Nature of the Incitor Element which is found in Heated Immune Serum after it has been Exposed to Heat.

Manifestly the plain teaching of our experiments is, that the opsonin which is found in the heated immune serum of a patient 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 the unheated normal serum.

A precisely similar conclusion with respect to the identity of the opsonins found respectively in unheated normal and heated immune sera was, we may note, arrived at by Dean in connection with his experiments on the sera of animals which had been immunised against staphylococcus.

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.

APPENDIX.

It may be convenient to subjoin here, in tabular form, the results of three experiments, similar to that set forth in Curve V, in which the opsonic power of a tuberculo-immune serum was measured in a series of dilutions made in the one case after the serum had been heated to 60° C. for 10 minutes, and in the other case before the serum was so heated.

Serial number of the experiment.	Source from which the serum was derived.	Phagocytic count obtained in the case of the heated undiluted serum.	Dilution in which the opsonic power was measured.	Phagocytic count in the case where the serum was heated before it was diluted.	Phagocytic count in the case where the serum was diluted before it was heated.
Expt. 1 ...	Pooled serum of six patients who had been inoculated with tubercle vaccine	2·4	2-fold dilution	1·9	3·3
			4-fold	2·7	1·7
			8- "	1·1	0·6
			16- "	1·0	0·45
			32- "	0·97	0·2
			64- "	0·75	0·08
Expt. 2 ...	Serum of a patient (E. M.) who had been inoculated with tubercle vaccine	—	4-fold	1·5	2·7
			8- "	1·4	1·9
			16- "	1·6	1·2
			32- "	1·5	0·3
Expt. 3 ...	Serum of a patient (J. B.) who had been inoculated with tubercle vaccine	1·4	4-fold	0·9	0·05
			2-fold	—	1·5
			4- "	0·85	1·5
			8- "	0·7	1·6
			16- "	0·7	0·2
			32- "	0·25	0·0
			64- "	0·0	—