Studies on immunisation : second series with appendices dealing with anti-typhoid inoculation, chemo-therapy, and statistical and other operations of induction / by Sir Almroth E. Wright.

RESEARCHES FROM

THE INOCULATION DEPT., ST. MARY'S HOSPITAL, LONDON W.2

IV

To H. H. Dale
with the author's kind regards

RESEARCHES FROM
THE INOCULATION DEPARTMENT, ST. MARY'S HOSPITAL
LONDON, W.2

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SECOND SERIES

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BY

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ACKNOWLEDGMENTS

The Author hereby expresses his grateful acknowledgments to the Council of the Royal Society of Medicine, to the Académie des Sciences, to the Proprietors of the *Encyclopaedia Britannica*, and to the *Lancet* and *British Journal of Experimental Pathology* for permission to reprint the papers which are here reproduced.

VACCINE THERAPY: ITS ADMINISTRATION, VALUE, AND LIMITATIONS

AN ADDRESS INTRODUCTORY TO A DISCUSSION ON THE SUBJECT

BY THE AUTHOR

(*Reprinted from the 'Proceedings' of the Royal Society of Medicine, 1910, Vol. III*)

I do not conceal from myself that in opening this debate I am undertaking a task of quite exceptional difficulty. I have to set out to you such new points in connexion with vaccine therapy as may seem to me to deserve attention. I have to attempt to take the measure of the achievements of this new therapeutic method. I have to discuss its limitations. And, above all, I desire to suggest to you in connexion with all these subject-matters certain canons of criticism.

Before I embark upon these tasks I may perhaps with advantage consider for a moment the rationale of vaccine therapy. The fundamental principle of vaccine therapy, as I conceive of it, is *to exploit in the interest of the infected tissues the unexercised immunising capacities of the uninfected tissues.*[1] Let me illustrate my meaning. I will take the case of a localised infection. We have here a condition in which the invading microbes are holding their own or getting the upper hand, and our object must be to turn the tables upon them. For this purpose we inoculate into some other part of the body microbes which are similar to those which the patient has to combat. Such inoculation is not, however, as the unthoughtful immediately assumed, a mere replica of the original infection. There are two points of difference. *First*, the microbes in the vaccine have been devitalized, so that their multiplication is impossible; *secondly*, the dose of vaccine is so regulated that the organism of the patient must inevitably win at the site of inoculation. Now a victory such as this is won by the elaboration of antibacterial substances, and these are generated in such a case on a scale which is more than adequate to bring about a destruction of the bacterial elements which have been incorporated. The surplus of antibacterial substances which have thus been elaborated will now find its way by the channel of the blood to the focus of infection. There it will bring aid to the defensive forces of the organism which before were ineffectually combating the invading microbes. The victory which the uninfected tissues have won over the microbes of the vaccine will in this way lead up to a victory of the infected tissues over the microbes they have to combat.

[1] If I were now rewriting this paper I should substitute for the phrase 'the unexercised immunising capacities of the uninfected tissues', the unexercised immunising capacities of the leucocytes.

A therapeutic method which proceeds on this new-found principle must, of course, make new demands. It will be well to realise what they are.

The medical man who has recourse to vaccine therapy ought to have a familiar acquaintance with the microbes which affect the human body. He ought to appreciate the fact that vaccines owe their efficacy to the reaction they set up in the tissues, and not to any action exerted by the vaccine directly upon the invading microbe. He ought to have mastered the general principles of immunisation. He ought to know in connexion with each vaccine the *directly effective dose*—i.e., the dose which gives an immunising reaction without any intervening negative phase—and the *medium*, or average, dose—i.e., the dose that gives, after a negative phase, a maximum immunising reaction. He ought to know the general conditions which affect the sensibility of the organism. He ought to understand how to adjust the dose to the requirements of the individual patient. And he ought to have a knowledge of the conditions which obtain in the focus of infection, and of the methods of circumventing the difficulties which are introduced by these local conditions.

If vaccine therapy demands all this of the medical man, it is clear that the discussion of vaccine therapy must inevitably lead on to the opening up of the question as to whether the medical practitioner must of necessity be a bacteriologist.

If, in the course of this discussion, it becomes clear that vaccine therapy is more effective than any other method of treating bacterial disease,[1] and if it is conceded that it holds out in very many cases the only real prospect of advantage to the patient, such concession will in reality be tantamount to a declaration that applied bacteriology is the essential and indispensable part of medicine, and that the practice of medicine must be reorganised upon that basis.

If, on the contrary, the conclusion is arrived at that vaccine therapy has, as compared with other methods, only a limited utility and a limited application, this will be tantamount to a declaration that the reorganisation of medicine on the basis of bacteriology may still a little longer be postponed. Such reorganisation, of course, becomes inevitable as soon as an effective application of bacteriology has been made.

Inasmuch as these are, if I understand the matter aright, the real issues which lie behind this debate, I think it will not be unprofitable if, before dealing specifically with vaccine therapy, I pass in rapid review the recent history of medicine and of its relations to bacteriology.

As one looks back on the history of medicine, and recognises that the successive applications of bacteriology stand out as great landmarks, one often wonders that medicine was not long ago swept along irresistibly into the channel of bacteriology. One wonders, for instance, in connexion with the advent of antiseptic surgery why this triumphantly successful application of bacteriology was not immediately followed up by a serious attempt on the part of our profession to study and turn to account this new science. Perhaps it was that the science of bacteriology was then

[1] The reader will here bear in mind that there was, at the date at which this lecture was delivered, no such thing as chemo-therapy for any bacterial infection; that there are still many bacterial infections for which there are no effective 'chemo-therapeutic' remedies; also that 'chemo-therapy' cannot protect against relapses or reinfection; and lastly that a fine in terms of constitutional poisoning has always to be paid for effective 'chemo-therapy'.

quite in its infancy, and perhaps that the thoughts of our profession were here too engrossed in experimentation with different antiseptics. But the dominant factor in the situation was, I feel sure, the consideration that since the action of antiseptics is exerted upon microbes without distinction of kind, the study of bacteriology appeared to the surgeon to be a mere work of supererogation.

When, following in his wake, the physician embarked upon the treatment of bacterial diseases by antiseptics, his thought followed the same road. He saw in antiseptics therapeutic agents which would be effective indiscriminately against all microbes, and seeing this, he, like the surgeon, held himself absolved from any study of bacteriology.

In the meanwhile the science was advancing with giant strides. One by one the pathogenetic microbes with which we are now acquainted were discovered and their rôle in connexion with the production of disease was elucidated. At the same time methods for identifying and making cultivations of each of these species of microbes were gradually elaborated. Then followed the discovery of agglutination, and with this the recognition of the possibility of diagnosing the nature of a bacterial infection by testing the properties of the blood fluids. After this came the discovery of the 'deflection of complement test' and the discovery of the opsonic power of the blood, and with this another and much greater extension of the method of serum diagnosis. Finally came the recognition of the possibility of inducing auto-inoculation for diagnostic uses,[1] and with this the serum tests which previously gave assistance only when spontaneous auto-inoculations were taking place became applicable also in connexion with strictly localised infections.

But the clinician has not yet been prevailed upon to reconsider his position. He still—in face of these discoveries—claims to be absolved from a study of bacteriology; and he is still bemused with the idea that the final appeal must always be to himself and to his methods of physical diagnosis.

Let me, in connexion with this fixed idea, invite you to consider the following points:—

(1) There was undoubtedly a time when the verdict of the pure clinician on a question of a diagnosis was incontestable. That was when the disease could be recognised and identified only by its clinical symptoms. Diphtheria then meant a condition where a particular kind of false membrane appeared on the throat; typhoid fever, a fever where the temperature ran a particular course; and pneumonia and phthisis, diseases in which certain noises could be heard down a stethoscope. With the discovery of the specific causes of these diseases came a quietus to all this. Diphtheria now means an invasion of the throat by diphtheria bacilli; typhoid fever an invasion of the blood by the typhoid bacillus; and pneumonia and phthisis an invasion of the lungs by the pneumococcus and the tubercle bacillus respectively. It is not, at this hour of the day, arguable that the verdict of a bacteriologist stands in need of confirmation from a clinician. In blunt language, 'the boot is on the other leg'.

Let me pass to a further point:—

[1] *Vide* these *Collected Researches*, Vol. III, Index, under 'Induced Auto-inoculations produced by various agencies.'

(2) While it must be conceded that clinical methods do furnish in many cases a bacteriological diagnosis, this is reached only by a process of inference, and the chain of inference is in this case made up of a series of unwelded links. I should not have thought it necessary to emphasise this so obvious fact were it not that I was recently assured by a reputable consultant physician that it had never crossed his thought that dullness over the apex of the lung did not supply proof of tubercular infection.

And there is yet one further important point :—

(3) A diagnosis of the nature of a bacterial infection which is based upon the physical signs comes short, not only in the respect that it can never be wholly trustworthy, but also in the respect that it must almost always be belated. For physical signs such as can be appreciated by clinical methods can, in the nature of things, be elicited only after comparatively gross physical damage has been done. I need not insist that bacteriological methods have from this point of view a great superiority. Pathogenetic microbes may, as you know, often be found in the secretions or the blood long before a diagnosis can be arrived at by clinical methods ; and when methods of direct bacterial diagnosis give negative results, we can nearly always by inferential methods—by the opsonic index,[1] or the agglutination test, or the deflection-of-complement test—arrive at the nature of the infection long before its clinical features have become distinctive.

While all this was, and is, uncongenial to the pure clinician, he did not fail to appreciate that there could be no question of ignoring bacteriological discoveries which were of fundamental importance for medicine. He was therefore face to face with the problem as to how to make provision for the carrying out of the necessary bacteriological tests and examinations. The proper way of confronting the situation would, of course, have been to look the future fairly and squarely in the face, and to enact that in the future no one should qualify as a medical man without some elementary knowledge of bacteriology, and that no one should proceed to any of the higher qualifications without a thorough training in this science.

If that course had been taken even a few years ago—and it has not yet been taken—the younger rank and file of the profession and the younger generation of clinical teachers would now be abreast of any work which might fall to them in connexion with vaccine therapy.

There was, however, one way of escape out of the situation. This was to delegate all bacteriological work in hospitals to a special bacteriological department, and in private practice to such public or private laboratories as might be disposed to undertake it. For a time—as in the case where the Merovingian Kings delegated all their functions to the Mayors of the Palace—everything smiled upon this arrangement. In particular the next great advance in bacterial therapeutics—I refer, of course, to the serum treatment of diphtheria—seemed to fit very harmoniously into this scheme. The medical man who had divested himself of all bacteriological work found here, to his joy, that it would be practicable for him to get his diagnosis from one bacteriological laboratory and his remedy from another, and that there would

[1] If I were rewriting this paper, here and elsewhere where I use the expression Opsonic Index, I would write Phago-incitor Index.

still remain for himself a dignified rôle as a middleman between the patient and the bacteriologist. Moreover, this rôle would, it was anticipated, continually grow in importance as serum therapy found new application in connexion with one acute disease after another. The medical man even dreamed dreams of polyvalent sera that would make everything in the nature of minute bacteriological diagnosis superfluous.

Upon this basis the march of bacteriological discovery seemed to promise advantage to the medical profession—advantage in the form of greater therapeutic success—and there would not be as counter-weight any added burden of unaccustomed labour.

The advent of vaccine therapy has disturbed this carefully thought out scheme, for it has brought home to us that bacteriological work is called for in connexion with nearly every case. And that is a fact which gives, as you will see, quite a new complexion to the proposal that the medical man should depute to others everything that relates to bacteriology. In point of fact we are here brought up against a very fundamental question—the question as to when, and under what circumstances and to what extent, the skilled work of a profession may legitimately be delegated. We may here confine ourselves to the case of the delegation of skilled work in connexion with our own profession.

It will prevent a confusion of issues if we at the outset discriminate between the case where a consultation is held over a patient and the case where a specimen is sent to a laboratory for report. In the former case the bacteriological consultant is brought face to face with the patient and with the problem which is to be resolved. He is asked to undertake any examination which may seem to him required for the elucidation of the case, and to discuss the whole problem with the practitioner; and the consultant and practitioner can then, as fellow experts, consider exactly where and to what extent '*functional errors*'—concerning which I shall presently [1] have something to say—may be affecting their several findings, and exactly how far these findings may be accepted as assured. And, finally, when, by such collaboration, a decision has been arrived at, the responsibility for any action taken rests upon the shoulders of both.

In the case where a specimen is sent to a bacteriological laboratory for report, we have utterly different conditions. The bacteriologist is not brought face to face with the problem. Instead of being afforded an opportunity of obtaining his own specimen in the way that he judges best, or even an opportunity of suggesting the proper way of approaching the bacteriological investigation, he is tied down to the examination of such specimens as may be submitted to him. Again, the bacteriologist is generally very imperfectly informed with respect to the nature of the problem which is to be resolved. It has even been suggested that it is wise to keep the bacteriologist, so that he should not be prejudiced, quite in the dark about the case. Moreover, when the written bacteriological report comes to hand the practitioner, who has not himself been a laboratory worker, will be quite unable to exercise any expert criticism upon it or to see where fallacies may come in. He will, in the ordinary case, assume that laboratory methods are not subject to fallacy

[1] pp. 22–23.

and 'functional error', and that the 'Yes' of the laboratory means definitely 'Yes', that the 'No' of the laboratory means definitely 'No', and that in the case of a laboratory result which is expressed in terms of figures, those figures must be an absolute mirror of reality. And even if it should so happen that the practitioner to whom the bacteriological report is sent has expert knowledge of laboratory work, he will, in default of personal knowledge of, and opportunity for questioning, the bacteriological worker, be quite unable to evaluate the 'functional error', and to assign in accordance with this its proper value to the report.

Having discriminated the case of the consultation which is, as we have seen, really the case of collaboration in skilled work from the case where a specimen is referred to a laboratory for report, we have now to try to arrive at a considered decision upon the morality of that kind of delegation. Three different cases of delegation must be distinguished.

The *first* is the case of a man who, being himself skilled in the whole of the work which he undertakes, has part of it carried out by an assistant, satisfying himself that it has been done in the proper manner and accepting the responsibility for this. It is the case of the delegation of duties by the honorary physician or surgeon to the resident staff of the hospital, by the director of a laboratory to his staff, and by the practitioner to the nurse.

The *second* is the case of a man who, when invited to undertake work which lies outside the range of his competence, hands over that work and the attaching responsibility to another who has the special kind of skill which is required. This is the ordinary case of a practitioner sending on a case to a specialist.

The *third* is the case of the man whose studies have not covered the whole of his professional work and who arranges to delegate to others that portion of the work which he has omitted to learn, while retaining for himself the responsibility for the whole, along with the higher scale of rewards which goes with that responsibility. You will, I think, recognise that this is the case of the medical man who, finding that bacteriological work is required for diagnosis and treatment, makes shift, without learning any bacteriology, to depute that work to others while retaining for himself the control of the case.

I have no doubt that you will adjudge the *first* of these forms of delegation to be both legitimate and necessary to the carrying out of work on any large scale; that you will commend the *second* while recognising that it can only exceptionally be resorted to; and I believe that you will see that the *third* case is not very clearly distinguishable from the case of a layman who should undertake to treat a case on condition of his being permitted to consult by correspondence competent medical authority whenever he might see occasion for doing so.

When I suggest that these cases are comparable, I do so because I want to put this issue before you. If we, as a profession, deprecate treatment by the unqualified on the ground of the dangers which may attach to the treatment of grave cases by the ignorant, can we then refrain from condemning, as perilous to the patient, the treatment of grave bacterial infections by those who are ignorant of bacteriology? And if we as a profession condemn consultation by correspondence on the ground that a trustworthy opinion cannot be based upon medical data which are furnished

by an ignorant patient, how then shall we refrain from condemning the system by which a medical man who is ignorant of bacteriology selects the bacteriological data upon which a diagnosis is to be based ?

I confess that I myself can find only one answer to that question. But let me assume that the question as to whether this kind of delegation is legitimate is still doubtful. There is then still another way by which we may arrive at a decision. Every tree is known by its fruits. We may therefore inquire into the practical working of the system. I would point out here—for we must judge everywhere by one and the same standard—that if we deprecate the treatment of cases by the unqualified on the ground that such treatment may sometimes be perilous to the patient, and if we condemn consultation by correspondence on the ground that this system would often work badly, we must, on the same basis, condemn the system by which the bacteriologically uninstructed delegate bacteriological work to the laboratories, if this system is occasionally perilous to the patient, and often works to his disadvantage.

I suppose that no one who has any experience of the practical working of this system of delegation has any doubt on this point. Every laboratory worker could tell a tale of opportunities of diagnosis missed, of misdirected searches, and of quite incredible solecisms committed by distinguished clinicians whose qualifications date back to pre-bacteriological days. It is probable that every worker in a clinical laboratory has frequently been sent blood in a capillary tube with a request that it should be examined microscopically with a view to the detection of pathogenetic microbes. Or he has been sent a specimen of desiccated blood in a capsule with a request to examine for malaria parasites. Or he has been asked to report on the opsonic index of a charred specimen of blood—the opsonic index being, in the view of the sender, a function which does not stand in need of any further qualification, and a function which is indestructible by heat. Or he has been asked to find the '*malaria bacillus*' in a specimen of urine, or has been sent a twenty-four-hour specimen of urine from a case of septicaemia to make a vaccine for the treatment of the case. Or he has exhibited a preparation showing phagocytosis and has been asked by high clinical authority to say which is the microbe and which is the phagocyte. In fact every bacteriological worker has been asked to place his services at the disposal of medical men who are in everything that appertains to bacteriology more uninstructed than the educated layman.

One ought perhaps here, you think, to accept as a counterpoise to the bad results which accrue from this system of delegation the good results which accrue from it in connexion with the diagnosis of diphtheria from swabs sent to the laboratory, and in connexion with the treatment of this disease by diphtheria antitoxin. I do not for one moment wish to overlook either these or the many other advantages which may have accrued from the establishment of laboratories for clinical bacteriology ; but if I take these into consideration, I am, by the same compulsion, obliged to take into consideration also that treatment by the unqualified and consultation by correspondence may also do good. And I put it to you that if we, as a profession, are under an obligation to call attention to the shortcomings of unqualified practice and consultation by correspondence, are we not under the same

obligation to exercise censure upon ourselves when we have recourse to a system of delegation which involves us in similar shortcomings ?

But let me deal with the suggestion that our present system of delegating bacteriological work, as exemplified in the case of the diagnosis and treatment of diphtheria, is an ideal system. I am very far, indeed, from taking this view of the matter. I regard our present system of dealing with the diagnosis and treatment of diphtheria as essentially defective. It is defective, first, in the respect that it takes into account only one bacteriological factor—the diphtheria bacillus—and one of its pathological effects—the intoxication by diphtheria toxin. It is defective, secondly, in the respect—and this defect attaches to nearly all our therapeutic efforts—that, instead of adapting itself to the particular requirements of the individual case, our present treatment of diphtheria aims only at securing a high average of successes. I hold it to be a great defect in our methods of diagnosis of diphtheria, that attention is concentrated on the presence or absence of the diphtheria bacillus. We simply close our eyes to the fact that there may be associated with the diphtheria bacillus other pathogenetic microbes, such as streptococcus, whose presence may perhaps involve almost as much danger to life as the diphtheria bacillus itself. This neglect of the associated infections is only a natural outcome of the delegation of bacteriological work to bacteriologists who are out of touch with the case, and of the faulty appreciation of bacteriological data by medical men who are uninstructed in bacteriology.

Nor even if we leave entirely out of sight the question of therapeutics of the associated infections, and confine ourselves to the question of the therapeutics of the diphtheritic infection, would it be possible to applaud the policy of delegating the manufacture of the serum to a bacteriologist who is not in personal charge of diphtheria cases. For the laboratory bacteriologist almost by necessity sets himself, in the manufacture of serum, an ideal which is merely a laboratory ideal. He aims at the production of a serum which will conform to accepted laboratory tests, and, in seeking to achieve the greatest possible antitoxic potency, he leaves out of sight the fact that a diphtheritic infection is something more than an intoxication by diphtheritic poison. In other words, he makes no provision to secure a serum which would promote the rapid casting off of the diphtheritic membrane and extermination of the diphtheria bacilli. So even in the case of diphtheria a policy of delegation which absolves the clinician from all concern with bacteriology, and commits his work to the purely laboratory bacteriologist, would seem to be merely a policy of the second-best.

(I) Limitations of Vaccine Therapy.

And now I pass to deal with the subject which is set down for me. That subject is vaccine therapy : its administration, value, and limitations. Let me begin with the discussion of the limitations of the method. To the man who is not in sympathy with vaccine therapy the discussion of its limitations must be pre-eminently welcome ; and to those of us who desire to see vaccine therapy employed in a scientific manner, such discussion must be equally welcome. With a view to facilitating the consideration of this question, I have here set out, in the form of a

tabular statement, certain salient points with reference to the limitations of vaccine therapy. I have, as you see, divided my table into two columns. In the first column I have set down a list of the objections which I have heard urged by pure clinicians. In the second column I have set forth the limitations which I myself, in common I hope with every bacteriological worker, would wish to insist upon (see p. 10).

When you have scanned the suggestions with regard to the limitations of vaccine therapy which are set out in Column 1 of the table on next page, you will, I think, recognise that we have there the objections of the practitioner who shuts his eyes and ears to the facts until it becomes absolutely impossible any longer to do so. In Nos. 2, 3, 4, and 5, we have the usual objections of the general practitioner, consulting physician, operating surgeon and specialist who desire to stand upon the old paths, and to be let alone. Lastly we have in No. 6 the objection of the man who has no conception of the rôle which bacteria play in connexion with disease.

The contention that therapeutic inoculation has a useful application only in connexion with staphylococcus infections finds its exact parallel in the contention that prophylactic inoculation finds a useful application only in connexion with small-pox. We all now know that prophylactic inoculation has achieved brilliant results in man in connexion with typhoid and plague, and in animals in connexion with anthrax and many other diseases, and we infer from this that the principle of prophylactic inoculation is a principle of general application. In the same way we all know—all of us who care to know—that therapeutic inoculation is every day applied successfully in connexion with every kind of pathogenetic microbe. And we know also—and those of us who are actually at work on the question have realised this from the outset—that we have here a principle of general application.

We pass now to the contentions 2, 3, 4 and 5, to the contentions of those who urge that their particular spheres of practice cannot usefully be intruded upon by vaccine therapy: to the objection of the consulting physician that vaccine therapy has no useful application to any of the graver bacterial infections which he is called in to see; to the objection of the operating surgeon that vaccine therapy ought never to take the place of operative procedures; and to the objection of each several specialist that useful application cannot be found for vaccine therapy within the particular speciality which he himself practises.

In reality there is no kind of necessity for combating these contentions. In so far as they are the outcome of hasty generalisations, and in so far as they are irrational and interested, they will crumble away of themselves; while in so far as they are based on reason and upon a real insight into facts of Nature they will, of course, hold good.

For the present I will content myself with pointing out that already vaccine therapy promises to be successful in pneumonia; that it holds out promise in typhoid fever and in many forms of rheumatism; that it supplies the only ray of hope we have in endocarditis; that it or a system of regulated auto-inoculation—such as Dr. M. Paterson has suggested and carries out at Frimley Sanatorium—is our only stand-by in phthisis; that it has already—pre-eminently in the case of tuberculous glands and many other forms of tuberculous infection—proved its

superiority over operative procedures; and that it has given very favourable results in treatment of diseases of mouth, nose, ear, and genito-urinary system.

There remains to be considered objection No. 6—the objection that vaccine therapy is of limited utility because it is applicable only in disorders which are produced by microbial infection. While the world in general has progressed up to the point of accepting from the bacteriologist the fact that epidemic disease is due to microbial (or virus) infection, the medical profession in general has not yet accepted it from the bacteriologist that we have in practically every disease a bacterial infection, or the result of a bacterial infection. It is still comparatively un-

Limitations of Vaccine Therapy

(I) Limitations as contended for by the clinician who regards vaccine therapy as an uncomfortable innovation.	*(II) Limitations as contended for by the bacteriological worker who looks forward to vaccine therapy being applied in conformity with scientific principle.*
(1) Vaccine therapy finds no useful application except in connexion with furunculosis.	(1) Vaccine therapy can be applied only where an exact and complete bacteriological diagnosis has been made, and where the diagnosis is kept up to date.
(2) Vaccine therapy is of limited utility, because it can be applied only by those who have devoted study to bacteriology and immunisation.	(2) Vaccine therapy can be applied only by those who have some acquaintance with bacteriology, some understanding of the rationale of vaccine therapy, and a knowledge of the dose of the particular vaccine which it is proposed to employ.
(3) Vaccine therapy finds no useful application in connexion with the graver infections, such as pneumonia, rheumatic fever, typhoid fever, phthisis, meningitis, and streptococcal endocarditis.	(3) A limit is placed to the efficacy of inoculations by the fact that there are definite limits to the responsive power of the patient.
(4) The proper sphere of vaccine therapy is not to take the place of any surgical operation, but to supplement it.	(4) Successful results can be obtained only where an efficient lymph stream can be conducted through the foci of infection.
(5) Vaccine therapy finds no useful application in connexion with the ordinary infections of those regions of the body which fall within the sphere of the particular speciality which the critic happens to practise.	(5) In long-standing infections vaccine therapy can give definite results only after a long succession of inoculations, and there is no security against a relapse until the infection has been completely extinguished.
(6) Vaccine therapy is of limited utility because it is applicable only to disorders which are referable to bacterial infection.	(6) In a not inconsiderable percentage of cases it is essential to success that the dose of vaccine shall be controlled by measurements of the opsonic index.

familiar matter to the rank and file of our profession that jaundice, bronchitis, common colds, many cases of asthma, infantile paralysis, and almost all cases of cardiac disease are referable to microbial infection.

And though it is made a reproach to the bacteriologist that he finds everywhere a bacterial infection, it is in reality the besetting sin of the bacteriologist to underrate, in common with every other man, the part played by microbes in disease ; and he constantly has the mortification of finding that he has failed to appreciate the microbial factor in a disease, and has therefore misapprehended its nature. I would invite you to reflect in this connexion how few of us are prepared even to give a hearing to Metchnikoff when he urges that atheroma of the arteries may quite well be the result of an intoxication by bacterial products absorbed from the intestine, or to Mr. Arbuthnot Lane when he urges—as I understand him to do—that where the breasts and ovaries of the middle-aged woman are not regularly flushed through with blood in the hyperaemia of the sexual orgasm, they tend to undergo cystic degeneration under the influence of toxins absorbed from the intestinal canal.

But perhaps I shall press home my point better if I tell you some of the awakenings that have only recently come to me, and if I show you how prone one is to be misled by tradition and nomenclature and to overlook everything that does not directly obtrude itself upon our senses. I may take these things in any order, just as they come to mind.

Formation of Scar Tissue.—It has been handed down to us as a dogma that where there has been very considerable loss of substance as the result of deep and extensive ulceration, or deep and extensive burns, the best result which one can look for is healing by scar tissue. I remember being wakened out of this belief by finding that very deep and extensive tuberculous ulcers may heal up under vaccine therapy with a complete *restitutio ad integrum*. In the first case I remember there was an ulcer on the back of the hand. It was more than 2 in. in diameter and it went down to the bone, and the *restitutio ad integrum* was here so complete that, a year afterwards, it was practically impossible to tell the hand which had been ulcerated from its fellow ; and the patient, who, as a furrier, had to undertake very delicate manipulations, found his hand as flexible as before. The second case was that of a patient who has now for two years been one of our laboratory men. In his case a tuberculous ulcer which measured not less than 10 in. by 4 in., and which extended from the ear to the point of the shoulder, and which was at one point so deep as to seem as if it must eat into the pharynx, has now for years been covered in with a soft white elastic skin which shows no sign of contraction and is not very different from normal skin. It would seem in view of these and other similar cases that it might perhaps be practicable to avoid the formation of scar tissue after burns by combating the supervening bacterial infections. We are trying to see what can be done on these lines.

X-ray Dermatitis.—It will perhaps seem to you that only a man who was riding a hobby to death could suggest that a bacterial factor entered into the pathology of X-ray dermatitis. I will confess that it had never occurred to me that this might be the case until I was asked to see an X-ray operator whose hands were in a terrible

condition with cracks and ulcers. Cultures here disclosed the fact that we had to deal with an extensive streptococcus infection, and the patient received great benefit from vaccine therapy, the intractable ulcers rapidly healing up as soon as the proper dose of his vaccine had been arrived at. I ask myself, in view of the burning quality of the pain in X-ray dermatitis, and of the course that the disease runs, whether a streptococcus may not often be an important factor in this complaint.

Inflammatory Trouble at the Roots of the Teeth and Toothache.—It probably has not occurred to the ordinary man to connect trouble at the root of the teeth and toothache with a bacterial infection. But no sooner does one make an effort to shake off 'the disease of not thinking', and to sit down to reflect upon the subject, than it becomes clear that trouble at the roots of the teeth must be due to microbes finding their way down. And a very simple bacteriological observation then suffices to show that we have in those cases where there is trouble at the roots of the teeth generally to deal with the ordinary streptococci of the mouth. We have, therefore, here a source of trouble which may quite well fall within the range of vaccine therapy; and as a matter of fact I have often seen such trouble quiet down under the influence of a vaccine. I would suggest here, as a point for investigation, whether the burning pain of actual toothache is not analogous to the burning pain of some streptococcus rashes and ulcers, and whether it may not be due to a quite similar infection.

Pruritus ani.—Pruritus ani is, again, one of those disorders which the ordinary man would not think of referring to a bacterial infection. At any rate, it had not occurred to me that it might be due to such an infection until a patient who was suffering from this condition was referred to me for the treatment of an associated furunculosis. I now find it difficult to understand how it is possible to look at pruritus ani from any other point of view than that of a bacterial infection. I have had under observation and treatment, in addition to the case just referred to, three very desperate cases of this affection. In each case I have found that a platinum loop applied to the seat of irritation brought away quite astonishing numbers of microbes, invariably staphylococcus and pseudo-diphtheria, and occasionally tetragenus [1]; and in each of these cases life has been rendered comfortable, or, if not quite comfortable, at any rate quite endurable, by the use of appropriate vaccines.

Hay Fever.—Although the brilliant researches of Dunbar have put it beyond doubt that hay fever is to be traced to the toxic action of pollen, it would none the less seem possible that bacteria play a rôle in connexion with it. On the one hand the consideration that the coryza which begins as hay fever may culminate in an asthma which may last for months after the season of pollen is over, and on the other hand the fact that cultures made from the mucous membrane of the nose in hay fever often furnish voluminous growths of staphylococcus and *Bacillus septus* and other organisms, make it probable that the action of the pollen may pave the way for a microbial infection, and that this may seriously aggravate the disorder. If this is so, and if the exaggerated susceptibility to pollen which is the prime cause

[1] If I were now rewriting this sentence I should say 'invariably streptococcic and generally also staphylococcic infections'.

of the disease cannot be successfully overcome, bacterial vaccines may quite well prove useful in these cases.

Urinary Calculus.—It is now perfectly well understood that the formation of biliary calculi stands in connexion with a coli infection of the biliary ducts and gall-bladder, and it has been known for very many years that phosphatic calculi form in the urine as the result of changes produced by bacteria. It is only going one single step further to search for a bacterial cause in connexion with every case of urinary calculus, and to try to identify the bacterial cause if such should exist. I have not undertaken any systematic observations along these lines, but, again, in the course of our daily routine of work a certain number of facts which all point in one direction have thrust themselves on my attention.

Case I.—A medical man who has suffered for years from a bacteriuria, which furnished in every case a pure culture of staphylococcus, developed a renal calculus and was operated upon.

Case II.—The daughter of a medical man, who had undergone an operation for renal calculus which resulted in the removal of twenty-one oxalic-acid calculi from the pelves of the kidneys, was brought to me with a view to something being done to prevent a recurrence of her troubles. A series of cultures made from her urine furnished in each case a copious growth of staphylococcus.

Case III.—A patient whose urine had for years furnished on each occasion a pure culture of a coliform bacillus, and who had undergone vaccine treatment for this, developed symptoms of renal calculus. The operation revealed the presence of a stone, and cultures made from the pelvis of the kidney furnished copious cultures of staphylococcus. A similar operation for calculus, undertaken twelve months later on the other kidney, again furnished a culture of staphylococcus.

Case IV.—A patient who had been admitted to the Inoculation Wards at St. Mary's Hospital for the treatment of a deep and extensive ulceration caused by a combined syphilitic, streptococcal, and staphylococcal infection, developed symptoms of renal colic. Radiographic examination showed the presence of a stone in the pelvis of the right kidney, and a similar calculus in the right ureter. An examination of his urine revealed the presence of staphylococci in fair numbers.

Indigestion, Vomiting, Flatulent Distension of the Stomach.—The fact that these are often prominent features in early phthisis is one of those facts which have been known so long that no one any longer asks for an explanation of them. I believe the answer to the problem may perhaps be found in the fact that pyorrhoea alveolaris is a frequent accompaniment of phthisis. I can hardly doubt, after what I have seen result from vaccine treatment in these cases, that the gastric disturbance must often be due to streptococci, swallowed with the food. I have, for instance, seen vomiting that had occurred regularly every day for six months disappear after the inoculation of a vaccine made from streptococci derived from the mouth. We must remember, in connexion with gastric disturbance which is associated with pyorrhoea, (1) that cooked food is for all practical purposes sterile food ; (2) that the admixture of microbes which gives rise to fermentation can only come from the mouth or stomach ; (3) that inasmuch as a more or less effectual process of sterilization takes place in the stomach, while the development of microbes goes on without such

check in the mouth, the cause of the gastric fermentation is probably to be found in the microbes of the mouth which are swallowed with the food.

Epilepsy.—It would seem as if we had in epilepsy a condition which could not by any possibility stand in any aetiological connexion with any bacterial infection. But even here the judicious will find ground for hesitancy. He will reflect that in infancy almost any infection which is associated with the setting free of bacterial toxins in the organism will produce convulsions. It may therefore not unreasonably be surmised that a bacterial focus which stood in connexion with the nervous system might even in the adult produce a like result. And I believe that I have seen epilepsy in tuberculous patients improve under inoculation treatment.

Cancer.—In spite of the fact that a moment's consideration would bring it home to anyone who had come into intimate contact with cancer that the microbic infections make a large part of the misery of the disease, both for the patient and for those that come in contact with him, the rôle which microbic infections play in connexion with cancer is as yet almost unrecognised. In reality that rôle is far more important than appears at first sight. Owing, no doubt, to that defective resistance which seems to be a feature of all neoplastic tissues, cancer, very early in its history, long before it has burrowed its way to the surface, becomes the seat of a staphylococcus infection—an infection by the so-called *Micrococcus neoformans* of Doyen. And there is reason to believe that much of the pain and swelling and inflammation in connexion with the tumour, and much, if not all, of the so-called cancerous cachexia, is due to bacterial infections. In view of this it is clear that, even though we cannot hope to strike at the root of the evil by anything in the nature of a vaccine treatment, cancer—and in particular inoperable cancer—comes within the range of vaccine therapy.

Diabetes.—It must over and over again have suggested itself that diseases which are due to the faulty functioning of some organ—diseases, for instance, such as Graves' disease and pancreatic diabetes—may ultimately be traced to bacterial infection. Countenance is given to this suggestion by such work as has recently been done in connexion with the vaccine therapy of diabetes. I may refer to a case of pancreatic diabetes which was treated by my colleague, Capt. S. R. Douglas, where the secretion of sugar and the symptoms of the patient were found to vary with the patient's resistance to a coliform bacillus which had been isolated from her faeces. I may refer also to the interesting facts which Dr. McWatters is bringing forward in this discussion in connexion with the treatment of glycosuria by staphylococcus vaccine. I think you will see that his facts suggest that glycosuria and carbuncle, which we have always supposed to be related as cause and effect, may perhaps in some cases be merely two different manifestations of a staphylococcus infection.

Enuria.—Finally, let me invite you to consider whether a bacterial infection may not in some cases turn out to be the cause of enuria. I can call to mind a case where enuria was apparently attributable to an unsuspected coli infection of the urine, and another case in which it was also associated with the same infection. Both cases yielded to treatment by vaccines.

I have perhaps said enough to rebut the contention that the range of vaccine

therapy is restricted. In point of fact it not only covers almost the whole range of our present medicine, but also much that lies altogether outside its borders. For it beseems every doctor to remember with humility that there are an infinitude of chronic or recurrent ills affecting mankind which are never seriously brought to his notice, because no one believes that there is any understanding of them in the medical profession, or any possibility of a cure being found for them. I have myself in the course of researches on haemophilia had a great deal to do with patients who, for the reasons given, sedulously avoid all contact with the medical profession.

Let me now just glance at another point which, perhaps more than any other, will bring home to you how extensive is the range which opens out before vaccine therapy. It is certain that we come into this world each with his individual susceptibility to microbic invasion. I am here thinking not of our susceptibility to those microbes which can pass from man to man in the form of epidemics—those microbes which alone come into the field of view of the hygienist. I am thinking rather of the fact that one man is by nature deficient in his resisting power to the staphylococcus, another to the pneumococcus, another to the bacillus of Friedländer, another to the influenza bacillus or to the acne bacillus, another again to some one of the different forms of streptococcus, or of the pseudo-diphtheria bacillus or of the coli bacillus, and another to the tubercle bacillus. And I would have you reflect, in connexion with these microbes, that, while their ravages may not be dramatic, they are collectively responsible for a very large fraction of human disease. And then I would have you reflect that while man makes efforts to guard himself against epidemic diesase, and while he enlists the help of the State in this task, he accepts it as if it were an unalterable law of Nature that he should be buffeted throughout his life by the particular microbe to which he individually is liable. Thus one man puts up with recurrent influenzal attacks, another man with a succession of sore throats, another man with continual recurrences of boils, another man with chronic bronchitis, another with perpetual trouble in the roots of his teeth, another with a continuous discharge from the ear, another with sycosis or acne, another with the coli infection of the urine, another with continual pruritus, another with tuberculous glands, another with phthisis, another with recurrent intestinal attacks, and so on through the whole gamut.

Vaccine therapy will, I believe, help every man to keep under the particular microbe which besets him.

I now pass to consider Column II of my table ; in other words, to consider the limitations of vaccine therapy, which must—if I see the matter aright—be contended for by every bacteriological worker who desires to see vaccine therapy applied in conformity with scientific principles.

I think that only the first and the last two of the contentions in Column II stand in need of anything in the way of amplification and defence. There is no one I conceive who would think of questioning that a complete and exact diagnosis is a necessary preliminary to a successful application of vaccine therapy. Nor will any one who has done work on a case of mixed infection have any doubt as to the absolute necessity of keeping the diagnosis up to date. It must none the less be

emphasised that as soon as ever a definite label has been placed upon a case, that label generally dismisses from view all further bacteriological possibilities.

The risk of accepting an incomplete for a complete diagnosis, and so imperilling the success of our treatment, is perhaps most conspicuous in connexion with syphilitic and tubercular infections. And yet in many cases the very clinical characters which serve as stigmata of tuberculous or syphilitic infection are characters which ought properly to go down to the account of other microbes ; and when we go into the matter it becomes clear that it is the presence of these microbes in the lesion which puts the clinician on the trail and furnishes him with the logical justification for the diagnosis of syphilis or tubercle.

A typical instance is furnished by *rupia*. The characteristic rupial scab testifies to the presence of a bacterial agent which induces a copious transudation of lymph. The bacterial agent which has a pre-eminent power of doing this is the streptococcus. But in the case of a typical pure streptococcal infection the exudate is wont to be a perfectly clear lymph which sets into an amber-yellow scab which crumbles into a powder like gum arabic. In rupia, however, the discharge is semi-purulent, and the heaped-up scab which suggests the idea of a streptococcus infection is very tough and opaque. It is, in fact, a scab that contains a large admixture of leucocytes. This suggests a superadded infection, and the commonest infective agent which leads to an emigration of leucocytes is the staphylococcus. The characters of the rupial scab thus furnish presumptive proof of the presence of streptococcus and staphylococcus—in reality both these are regularly to be found in rupia—and, little as the pure clinician appreciates the fact, it is this twofold infection which leads him to the diagnosis of syphilis. For we have apparently in the spirochaete a microbe which brings about a general lowering of the resistance of the body to microbic infection. I may say, in passing, that the inference that a multiple infection such as we have in rupia is *always* due to syphilis is not correct.

It is, however, certain that there is no tuberculous or syphilitic ulceration of a mucous membrane, and no extensive tuberculous or syphilitic ulceration of the skin, which is not complicated by secondary infections. It follows that it is improper in any case of tuberculous ulceration of the larynx or tongue to depend entirely upon tuberculin and to overlook the associated infections. Similarly, it is important, in connexion with every syphilitic ulceration, to keep in mind the possibility that chemotherapy may be ineffective if we do not turn our attention to the associated microbes. And in such cases it often does not suffice to combat only one variety of associated microbes. One comes across case after case where, owing to the fact that one of a batch of infecting microbes has been overlooked, a wound obstinately refuses to get well. I have, for instance, in mind a case of tuberculous ulceration of the chin which failed to make progress when treated with a combination of tubercle and staphylococcus vaccine, and which healed up rapidly when, after the discovery of a streptococcus in the wound, a combination of tubercle, staphylococcus, and streptococcus vaccine was employed. The subsequent history of this case is interesting also, as showing the necessity of keeping the diagnosis up to date. When some months after the patient had gone back to the country the ulceration broke out again, the same triple combination proved quite ineffective.

The ulceration spread in an alarming manner, and the patient returned to hospital for treatment. It was then found that the *Bacillus proteus* had established itself in the wound, and that his blood gave a positive reaction with Wassermann's test. A proteus vaccine was now administered, and under the influence of this, in combination with a few doses of iodide of potassium, the wound healed up rapidly.

The fact that long-standing infections cannot be got under save by a long succession of inoculations is obvious to anyone who understands anything of the rationale of vaccine therapy. I, however, specially emphasise it here because I find that the most unreasonable expectations are afloat as to what may legitimately be expected from vaccine therapy. In view of these, I must try to make it clear what we can and what we cannot hope from the method, in a case of long-standing infection.

Perhaps the easiest way of bringing home some appreciation of the conditions is to compare the human body to a garden, the vaccine-therapist to the gardener, and the pathogenetic microbes to weeds which can be thinned out, but which, so long as any of them remain over, retain their power of multiplying and reoccupying lost ground.

This analogy makes it plain that the most that can be achieved in a long-standing infection by one dose of a vaccine is a temporary reduction in the number of the infecting microbes, and that it is not worth while, in such a case, to embark upon anything less than a systematic campaign. And it also makes it plain why it should be necessary to inoculate again and again for an unlimited time when a vaccine is employed for therapeutic purposes, and once or twice only where it is employed for prophylaxis. Clearly where inoculation is resorted to for therapeutic purposes, the surplus of anti-bacterial substances, which is elaborated, is immediately expended in the destruction of microbes. There is, in prophylactic inoculation, though the protective substances elaborated are not retained in the circulation, no such active drain upon the bacteriotropic substances engendered.

When a patient is informed of these facts, and when he is told that it will be necessary to inoculate again and again for an unlimited time, he will inevitably ask how long it will take to accomplish a *cure*.

It will be profitable, therefore, to clarify our ideas about this question of the achievement of a cure.

Bacterial infections fall in reality into two classes :—

(*a*) Into the first class would fall ' surface infections ' by microbes which are normally saprophytic on the affected surfaces. The pneumococcal or streptococcal infections, which are the commonest causes of bronchitis, are instances in point.

In this class of infection it is unreasonable to aim at a complete extinction of the infecting microbe. What we can here hope for in the way of a cure is to keep the number of microbes within bounds, and to minimise the chance of a recrudescence by keeping the patient's resisting power up to the mark.

(*b*) Into the second class would fall infections by microbes which are extraneous to the normal organism, which are always pathogenetic, and which can only be acquired by infection from the sick. Tuberculous infections are instances in point.

In this class of cases the extinction of the infection is quite a reasonable ideal. But if now, in connexion with this class of infections, the question is pressed as to

how soon this can be achieved, the vaccine therapist is bound to reply that he can never say beforehand, and can never guarantee the attainment of this result. But, though he cannot prophesy, he can from time to time take stock of the patient's condition, and tell him whether or no he has made progress, and whether or no the infection has been extinguished.

In reality, we have in addition to the clinical condition—which, of course, cannot tell us whether an infection has been extinguished—not less than four different methods by which we may see how we have progressed. Let me explain what these are :—

(1) In the case where the patient's index is being regularly taken, let us say at the expiration of ten days after inoculation, we have an automatic check upon the progress of the case. For if we find that, instead of sinking rapidly away to below normal, as it did at the outset, the opsonic index now maintains itself at the level of the normal, or a somewhat higher level, we may—if we have excluded the fallacy of auto-inoculation—confidently conclude that the patient is making good progress. For clearly as the pathogenetic microbes in the body diminish, there must be a proportionately slower expenditure of anti-bacterial substances. When we have satisfied ourselves that this is the case, and if also all the overt signs of infection have disappeared, we may proceed cautiously to apply the next test.

(2) Where we have reason to believe that the infection may have been extinguished, and want to make certain that this is really the case, we may tentatively give up the inoculations but continue to make measurements of the opsonic index. And if, under these circumstances, we still find the opsonic index remaining normal month after month, we may conclude that the drain upon the patient's anti-bacterial resources—it is probably that drain which accounts for the subnormal indices found in the early stages of tuberculous infection—has been arrested. This will signify the complete, or all but complete, extinction of the infection. A table showing the results obtained by this method of testing on a series of patients is subjoined (see p. 19). If we desire an even more searching test, we may take either the next or the one following.

(3) This test is based upon the consideration that if we send a lymph stream through a limb or any other region of the body which has been the seat of a bacterial invasion, that lymph stream will, as it returns to the blood, carry bacterial poisons back into the blood, with the result that a characteristic fluctuation will occur in the opsonic power of the blood. This test furnishes information which is specially valuable where the decision of the question as to whether the patient may use his limb depends upon the question of the extinction of the infection in a joint. For the purpose of this test, active hyperaemia is induced in the suspected focus of infection, a bandage is then applied to the vein in such a way as to obstruct the returning blood current, and when a transudation of lymph has in this manner been obtained, the lymph is driven back into the circulation by massage or active movements. In order that the test may yield conclusive results, it is advisable, in cases where there can be only very little remaining infection, to keep the limb at rest for a day or two before undertaking the test, in order to allow of an accumulation of bacterial toxins in the focus (Chart I, see p. 20).

Table of Cases in which the Infection is inferred to be Extinct from the Maintenance of a Normal Opsonic Level, and in which Confirmation of this Inference has been afforded by the fact that the Patients have remained quite free from all signs of Infection.

Case initials	Nature of tuberculous infection	Length of treatment up to complete disappearance of all evidence of infection	Measurements of opsonic index undertaken after cessation of treatment — Months										
			1	2	3	4	5	6	7-8	9-12	13-16	17-20	21-24
A. H.	Extensive ulceration of skin and subcutaneous tissues	3 months	—	·86	—	—	—	1·06	—	·93	—	—	—
F. M.	Peritonitis	39 ,,	1·13	—	·95	·91	—	—	1·05	1·05	—	—	—
M. B.	Adenitis	6 ,,	1·11	—	·98	·94	·94	—	*·84 1·07	—	—	—	—
W. C. P.	Cystitis	5 ,,	1·17	—	—	·88	†·69 ·96 ·88	—	—	—	1·13	—	—
G. E. G.	Epididymitis	2 ,,	·96	—	—	—	—	—	1·02	—	—	—	—
W. M. P.	Tubercular mass in vitreous ..	23 ,,	—	—	—	—	—	1·01	—	·96	—	—	—
E. P.	Adenitis	11 ,,	1·13	—	1·30	1·06	—	1·15	—	—	·96	1·12	—
L.	Ulceration of hand	7 ,,	1·18	—	·94	—	1·00	—	—	·86	1·04	—	1·20
G.	Adenitis	14 ,,	—	·83	—	·94	—	1·12	—	—	—	—	—
R. M.	Arthritis of knee	25 ,,	1·17	—	·98	—	1·06	—	1·11	—	1·14	1·19	—
R. W.	Adenitis	9 ,,	·80	—	·97	—	·96	—	·87	—	1·05	—	—
W. S.	Arthritis of knee	34 ,,	1·00	·93	·92	—	—	—	1·04	—	—	—	—
G. L.	Adenitis	22 ,,	1·12	—	—	1·18	·93	—	·84	1·07	—	—	—
G. M.	Arthritis of knee	13 ,,	1·21 1·08	1·07	1·02	—	—	—	—	—	—	—	—
G. S.	Adenitis	21 ,,	·92	—	—	1·28	—	—	·90 1·24	1·37	1·11	1·04	—

* Patient suffering from a temporary indisposition; and gave a normal result when retested a few days afterwards. † There is a doubt as to the accuracy of this estimation, as the patient was in perfect health, and the blood, when tested three days later, gave an index of ·96.

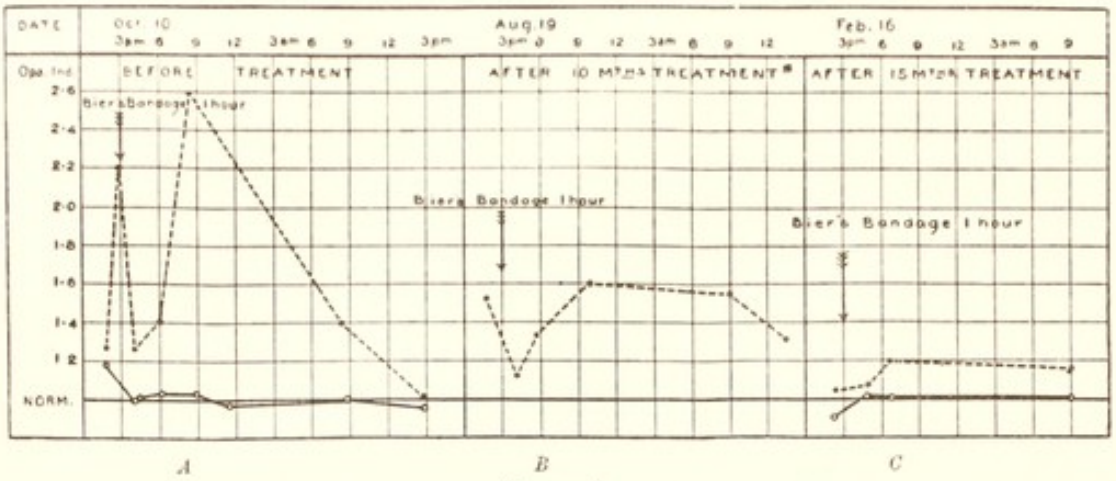


CHART I.

Chart showing results of auto-inoculation tests carried out in a case of gonococcal arthritis of wrist. Broken line, Gono-opsonic Index. Unbroken line, Tuberculo-opsonic Index. *A*, before treatment; *B*, after 10 months' treatment with gonococcic vaccine; *C*, after 16 months of that treatment.

(4) While the test which has just been described resolves the question as to whether there is still lurking infection in a suspected focus, consideration will show that it will not resolve the question as to whether there may not be elsewhere in the patient's body an unextinguished focus. If this question should not be held to be sufficiently resolved by such a series of tests as is described under (2), it can be further put to the proof by testing the patient's blood before and after severe

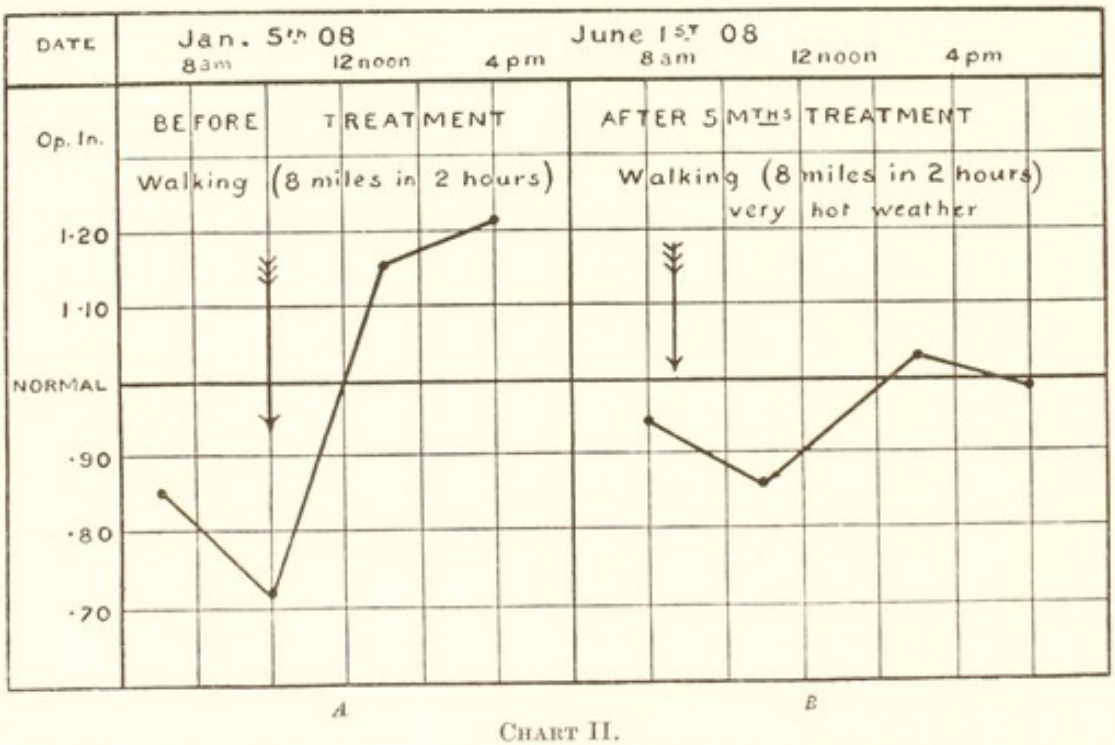


CHART II.

G. D. O. Phthisis. Auto-inoculation tests carried out; *A*, before treatment; *B*, after 5 months of vaccine treatment with B.E. tuberculin.

exercise. The routine procedure which we employ in such a case is to test four samples of the patient's blood—the first drawn off immediately before exercise, the second immediately after, the third six hours after, and the fourth twenty-four hours after. The reader has already on the previous page seen a pair of companion charts showing the results obtained by this method of testing.

The only one of the expert contentions set out in the table on p. 10 which is in any sense of the word controversial is the contention that it may often be essential to success that the doses of vaccine shall be controlled by measurements of the opsonic index. We have in this connexion to consider, *first*, the question as to whether the opsonic power of the blood can be accurately measured; *secondly*, the question as to whether there is a correlation between the rise and fall of the opsonic index and improvement and aggravation in the condition of the patient; and, *thirdly*, the question whether the measurement of the opsonic index can be dispensed with, and whether any other guide can take its place.

(i) *Question as to whether the Opsonic Power of the Blood can be accurately Measured*

I may point out, *in limine*, that the controversy which has taken place on the subject of the accuracy of the opsonic index is only what might have been expected, seeing that it is in connexion with the measurement of the opsonic index that the capacity of bacteriological workers for accurate quantitative work has for the first time been seriously put to the test.

I may very briefly refer here to three schools of criticism.

The *first*, represented by Pigg-Strangeways and his collaborators, asserted that differences such as emerge in connexion with the counting of phagocytic preparations of different bloods might quite well be due to the operations of chance, which brought into the field of view of the observer in one specimen of blood a group of leucocytes which happened by chance to contain more microbes, and in the other specimen of blood a group of leucocytes which happened to contain less microbes. These critics further suggested that if a sufficient number of leucocytes—1000 leucocytes was the proposed number—were counted, it might be found that there was never any difference between tuberculous and non-tuberculous bloods.

A *second* school of criticism declared that, while there could be no question but that there were often marked differences between the blood of infected patients and normal blood, the margin of error in the method is so considerable as to deprive the method of any practical value. In support of this thesis some observers brought forward discordant results obtained by themselves on duplicate samples of blood. Other critics again brought forward discordant results obtained by a series of observers in different laboratories who were all supplied with the same bloods. In connexion with the last-mentioned results, it is to be observed that in tests thus organised the results of inaccurate workers throw doubt upon the work of accurate workers. Moreover, as I have already elsewhere [1] pointed out, results obtained in

[1] *Collected Researches*, Vol. III, p. 390, footnote 2.

different laboratories with different strains of microbes are not, and cannot be expected to be, comparable *inter se*.

A *third* school of criticism proclaimed that, inasmuch as the average ingest of a certain number of leucocytes is taken to represent the opsonic power of a blood, there here enters into the method a certain factor of chance—a factor whose magnitude can be calculated only by a statistician. Again, this school of criticism contends that, inasmuch as the phagocytic power of the individual leucocyte varies within certain limits, there here enters again into the method another factor of chance which also is a proper subject-matter for mathematical evaluation. On this basis the statistician puts forward a claim to determine the limit of error of the opsonic method.

In reality, however, in addition to the two factors which have come within his purview, there are many others which have an important influence on the result.

In the first place, the range of variation in the phagocytic power of the leucocytes, which is by the statistician assumed to be a constant, is a factor which is in reality profoundly modified by the treatment to which the leucocytes are subjected. While we have in some cases a comparatively small range of variation, in cases where the leucocytes have been maltreated[1] we have a much larger range of variation.

Again, though the statistician assumes that a record of bacteria counted in a series of leucocytes is as unambiguous and as little open to error as, let us say, a record of pips counted on a series of cards, this is very far from being so.

Microbes cannot be satisfactorily counted unless the leucocyte has first been spread out flat as a card. Again, the microbes must have been differentially stained so as to stand out perfectly clear against the background of the leucocyte. Again, the bacteria must not be fragmented or gathered together into groups. They must not be so numerous as to make accurate counting difficult. And there is also another requirement ; the observer must bring to the task of counting exceptional concentration, and he must display no little judgment in the avoidance of pitfalls and fallacies.

It follows that the figures which represent the number of bacteria counted in the individual leucocyte are affected by the mechanical treatment to which these have been subjected *in vitro*, and these figures are also affected by the degree of skill and attention which has been brought to bear upon the preparation of the bacterial suspension, the spreading of the blood film, the fixing and staining of the preparation, and the counting.

Figures which are the resultant of all these factors, which are personal to the individual worker, cannot, I submit, possibly furnish a '*mathematical limit of error*' for a method. What they may, perhaps, furnish is a value for the 'mathematical limit of error' in combination with what I may perhaps call the '*functional error*' of the particular observer, or groups of observers, whose work is under review.

Let me try to make clear to you—in so doing I shall only be elaborating what is familiar matter to us all—the importance of the '*functional error*' in connexion with our work.

The '*limit of error of a method*' is, if I understand the expression aright, a

[1] The same applies to leucocytes of auto-innoculating patients and to leucocytes innoculated *in vitro*. *Vide infra* p. 197.

function which can attach only to quantitative methods in which skill does not enter. It is a value which is unalterably fixed by mathematical laws, which can be arrived at only by a mathematician. It is a value which is exactly the same for every worker. No one can emancipate himself from it, or do anything to diminish it ; and it is indissolubly attached to the *method*.

The '*functional error*' is an error which attaches only to methods which involve skill. It attaches to the *operator*. It has a different value for every operator. It may in the case of one and the same operator vary with his physiological efficiency. Its magnitude can be diminished by practice and attention. It cannot be evaluated by a mathematician. It can be pretty accurately gauged by the operator himself.

The '*working error*' corresponds sometimes to 'the limit of error of the method', sometimes to the 'functional error of the operator', and sometimes to the sum of these values.

In the case of such an exercise as counting of the number of pips on a series of cards and taking the average of these, or in going through the same operations with a series of throws of the dice, nothing in the nature of skilful functioning is required. The 'functional error' is therefore here negligible ; and the 'working error' may be identified with the 'limit of error of the method'.

In such a case as the auscultation and percussion of a chest, or in billiards, the whole 'working error' is the 'functional error of the operator'.

In the case of the measurement of the opsonic index 'the working error' is the sum of 'the limit of error of the method' and 'the functional error of the operator'. Of these, by far the most important factor is the 'functional error'.

I have satisfied myself, and all my fellow-workers have satisfied themselves, and I am glad to say a very large and increasing number of bacteriological workers all over the world have satisfied themselves, that when the 'functional error' has been reduced, as it can be by practice and patience, to small dimensions, and when, in connexion with tubercle, the customary counts of 100 or more leucocytes are made, the 'mathematical limit of error' of the opsonic index is such as need not seriously be taken into account. In view of this, I suggest that those critics who have put forward figures showing enormous working errors in opsonic estimations may have supplied to the world data with regard to the magnitude of their own functional errors, instead of—as self-esteem assured them—data with regard to errors inherent in the opsonic method.

(ii) *Questions as to whether the Rise and Fall of the Opsonic Power of the Blood is correlated with Improvement and Aggravation in the Condition of the Patient.*

I have elsewhere [1] discussed at some length the questions as to whether it is a practical ideal to make a *complete evaluation* of all the factors which make up the resisting power of the organism to bacterial invasion. I have pointed out there that inasmuch as it would be necessary for a complete evaluation of the antibacterial power of the organism to enumerate all the leucocytes, to measure their individual phagocytic activity—and, I may add, their power of digesting the ingested microbes

[1] These *Collected Researches*, Vol. III, pp. 287–291.

—and to consider the question as to how far the leucocytes which are available could be brought into application ; and, further, inasmuch as it would be necessary for a complete evaluation to evaluate all the known antibacterial elements which may affect the microbe from which the patient is suffering, and to make allowance for all the antibacterial elements which have not yet been discovered ; and, lastly, inasmuch as there is no common denominator to which we can refer these different classes of defensive elements so as to add the one to the other or to set off the one against the other, it must for ever be impracticable to make a complete evaluation of the antibacterial forces of the body. And I have pointed out [1] that it is for these reasons absolutely necessary—if we are to have any direct guide in our immunisation proceedings—to content ourselves with a confessedly *partial evaluation* of the antibacterial powers of the blood. Again, I have pointed out that we can quite well turn to account a partial evaluation if the antibacterial element which has been selected for measurement is an element which can be accurately measured, which decreases and diminishes in the blood in response to inoculations of vaccine, and which increases and diminishes in correlation with the clinical condition of the patient.

The measurement of the opsonic power of the blood is *confessedly* and *in intention* a partial evaluation of the antibacterial defences of the body ; it can be accurately measured, it is an element which decreases and diminishes in the blood in response to inoculations of vaccine ; and the question for discussion is whether it increases and diminishes in correlation with the patient's clinical condition.

I have affirmed that there is such correlation. If anyone desires proof of this, he has only to watch the effect which is produced on the clinical condition and the opsonic power of a tuberculous patient by an excessive dose of tuberculin or an excessive auto-inoculation. Or if he wants to see that a rise in the opsonic index is correlated with an improvement in the patient's condition, he has only to take a tuberculous patient who has a chronically low index and watch the improvement that goes hand in hand with an improvement in the index. Or if he wants to watch the way in which the clinical condition varies with the opsonic index, he has only, in a tuberculous person, to inoculate somewhat smaller doses than are required and he will see the patient's condition improve and his opsonic index increase for a few days after inoculation, and then regularly fall away again before the next inoculation is undertaken.

I have hitherto always emphasised this generalisation and said little about the exceptions, *first*, because in every new scientific departure our business is with the rule and not with the exception ; and, *secondly*, because when it has once been stated that a measurement of the opsonic power of the blood is confessedly and in intention a partial evaluation of the antibacterial defences of the body, that inevitably brings it home to the thoughtful that there cannot by any possibility be a perfect correlation between the opsonic power and the clinical condition.

I leave the matter there for your consideration. I would, however, point out to you that the difficult thing in this intricate web of things in which we are entangled is to possess ourselves of the broad generalisations. It is no very difficult

[1] *Collected Researches*, Vol. III, *loc. cit.*

task to find an exception to a generalisation when it confessedly gives only the general rule. The man who, meeting an exception which might have been foreseen, straightway throws up the sponge is like the man ' who encountered a corpse and retreated to bed, announcing that all life was contradicted '. I suppose that not even the correlation that exists between the readings of the clinical thermometer and the condition of a patient can be accounted a perfect correlation.

(iii) *Question as to whether the Measurement of the Opsonic Index can be dispensed with, and whether there is any other Guide which can take its place.*

I have said elsewhere [1]—weighing my words very carefully as I did so—practically everything I have to say on the question of the importance of controlling the dosage by measurements of the opsonic index. I pointed out there that in a large class of cases it is impossible within the short interval which normally elapses between one inoculation and another to discover by any clinical manifestation whether the preceding dose of vaccine has elicited a satisfactory immunising response. We have in chronic tuberculous infections typical examples of this class of case, and there are also many obstinate cases of infection by other micro-organisms—cases of empyema, sinusitis, middle-ear disease, urinary infections, streptococcal infections of bone, &c.—where a definite clinical improvement can be hoped for only after a consecutive series of effective inoculations. In all these cases we have either to work entirely without a guide or to rely upon the opsonic index as our guide. I have also emphasised that even where we have more or less definite clinical symptoms to guide us at the outset there will, if the patient improves, inevitably come a time where the clinical symptoms will fail us as a guide, but where the inoculations ought to be continued for the purpose of extinguishing the infection. Here, again, the opsonic index is our only possible guide. And we cannot dispense with it if we are to diagnose our obscure cases, to take accurate stock of the progress of the cases we have under treatment, and to satisfy ourselves that we have extinguished the infection and that we may suspend our inoculations.

If any of these statements are controvertible, it would be well that they should now be controverted. For, up to the present, what I have urged in favour of the importance of controlling dosage by the opsonic index has been met only by insistence that the correlation between the opsonic index and the clinical condition is not a perfect one—if this were a reason for dispensing with the opsonic index, it would also be a reason to dispense with the thermometer—and by the iteration of the formula that the clinical signs give to the clinician sufficient guide in the regulation of his dose. When I find a speaker obsessed with this formula ; when I find him ignoring the fact that there are cases where no guidance can be obtained from the clinical symptoms ; when I find that he refuses to face the problem as to how in such cases the dose of vaccine is to be regulated ; when I find him citing cases which he has inoculated with success under the guidance of definite clinical symptoms, and adducing these as proof that the opsonic index can be dispensed with in cases where guidance cannot be obtained from the symptoms, I ask myself whether

[1] *Collected Researches*, Vol. III, p. 379, *et seq.*

I am not perhaps listening to ' one of those sages whom a man should understand less as he heard him longer '.

While I have insisted, and continue to insist, that there are many cases where we are not doing the best for the patient if we are not controlling the effect of our inoculations by the opsonic index, I have from the very outset recognised that vaccine therapy can in many cases be carried out with success without its aid. I recognise that more fully every day, and I rejoice, with all those who understand what vaccine therapy may mean to the world, that it should be so.

Let me briefly describe to you what our practice is in connexion with the control of inoculation by the opsonic index in the case of the out-patients and in-patients in the Inoculation Department of St. Mary's Hospital. In an ordinary case of localised streptococcus or staphylococcus infection we practically never have recourse to the opsonic index. In connexion with these infections we know the appropriate doses of vaccine, and the clinical symptoms furnish any further guide that may be required. The same holds true of acne. It holds true again of croupous pneumonia.

Where we have to deal with a case of staphylococcus infection, such as sycosis, which has obtained a firm hold upon the patient and which offers considerable resistance to the treatment, and which we can only hope to overcome by a succession of effective inoculations, it is often necessary to regulate the dose by means of estimations of the opsonic index.

The same holds true of the very chronic streptococcus infections which are associated with tuberculous disease of bone. It holds true again of the chronic coliform infections.

In the case of tuberculous infection we make a distinction. We make it a practice in every case of phthisis to control the effects of the inoculations by the opsonic index, but employing, as we do in the case of phthisical out-patients, only doses which give no negative phases, we find it sufficient to determine by blood examination, undertaken on the day before the patient returns for inoculation, whether the dose has been adequate to keep the opsonic index up to the normal. In the case of phthisical patients who are treated in the wards, more frequent examinations are undertaken. In the case of tubercular adenitis and other localised forms of tubercular infection we limit our opsonic examinations if satisfactory progress is being made. As a rule, we undertake these only where the question of increasing the dose presents itself. In cases which do not make such satisfactory progress the opsonic index is estimated much more frequently.

In cases of septicaemia and in cases of advanced phthisis, and, in short, all cases where the condition of the patient is undergoing constant and rapid changes under the influence of continuous auto-inoculations, we find that the measurement of the opsonic index does not render any very valuable services.

In conclusion, I may mention, in connexion with the question as to whether the temperature in a pyrexic case can be taken as a guide to the opsonic index, that we have over and over again verified that, except in those unfortunately more or less rare cases where a pyrexic infection is being definitely got under by inoculation, we do not find any of that inverse correlation of temperature to the opsonic index

which is illustrated in some of our published charts [1] and which Dr. Latham, generalising apparently from very few cases, has alleged to constitute the general rule. The temperature cannot therefore be depended upon as a guide in immunisation.

I pass on from the consideration of the limitations of vaccine therapy to its results.

(II) Value of Vaccine Therapy.

It would clearly be an impossible task to attempt here even a summary of the results of vaccine therapy. All that I would propose to do is to suggest for your consideration certain general canons of criticism which ought, I think, to be kept in view when we set ourselves to appraise the results of any therapeutic procedure.

The question as to how we are to give our verdict upon the success or failure of vaccine therapy in the individual case ought obviously to take precedence over the question as to how, when we have passed our verdict upon the success or failure of vaccine therapy in a series of cases, we can bring these separate verdicts together into the form of a general verdict.

Let me then begin with the former of these questions. Now I would put it to you that we have in connexion with vaccine therapy to consider two entirely different classes of cases.

We have, first, the simple and unambiguous case. This is the case where the treatment consists of a single inoculation of vaccine, and where the verdict takes into account only the results of that one inoculation. We may take as an example the case in which the vaccine therapist sets himself the task of aborting a stye on the eye, or of arresting a streptococcus infection in a poisoned wound. To abort the stye, and to stop the streptococcus infection promptly, is to succeed—for these things do not happen of themselves; to do less than this is to fail. The game consists here in the winning of one trick, and there can be no two opinions as to whether that trick has been won or lost.

We come now to the more intricate case. This is the case where the treatment comprises a whole series of inoculations, and where what has to be adjudicated upon is the success or failure of the whole series of operations. Consideration shows that the conditions which here present themselves may be compared with a game which consists in winning, not, as in the case last considered, a single trick, but a whole series of tricks. Among the problems which here present themselves there is again a simpler and a more difficult one.

Let us begin with the former. It is clear that if the patient gets steadily worse under inoculation, the case must be counted as a failure to vaccine therapy. And if the case gets steadily better and has ended in the extinction of the infection, it must be classed as a success to vaccine therapy, *if* it is conceded that the case offered no prospect of a spontaneous cure. If that is not conceded, the case becomes, for those who have either a bias in favour of, or a bias against vaccine therapy, a case for wrangling over. For the judicious and unbiased person it becomes a case which is to be set down as a more or less probable success for vaccine therapy. In other

[1] *Collected Researches*, Vol. III, pp. 350 and 353, charts 20 and 21.

words, if the game is won it may be a matter of dispute whether it is the vaccine therapist or not who has won the tricks, and the dispassionate man will decide on the probabilities.

I pass to the really difficult cases. We have such a case where the vaccine therapist begins by winning a series of tricks and then begins to lose, and the game is broken off. We have again such a case where the vaccine therapist wins a series of tricks and it is assumed that this wins the game, and then the game is broken off, and then, after abandonment of treatment, there comes a relapse. And we have again such a case where the game is never definitely won, but where the vaccine therapist reinoculates whenever the condition of the patient requires it, and each time temporarily ameliorates matters.

In these cases the judgments that are passed are often absolutely reckless. It is here that the man who is blinded by a bias in favour of inoculation claims credit for winning the whole game when he has won only a very few tricks. It is here that the man who is blinded by a bias against inoculation contends that the game has been lost when only one trick or a very few tricks have been lost.

I will leave it to others to illustrate the effects of the bias in favour of inoculation. I think the following verdicts will illustrate the effects of the bias against it. They relate to cases which were treated by me, which then passed out of my hands into the hands of others, furnishing to them material for controversial uses :—

The first was a case of tuberculous epididymitis. An eminent surgeon had arranged to operate in this case, and the day had been fixed. On the advent of that day he, however, found that the disease had progressed so rapidly that the operation had to be abandoned. The patient was then referred to me for treatment by vaccine therapy. For a long time the patient made very satisfactory improvement under vaccine therapy. He then lost ground. If I remember aright, the epididymitis began to break down. He then went back to the surgeon, and the operation for amputation of the testicle, which had before been abandoned, was undertaken. The case was then controversially cited as an example of the failure of vaccine therapy.

I do not demur. I merely point out that this was a case where, in the language of my metaphor, I had won a series of tricks ; I then began to lose, and the fact that I had failed to bring about a cure was the only fact which was taken into account, although, if I appreciate the case aright, the success which attended vaccine therapy had made it possible to undertake the operation with a prospect of success. But I ask myself what would be the verdict of the judicious upon such a case. Would *they* pronounce it a failure or a success ?

The second case was that of a patient who had been suffering for several months from a chronic oedematous inflammation of the pharynx and soft palate, which had been treated unsuccessfully by an eminent laryngologist. I was asked to see the patient, and found that the cause of the trouble was a mixed pneumococcus and catarrhalis infection. A striking improvement manifested itself immediately after the inoculation of the first dose. The patient was easier and the oedema and cyanosis of the affected parts had markedly diminished. A few day later, however, a small perforating ulcer made its appearance in the central line of the soft palate,

and inoculation-treatment was discontinued. The case was published by the laryngologist who had been in charge of the case as a failure for vaccine therapy.

Again I ask myself what the verdict of the judicious would be. It would perhaps have been that one trick had been won by vaccine therapy, or perhaps—in view of the development of the ulcer—that one trick had been lost. But I think the decision would not have been that the game had been played to a finish and that vaccine therapy had proved a failure.

The third case was that of a patient with fairly advanced phthisis who was treated as an out-patient in the Inoculation Department of St. Mary's Hospital. Considering the fact that she was a frail little woman and had a very considerable distance to come for treatment, this patient had, we thought, made very fair progress under vaccine therapy ; and she, of course, assured us that she had. Some time in the late autumn of last year she fell seriously ill, discontinued her attendance, and saw in consultation with her medical attendant an eminent physician. By his advice she was placed upon continuous inhalations of an antiseptic. A letter published by her medical attendant in one of the medical journals early in this year recounts the brilliant success of this method and the failure of vaccine therapy.

Only two or three days ago that patient was admitted to St. Mary's suffering from pneumothorax and in a very serious condition.

I do not know what the commentary of the judicious would be upon this. He might, of course, decide that the case was a success for vaccine therapy, and a success also for the method of the continual inhalation of antiseptics. Or he might decide that it was a failure for both methods. But I think he would not decide that it was a failure for vaccine therapy and a success for the method of the continuous inhalation of antiseptics. I think he would remind the letter-writer that ' with what measure you mete it shall be measured to you again '.

I have suggested all these difficulties to you. I confess I do not see my way out of them, unless perhaps we could content ourselves, in cases like those which have been cited above, to abstain from pronouncing a verdict on the summarised result of the whole. In such cases we might, it seems to me, content ourselves with giving a verdict on each successive movement in the game.

If I have succeeded in making you see eye to eye with me in this matter, if I have succeeded in making clear to you how in many cases almost insurmountable difficulties stand in the way of a final decision on the results obtained in the individual case, it will not be necessary for me to emphasise the fact that it must be hopeless to attempt to summarise verdicts on vaccine therapy in any statistical form.

I have, in conclusion, only one or two passing remarks to make in connexion with the question of the mode of administration of the vaccine.

(III) Mode of Administration of the Vaccine.

Probably to those who are familiar with Dr. Arthur Latham's work the issue as to whether the administration of vaccine by the mouth is an improvement over the method of hypodermic inoculation will be the first issue to present itself for consideration. For all of us, however, who approach it from the standpoint of the

laboratory, this issue was already *res judicata* before it was brought into prominence by Dr. Latham. I had satisfied myself long ago—and the results of my experiments are embodied in my *Short Treatise on Anti-typhoid Inoculation* [1] — that, though typhoid vaccine may be absorbed by way of the intestinal canal, it is only badly and incompletely absorbed, and above all that its action is uncertain. My friend and successor at the Royal Army Medical College, Sir W. B. Leishman, has carried out far more extensive and more careful experiments in connexion with the same question, and has arrived at the same result. He finds that inoculation by way of the alimentary canal is an extremely uncertain process. I do not think it is necessary to go any further than that, for science never sanctions a more complicated and more uncertain method being employed where a simple and certain one, such as subcutaneous inoculation, is available. And I would submit that the idea that a *via media* can be found between the antique system of prescriptions—with doses taken t.i.d., before or after meals—and scientific applications of bacteriology, and the idea that that *via media* can be found in the administration of vaccines by the mouth, ought steadily to be put away from us. The new wine of bacterial vaccines cannot with impunity be poured into the old bottles of traditionary medicine.

There is, in conclusion, just one other issue which I should like to put before you for consideration. The problem has been before my mind for a long time, and I understand that it has also suggested itself to others.

Let me introduce it to you by recalling to your mind the mental picture which I dwelt upon at the outset of this discourse. You will remember that I suggested to you that the rationale of vaccine therapy was to be found in the exploitation of the unexercised immunising capacities of the uninfected tissues. In other words, I suggested that the antibacterial substances which are elaborated in the organism in response to inoculation are elaborated by the tissues into which the vaccine is introduced. Now inasmuch as we may make our inoculation into any and every part of the subcutaneous tissue, it would, upon the theory that antibacterial substances are produced at the site of inoculation, seem to follow that we ought, by inoculating in a series of different places, to get a summation effect. The idea that such a thing might be opens up magic vistas of therapeutic possibilities.

But at present the achievement of a summation effect from a series of simultaneous inoculations undertaken in different parts of the body belongs, like the achievement of a summation effect from a series of consecutive inoculations undertaken each after the previous one has evoked its immunising response, to the domain of unrealised things.

There lies before us here a wide and unexplored field of work.

[1] Constable, London, 1904.

ON PROPHYLACTIC INOCULATION AGAINST PNEUMONIA, AND ON THE RESULTS WHICH HAVE BEEN ACHIEVED BY IT.

(Being an Excerpt from the Author's 'Drugs and Vaccines in Pneumonia', Constable, London, 1914)

It will not be amiss to begin by setting out the evidence which establishes that the pneumococcus is the microbe which is the causal agent of the pneumonia on the Rand.

TABLE I.—*Data of Blood Cultures and Lung Puncture Cultures undertaken upon Cases of Pneumonia in Tropical Natives treated in the Hospital of the Witwatersrand Native Labour Association.*[1]

Nature of the bacteriological observation	Number of cases examined	Number of cases in which the pneumococcus was found	Number of cases in which the cultures remained sterile	Number of cases in which a microbe other than the pneumococcus was found
Blood cultures (1 to 20 c.c. of blood)	380	99 [2]	277 [3]	4 [4]
Lung-puncture cultures	53	34 [5]	18 [6]	1 [4]

[1] It may be explained that this Hospital is attached to the depot to which all Tropical Native Labourers arriving at Johannesburg are drafted for subsequent distribution to the mines.

[2] Of these, 56 (i.e., 56 per cent.) succumbed. [3] Of these, 76 (i.e., 27·5 per cent.) succumbed.
[4] The micro-organism which grew was in each case one of the coli group. [5] Of these, 10 succumbed.
[6] Of these, 6 succumbed.

The nature of the bacterial infection which was to be combated having been set at rest, our principal task stood out clearly before us. It was to prepare vaccines of the pneumococcus; to ascertain, if possible, what was the best form of vaccine; and above all to determine the effective and, so far as might be possible, the *optimum* dose of the vaccine, and the best method of application.

We may with advantage begin with a brief description of the methods we adopted in preparing the vaccine; and we may then, before dealing specifically with immunisation against the pneumococcus, call attention to those broad general principles with regard to dosage, and the *modus operandi* of vaccines, which would appear to be deducible from the experience won in connexion with immunisation against other bacterial infections.

Section I.—Preparation of Pneumococcus Vaccines.

We experimented somewhat extensively with a view to selecting for use a medium which should give a very copious culture of pneumococcus.

Varying in a methodical manner the peptone, extractives and alkali of ordinary peptone broth, and then making to such media graduated additions of human serum, we found that very copious cultures of pneumococcus were regularly obtained with a medium which contained (*a*) 1 per cent. of peptone and 1 per cent. of lemco ; (*b*) 2½ to 5 per cent. of human serum ; and (*c*) an amount of alkali fixed by neutralising to phenolphthalein, and then adding 6 c.c. of normal acid solution to each litre of medium.

During the first couple of months of our work in Johannesburg we worked with a medium composed as above, but finding that we obtained much more copious cultures by adding up to 1 per cent. of glucose to the above mentioned medium we employed such serum glucose broth in most of our subsequent work. We would, however, point out in this connexion that where such a serum glucose broth is employed it is necessary to be constantly on the watch against the occurrence of autolytic processes which go, it would seem, hand in hand with the development of acid in the culture medium. For these autolytic processes [1] may, even when they stop short of an actual disintegration of the microbes, deprive the bacteria of their power of staining with aniline dyes, and so give rise to formidable fallacies in standardising the vaccines.

Our vaccines were in all cases made from cultures which had grown for twenty-four to thirty-six hours. They were standardised by enumeration by the *Ratio Method*, devised by one of us.

The cultures were killed by exposure to a temperature of 55° to 56° C. for half an hour.

An addition of ½ per cent. of carbolic acid was then made to the vaccines.

Statistical Results of Prophylactic Inoculation against Pneumonia.

We pass now to study the results which were obtained by prophylactic inoculation. The inoculations which we carried out or initiated range themselves under six mass-experiments.

Mass-experiment No. 1

This mass-experiment was undertaken in the compound of the Witwatersrand Native Labour Association. This compound, familiarly known as the W.N.L.A. Compound, is the receiving station at Johannesburg where the gangs of native labourers, who are recruited for the general service of the mines, are housed for three to four weeks before they are drafted off to their respective mines.

We confined our operations in this and the four next mass-experiments to the Tropical Natives. For it is especially these who are ravaged by pneumonia.

[1] It may be observed in passing that we found that the culture fluid in which autolysis has occurred did not exert any bactericidal power upon the pneumococcus.

In each case, two or three days after the arrival of a new gang, the natives were lined up. We then, dismissing any who showed symptoms of illness, inoculated as they filed past us every alternate man, reserving in this way half of each gang to serve as controls.

As the men came up to us, the inoculated were made to pass on one side, and the uninoculated on the other; and lists were made of the depot numbers of the two groups—each man's identification docket being at the same time ruled across with a diagonal line, which was red or blue according as he was inoculated, or reserved as a control.

After the lapse of eight to ten days the natives were again ranged up, and every man who had on the first occasion been inoculated was now reinoculated, while the controls were still kept as controls. The red or blue diagonal line on each man's docket was at the same time converted into a red or blue cross; and a corresponding entry was made upon the lists.

Proceeding in this manner between the months of October, 1911, and April, 1912, with each gang of Tropical Native labourers as it arrived, we inoculated a total of 5963 and reserved 5671 to serve as controls.

The doses which were employed varied with each gang and sub-group—for we were, as has been indicated above, engaged in following out from day to day the effect exerted on the blood by different doses and different schemes of administration.

The average amount of vaccine given in the two successive inoculations taken conjointly was something like 300 million of pneumococci. It in no case exceeded 600 million.

The results of the inoculations are furnished, so far as relates to the three or four weeks dating from the first inoculation, in the register of pneumonia cases and deaths kept in the hospital attached to the W.N.L.A. Compound. The clinical diagnosis was in each case made by the Medical Officer, Dr. George A. Turner, or the Assistant Medical Officer, and by one of us (W. P. M.). In the fatal cases the cause of death was controlled by post-mortem examination carried out by the Medical Officer.

Information with regard to the subsequent history of the inoculated and uninoculated is furnished in two sets of returns.

We have, first, the special monthly returns made to us from the mines—the so-called *red and blue monthly returns*. These record the number of cases of pneumonia and deaths from pneumonia occurring respectively in the red and blue group of Tropical Natives.

We have, secondly, the returns of deaths, and specifically of deaths from pneumonia, rendered thrice monthly from the mines to the Chamber of Mines for the information of Government.

The 'red and blue' returns proved unserviceable for our purpose, inasmuch as the depot numbers of those who contracted, and died from, pneumonia were not supplied; and also because the results applying to the separate gangs were not kept apart.

The Governmental returns, since they furnish the depot numbers of those who

succumbed to pneumonia, are those we depend upon for the later results set forth in the table below. The results of the mass-experiment, ascertained as explained above, are as follows :—

TABLE II.—*Showing the Number of Pneumonia Cases and Deaths from Pneumonia which occurred in the W.N.L.A. Compound between the date of the first Inoculation and the Departure of the Natives to the Mines.*

	Number of men in the group	Cases		Deaths	
		Number	Percentage	Number	Percentage
Inoculated	5963	147	2·6	50	0·83
Uninoculated	5671	198	3·5	87	1·53

TABLE III.—*Showing the Number of Deaths from Pneumonia reported from the the Mines* [1] *in each successive month.*

	Number of men in the group	Second month	Third month	Fourth month	Fifth month	Sixth month
Inoculated	5963	11	19	12	10	7
Uninoculated	5671	29	19	15	13	8

[1] In compiling this table the fact that the native whose death was recorded in the Report from the Mines had passed before us, and had been inoculated or set aside as a control, was verified by reference to our records ; the period which had elapsed since the date of the first inoculation and his death being at the same time ascertained.

It will be seen that the inoculation exerted a marked effect for two months, but that after the end of the second month the inoculated had no advantage over the uninoculated.

To obtain the measure of the total effect of inoculation on the death-rate from pneumonia we may combine the figures for the deaths in Tables II and III.

TABLE IV.—*Showing the Effect which was exerted upon the Death-rate from Pneumonia in the first two months after Inoculation.*

	Number of men in the group	Deaths	
		Number	Percentage
Inoculated	5963	61	1
Uninoculated	5671	116	2

Mass-experiment No. 2

After Mass-experiment No. 1 had been in progress for a certain time, and after it had appeared from the returns that favourable results were being obtained, we

endeavoured to procure larger figures, such as would be really serviceable for statistical purposes, by undertaking ' general inoculations ' at the mines.

We accordingly in December, 1911, and January, 1912, organised and ourselves took part in a number of ' general inoculations ' at the Mines, and Dr. Turner who helped us in this work afterwards took independent charge of a number of others.

We compute that in all 20,000 to 30,000 natives were in this way inoculated.

Our intention was that the inoculated and the controls should, in connexion with these inoculations, have been chosen and registered in exactly the same way as had been done in the W.N.L.A. Compound ; and we arranged for a special form of return in which the population of the mines should be divided up into (*a*) inoculated natives, (*b*) natives who were set aside as controls, and (*c*) natives who were excluded from the experiment either because they had joined later, or because they had for other reasons failed to put in an appearance at the inoculation parade.

All sorts of difficulties however presented themselves in connexion with working out this scheme.

It was found very difficult to get the records of the inoculated and controls accurately made when such large bodies of natives as were here in question were being dealt with.

Again the arrangements made for keeping track of the inoculated and uninoculated left in many of the mines very much to be desired.

Complications also presented themselves with regard to making proper allowance for natives who left the mines ' time-expired ' within the period of observation.

Lastly, the inoculated turned out to be non-homologous with the controls, and there was—in contrast with what obtained in our other experiments—a sensible defect of homogeneity in the population of natives we were dealing with.

In connexion with the comparability of the inoculated and the controls it may be explained that the Native labourers on the Rand come from as far south as the Cape, and from as far north as the Equator ; and that there are between the different races of natives at the mines great differences with respect to tractability and susceptibility to pneumonia—the Tropical Natives being at once the most tractable and the most susceptible to pneumonia ; and the East Coast Natives, as we are informed, much more difficult to handle, and at the same time more resistant to pneumonia.

It will be readily understood that under these circumstances the inoculated group would tend to be more largely composed of the more tractable and, as it happens, more susceptible Tropical Natives ; and the uninoculated group to be composed of the less tractable and, as it happens, less susceptible East Coast Natives.

In connexion with the homogeneity of the population operated upon, it is to be observed that the fresh arrivals are (as will be seen from the figures for the uninoculated in Tables II and III, V and VII) the most susceptible to pneumonia, and that those who have been longer upon the mines have become more resistant to pneumonia. By consequence every group which is chosen from the native population, without reference to length of residence on the mines, will comprise persons of very different degrees of resistance.

As soon as these considerations had, one after the other, come home to us, we decided to bring this particular mass-experiment to a close, and to jettison, as open to fallacy, such statistical returns as had already been sent in to us in connexion with the experiment.

If, as we cannot in view of our other experience doubt, this mass-experiment gave good results we suggest that the advantage was reaped in the form of a diminution in the general death-rate from pneumonia on the Rand.

Mass-experiment No. 3

Having learned from experience in Mass-experiment No. 2 the difficulties which have to be confronted and the fallacies which have to be avoided in connexion with a general inoculation, we set ourselves to organise such an experiment upon better lines. The mass-experiment here in question was begun in May, 1912. It consisted in a series of general inoculations undertaken in twenty mines [1] and limited to Tropical Natives who had, in the season 1911–1912, passed out to the Mines from the W.N.L.A. Compound. In view of the fact that it had been shown in Mass-experiment No. 1 that there was, after two months, no difference between inoculated and uninoculated, the whole of the Tropical Natives of the year, with the exception of those few who left the compound for the mines in March and April,[2] were regarded as, for our purposes, homologous.

In each mine three general inoculations were undertaken, approximately a month intervening between the first and the second, and again between the second and the third inoculation; and the dose of vaccine was uniformly 150 million pneumococci.

On the *first* occasion approximately half of the Tropical Natives were inoculated, and half were left as controls—giving a group of 3975 inoculated and a group of 4769 uninoculated. Among these last were counted in a certain number who were actually at work on the mine, but who for one reason or another, were not actually forthcoming at the first parade.

At the *second* general inoculation, half of those who had on the first occasion served as controls were inoculated; and of those who were on that occasion inoculated one moiety was reinoculated, while inoculation was withheld from the other, with a view to find out how long the effect of the previous inoculation would make itself felt.

At the *third* general inoculation, again, half of those who functioned at the second inoculation as controls were inoculated; and nearly all those who were previously inoculated were reinoculated.

Quite special care was devoted to ensuring accuracy in the records—the procedure adopted being as follows: For each mine two lists were prepared. On the first (hereafter called the ' depot list ') the ' depot numbers ', with which the natives leave the W.N.L.A. Compound, were arranged in serial order, the corresponding ' mine numbers ', which are given to the natives on arrival at their mines, being

[1] The mines chosen for the experiment were those which had received the largest number of Tropical Natives.

[2] These were excluded from the experiment and left out of the record.

entered in the second column. On the second list (hereafter called ' the mine list ') the mine numbers were set down in serial order, and the corresponding depot numbers were entered in the second column. This done the depot list was taken in hand by us, and we inserted in the third column, opposite the numbers, alternately a red or a blue diagonal line—the red line to indicate that the native was to be inoculated, the blue that he was to serve as a control. Corresponding entries were then made on the mine list.

When visiting the mine for the purpose of the general inoculation the natives were identified by their mine numbers as they passed up from their work, and they were inoculated or sent on uninoculated, according as their numbers were marked in red or blue, and the diagonal lines on the list were at the same time converted into crosses to show that these natives had been dealt with. Corresponding crosses were afterwards made upon the depot list.

The returns were made monthly by the Medical Officers of the mines on printed forms specially issued for this purpose. The return gave in connexion with each patient admitted to hospital his depot number, the date of his admission, the diagnosis, and the date of his discharge or death.

In the table given below we have grouped the results under the headings of results in the *first*, *second*, *third*, and *fourth* months.

We have included in the results obtained by inoculation in the first month, not only the results which refer to the 3975 inoculated in the first general inoculation, but also the figures which refer to the natives who were inoculated for the first time at the second and third general inoculations. Moreover, in the case where a man was inoculated more than once we have set down in the ' results obtained in the first month after inoculation ' those which apply to him for the month following each of his several inoculations. For we find by analysis of the figures that the inoculation of men who have been inoculated one month previously with 150 millions gives neither better nor worse results than are obtained where men are inoculated for the first time.

We have dealt in a precisely similar fashion with the results which apply to the uninoculated. For instance, when in making up our results for the first month, we come upon a man who was at each of three successive general inoculations taken as a control, we enter him in our statistics three times over (once for each several month) in order that he may serve as a control for the men who were inoculated at the first, second and third inoculations respectively. Similarly when we come upon a man who was inoculated for the first time at the third general inoculation, we include him twice over in our controls. And when we come upon a man who was inoculated for the first time at the second general inoculation, we include him once in our controls. We follow, *mutatis mutandis*, precisely the same procedure when dealing with the returns which apply to the second, third and fourth months.

It will be seen that with the very small dose of vaccine which here came into application a very striking advantage was achieved in the first month after inoculation, but that advantage had nearly passed away by the end of the third month. The records are not continued beyond the fourth month because the mass-experiment next on our list was then begun.

TABLE V.—*Showing results for Four successive Months after Inoculation*

	Number of men in the group[1]	Cases of pneumonia		Deaths from pneumonia	
		Number	Percentage	Number	Percentage
		Results for the First Month			
Inoculated	10,626	125	1·1	22	0·21
Uninoculated	10,508	216	2·05	40	0·38
		Results for the Second Month			
Inoculated	6,787	76	1·12	15	0·22
Uninoculated	8,380	128	1·5	25	0·3
		Results for the Third Month			
Inoculated	6,103	59	0·96	13	0·21
Uninoculated	7,823	92	1·2	22	0·28
		Results for the Fourth Month			
Inoculated	6,103	44	0·72	16	0·26
Uninoculated	7,823	68	0·87	20	0·25

[1] In view of the fact that the corrections which would be applicable under the heading of deaths and repatriations would be quite insignificant, we have not troubled to deduct them from the numbers of the inoculated and uninoculated.

Mass-experiment No. 4

This experiment consisted in a general inoculation carried out, in November, in eighteen out of the twenty mines which were in question in the last experiment. It relates to those Tropical Natives who belonged to gangs which passed through the W.N.L.A. Compound in 1912, and who were accordingly due to remain in the Mines till at least the end of January, 1913. Those who had in the last experiment served as controls were retained as such, and the rest were reinoculated—in each case with a dose of 1000 million of pneumococci, grown in part on serum broth, and in part on glucose serum broth. In this way there was obtained a group of 610 uninoculated, which for the purpose of such comparisons as those we are here making was undesirably small, and a group of 2322 inoculated. The results as ascertained from the returns rendered on the printed forms referred to in connexion with the last experiment were as follows :—

TABLE VI.—*Setting forth the Result obtained for the Period of Five Months after the date of the Inoculation*

	Number of men in the group	Cases of pneumonia		Deaths from Pneumonia	
		Number	Percentage	Number	Percentage
Inoculated	2,322	70	3·0	20	0·86
Uninoculated	610	21	3·4	8	1·3

The question as to why larger advantage was not here reaped from inoculation is reserved for discussion in our summary of results (Section II, *infra*).

Mass-experiment No. 5

This experiment was undertaken upon the Tropical Natives who arrived in the Compound of the W.N.L.A. between the middle of August and the end of November, 1912.

The chief objects that it had in view were : (*a*) the determination of the optimum dose of pneumococcus vaccine for prophylactic uses, and (*b*) the decision of the question whether cultivations on glucose serum broth furnished a better vaccine than cultures on the ordinary serum broth.

The inoculations were carried out exactly as in the case of Mass-experiment No. 1, with the difference that only every fifth native was taken as a control.

The procedure adopted with regard to dosage was to take for the general inoculation in each case doses which had been previously ascertained to be perfectly safe ; and then to reconnoitre by inoculating a small group with larger doses.

Advancing in this manner we obtained for study a series of six groups.

The general results may be summarised as follows : Employing a *vaccine prepared by cultivating the pneumococcus on blood broth* and a dose of 250 millions, we obtain, for a period of one month after inoculation, a reduction of the incidence from 7 per cent. in the controls to 3·7 per cent. in the inoculated ; and a reduction of the death-rate from 2·9 per cent. in the controls to 1·5 per cent. in the inoculated, that is to say, in each case a reduction equivalent to about 50 per cent. With a dose of 500 millions of the same vaccine we obtain, for the first three months after inoculation, a reduction of about 25 per cent. in the incidence and nearly 50 per cent. of the death-rate. With 1250 millions of the same vaccine we obtain a much less favourable result : a reduction, on a period of three months, of 30 per cent. in the incidence, and of only 20 per cent. in the death-rate. We may presume that the dose here employed was too large. Employing *a vaccine prepared by cultivating a pneumococcus on glucose blood broth* we obtain, for a period of two months, with a dose of 500 millions a reduction of 50 per cent. in the incidence, and of 40 per cent. in the death-rate. With a dose of 1000 millions we obtain, over a period of three months, a reduction of 30 per cent. in the incidence, and of over 60 per cent. in the death-rate. With a dose of 2500 millions we again obtain less satisfactory results ; a reduction of just over 20 per cent. in the incidence, and a reduction of 35 per cent. in the death-rate.

TABLE VII.[1]—*Showing the results obtained by the Inoculation of various doses of Pneumococcus Vaccine grown on Blood Broth and Glucose Blood Broth respectively.*

	Number of men in the group	First month		Second month		Third month		Fourth month		Fifth and Sixth month	
		Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
(A) *Results obtained by the Inoculation of 250 millions of Pneumococci grown on blood broth*											
Inoculated	646	24	10	17	5	14	4	9	2	16	5
Uninoculated	626	44	17	17	5	12	4	15	5	16	6
(B) *Results obtained by the Inoculation of 500 millions of Pneumococci grown on blood broth*											
Inoculated	759	25	8	16	4	17	2	14	4	19	8
Uninoculated	764	46	19	21	6	17	5	18	5	20	6
(C) *Results obtained by the Inoculation of 1250 millions of Pneumococci grown on blood broth*											
Inoculated	1582	37	17	40	10	28	10	17	6	44	13
Uninoculated	791	23	10	31	8	21	6	12	3	14	7
(D) *Results obtained by the Inoculation of 500 millions of Pneumococci grown on glucose blood broth*											
Inoculated	463	18	10	7	1	9	3	7	2	7	5
Uninoculated	457	35	14	14	5	10	3	12	4	10	4
(E) *Results obtained by the Inoculation of 1000 millions of Pneumococci grown on glucose blood broth*											
Inoculated	650	20	6	20	3	10	2	6	3	14	3
Uninoculated	595	37	16	18	6	15	4	15	4	14	4
(F) *Results obtained by the Inoculation of 2500 millions of Pneumococci grown on glucose blood broth*											
Inoculated	1582	47	17	42	8	28	5	21	10	35	8
Uninoculated	791	23	10	31	8	21	6	12	3	14	7

[1] It is to be observed that the results of those inoculated with reconnoitring doses are excluded from consideration, and that the uninoculated who serve for controlling the effect of one dose are often the selfsame individuals as serve for the controlling of another dose.

There can be extracted from the statistical data which are available in connexion with this mass-experiment, not only conclusions bearing on the question of the relative efficacy of the various doses of vaccine which were administered, but

also valuable information bearing on the question of the production of a negative phase—information which has, as we shall see, an important bearing on the question of the utilisation of vaccine in the treatment of pneumonia.

The data which seem to us important from this point of view are set out below in the form of a table.

TABLE VIII

	Number in group	Number of cases of pneumonia which developed													
		First day		Second day		Third day		Fourth day		Fifth day		Sixth day		First six days	
		Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Group A (inoculated with 250 millions)	646	—	—	—	—	1	1	2	1	—	—	—	—	3	2
Control group	626	1	1	4	1	3	1	2	2	—	—	2	2	12	7
Group B (inoculated with 500 millions)	759	—	—	2	—	1	1	—	—	3	1	1	—	7	2
Control group	764	1	1	5	2	3	1	2	2	—	—	2	2	13	8
Group C (inoculated with 1250 millions)	1582	8	4	2	—	1	1	1	—	—	—	—	—	12	5
Control group	791	1	1	2	2	—	—	—	—	—	—	1	—	4	3
Group D (inoculated with 500 millions glucose vaccine)	463	1	1	—	—	1	—	—	—	—	—	—	—	2	1
Control group	457	1	1	3	1	3	1	1	1	—	—	2	2	10	6
Group E (inoculated with 1000 millions glucose vaccine)	650	—	—	1	—	2	—	2	—	—	—	1	—	6	—
Control group	595	1	1	4	2	3	1	1	1	—	—	2	2	11	7
Group F (inoculated with 2500 millions glucose vaccine)	1582	7	3	11	2	1	1	3	—	2	2	—	—	24	8
Control group	791	1	1	2	2	—	—	—	—	—	—	1	—	4	3

The facts which are set forth in this table are, as will be seen, very remarkable. Associating together the figures which apply to Groups A, B, D, and E: i.e., the groups which received doses up to 1000 million of pneumococci, we find that, in the first four days after inoculation, 2500 inoculated had an incidence-rate of 0·52 per cent., and a death-rate in connexion with these cases of 0·16 per cent.; while

750 [1] controls had an incidence-rate of 1·4 per cent., and a death-rate in connexion with these cases of 0·84 per cent. In other words, the uninoculated had an incidence-rate nearly three times, and a death-rate five times greater than the inoculated.

Again, associating together the figures which relate to Groups C and F—groups which received doses of over 1000 million of pneumococci—we find that 3200 inoculated had for the same period an incidence-rate of 1·1 per cent., and a death-rate in connexion with these of 0·32 per cent., while 800 controls had an incidence-rate of 0·4 per cent., and a death-rate also of 0·4 per cent.

Two important conclusions follow: the *first* is that pneumococcus inoculation undertaken with doses up to 1000 millions had a marked effect in aborting pneumonia, and in diminishing the case mortality.

Or we may phrase it otherwise. Vaccine therapy as applied to the treatment of pneumonia is successful when doses of 250 to 1000 millions are given in the incubation stage of the disease. The *second* conclusion is that inoculation undertaken with doses of over 1000 million of pneumococci may perhaps temporarily increase the incidence-rate of pneumonia.

It is perhaps of interest to point out that these conclusions are essentially the same as those formulated in connexion with plague vaccine by Mr. Haffkine, immediately after he had carried out his first mass-experiment in the Byculla Jail, Bombay, in 1898. In that experiment, as in the mass-experiment we are here dealing with, a decisive difference in favour of the inoculated half of the population manifested itself already within twenty-four hours. And the view that Mr. Haffkine maintained (in contravention to that held by one of us) that plague vaccine does not produce a negative phase, and that it has the power of aborting an incipient attack, was afterwards established by evidence accumulated by Miss Alice Corthorn, M.D.,[2] and Surgeon-General Bannermann, I.M.S.[3]

In connexion with this all that requires to be said is that the generalisations set forth in Section II of this Report—generalisations which have been reached only after years of further work—have made it intelligible that a negative phase should manifest itself with large doses of typhoid vaccine, a vaccine which is easily broken down in the normal organism, and again with all vaccines after the organism has, by foregoing immunising response, acquired bacterioclastic power, and that this phase should make default in the uninfected organism, and in the early stages of infection when vaccines, such as plague vaccine and pneumococcus vaccine which are with difficulty broken down in the body, are inoculated.

In concluding this account of the results obtained in Mass-experiment No. 5 we may profitably advert to one more general consideration. It is, as will presently be brought out more fully in Section II, reasonable to expect that an effective inoculation will give an additional bonus in the form of a diminution in the morbidity which comes upon the record under the heading of 'Other Diseases'. And in point of fact the records which relate to the particular mass-experiment we are here discussing show such a reduction. We have our bonus in the form of a 15 per

[1] This figure is arrived at by taking the actual number of men who served as controls for groups A, B, D, and E.

[2] *Med. Brit. Journ.*, Jan, 25, 1902. [3] *Ibid.*, Sept. 14, 1901.

cent. reduction in the 'other diseases' of the inoculated, the figures being: *inoculated*, 6224; *uninoculated*, 1545. Cases of sickness other than pneumonia: in *inoculated*, 2154; in *uninoculated*, 620.

We now pass to our sixth and last mass-experiment.

Mass-experiment No. 6

This mass-experiment was undertaken upon the natives employed in the Premier Diamond Mine—a mine which, despite the fact that it employs no tropical labour, has always suffered very severely from pneumonia.

We began with a general inoculation on January 21, 1912, inoculating at a sitting about four thousand natives.

The experiment thus begun was followed up by a methodical inoculation of the recruits who joined the mine. These inoculations were very admirably organised, carried out, and recorded by Dr. J. C. A. Rigby, who was very ably assisted by Mr. Sheridan.

From January 21 onwards till March 15, 1912, every second native was inoculated as he arrived at the mine. From that date onwards only every third man was kept as a control, and gradually as it became more and more evident that good results were being obtained an increased proportion of the recruits were inoculated.

At the outset the dose which was employed was 200 million pneumococci; and gradually as it became clear, from the results that were coming in from Mass-experiment No. 5, that the employment of larger doses was indicated, the doses were gradually increased to 250 million, then to 500 million, until finally a dose of 1000 million was reached.

Returns which were very carefully prepared were furnished to us every month, and we were further, at the end of the first six months, and again at the end of the first year of the experiment, furnished with a detailed synopsis of the results. These returns are our authority for the facts set forth below.

TABLE IX.—*Showing the Results for the Whole Native Population of the Mine for the Year beginning January 22, 1912, and ending January 21, 1913*

	Daily average strength of the group	Cases of pneumonia		Deaths from pneumonia	
		Number	Percentage	Number	Percentage
Inoculated	9909	508	5·13	154	1·55
Uninoculated	4520	467	10·33	145	3·21

We subjoin also, for reasons that will presently appear, another statistical record which excludes all those who were on the mine on January 21, 1912, and takes into account all the natives who came to work on the mine during the next twelve months.

It may be remarked that the fact that the native at this mine usually works on a six months' contract would make the daily average population equivalent to about half the number of the natives who join in the year.

TABLE X.—*Showing for the period January 22, 1912, to January 22, 1913, the Results for the Natives who came in that year to work on the Mine*

	Number of natives who joined the mine	Cases of pneumonia		Deaths from penumonia	
		Number	Percentage	Number	Percentage
Inoculated	17,431	376	2·16	120	0·7
Uninoculated	6,771	348	5·14	120	1·77

Finally, in connexion with the mass-experiment here in question, we may give the figures for the corresponding period relating to the incidence and death-rate of 'other diseases' in the inoculated and uninoculated sections of the population. These figures are as follows: *Inoculated*, average daily strength, 9909; incidence rate, 47·2 per cent.; death-rate, 0·93 per cent. *Uninoculated*, average daily strength, 4520; incidence rate, 106·6 [1] per cent.; death-rate, 1·90 per cent.

Section II.—General Survey of the Results obtained by the Prophylactic Inoculation of Pneumococcus Vaccine; and Critical Comment

Prophylactic Inoculation against Pneumonia.

The comparative statistics which have been set forth above testify, as has been seen, in every case to a reduction in the incidence-rate and death-rate of pneumonia in the inoculated.

In adjudicating upon a procedure of prophylactic inoculation the following general theses must be taken into account.

(1) The advantage derivable from a prophylactic inoculation will be limited by the patient's power of immunising response.

In accordance with this, one would not expect to achieve as much by inoculation in a naturally non-resistant population—such as that represented by the Tropical Native Labourers on the Rand—as in a more resistant population—such as is, by common repute, represented by the Native Labourers on the Premier Mine.

We may, perhaps, bring into relation with this consideration the fact that we have, as the result of the application of prophylactic inoculation to the Tropical Natives on the Rand, in Mass-experiment No. 1 (*Tables II and III*) a reduction of 37·5 per cent. in the death-rate of the inoculated upon an observation-period of six months; in Mass-experiment No. 3 (*Table V*) a reduction of 31 per cent. in the death-rate of the inoculated upon an observation-period of four months; and in Mass-experiment No. 5 (*Table VII, Group E*) a maximum reduction of 50 per cent. in the death-rate of the inoculated upon an observation-period of six months; while we have upon the Premier Mine Mass-experiment No. 6 (*Table X*) upon a

[1] The figures are here printed as received from the mine. One may take it that there were 106 'admissions to hospital', or that 106 men 'reported sick', during the year out of 100 uninoculated men working in the mine.

six months' observation-period a reduction of 60 per cent. in the death-rate for the inoculated.

(2) The advantage derivable from prophylactic inoculation will depend upon the degree to which the population to which it is to be applied has already run the gauntlet of the particular microbic infection in question.

In accordance with this, one would expect greater advantage from inoculation when applied to a population newly arrived in the field of infection, than from inoculation applied to a population which had been long in such a field *continuously* exposed.

It falls in with this, that while we have in the inoculated in Mass-experiment No. 5, Group E—an experiment in which the uninoculated had an incidence-rate of 15 per cent., and a death-rate of 5 per cent. over a five-month period—a reduction of 35 per cent. in the incidence and of 55 per cent. in the deaths; we have in the inoculated in Mass-experiment No. 4 [1]—an experiment in which the uninoculated had an incidence-rate of 3·4 per cent. and a death-rate of 1·3 per cent. for the same period—a reduction of only 10 per cent. in the incidence-rate and of 34 per cent. in the death-rate.

We may further note that we have, in connexion with Mass-experiment No. 6, in the case of the inoculated a reduction in the incidence-rate of 50 per cent., or 58 per cent.; and a reduction in the death-rate of 53 per cent., or 61 per cent.; according as we take as our controls (as in *Table IX*) the population already on the mine, or (as in *Table X*) the population of new arrivals.

(3) Where in comparative statistics we find that the difference between the inoculated and the uninoculated is after a certain time effaced, this does not necessarily indicate that the immunity of the inoculated is diminishing. *We may be witnessing, instead of a descent of the inoculated to the level of the uninoculated, an ascent of the uninoculated to the level of the inoculated.*

This is, presumably, the true interpretation of the fact that our inoculated natives displayed only for a comparatively short period an advantage over the uninoculated. For we have uniformly, in all our records (Mass-experiments Nos. 1, 3, and 5; *Tables II* and *III*, *V* and *VII*), and therefore presumably independently of any change in the external conditions—instead of a progressive falling off in the incidence-rate and death-rate of pneumonia in the inoculated, a progressive improvement in the incidence and death-rate in the uninoculated.

The point of interest which here suggests itself is the question as to how the ascent of the uninoculated to a higher level of immunity, and the accompanying progressive improvement in the figures for the inoculated, which is a feature of all the statistics, is to be accounted for. Two alternative hypotheses here suggest themselves. The attainment of a higher level of immunity may be explained by supposing that the most susceptible individuals have been weeded out by death from each group. But this is not a very acceptable hypothesis, for it is hard to believe that infection can ever be distributed over a population in so uniform a manner as punctually to pick out and kill all the more susceptible individuals. It

[1] In this mass-experiment the dose of vaccine employed was the same as that in Mass-experiment No. 5, Group E, and the culture medium employed in making the vaccine was also largely the same.

is much more probable that the progressively increasing resistance in both the inoculated and uninoculated groups is achieved by processes of self-immunisation consequent upon minor infections which affect practically the whole mass of the population. We have what we think presumptive proof that such agencies are at work in the fact, already adverted to above, that there were, in practically every group of four to six uninoculated natives whom we employed as controls, some who were almost certainly suffering from minor pneumococcus infections.

Not only does this theory furnish, as it seems to us, a satisfactory explanation of the progressive improvement in the figures for both inoculated and uninoculated, but it puts into our hands, as we think, a clue which may guide us in our search for the optimum dose of a vaccine for prophylactic use. In the case where we institute a comparative experiment—and we have the results of such a comparative experiment set out in *Table VII, Groups A* and *E—employing in* one group (let us call it *Group No. 1*) *a smaller*, and in another group (let us call it *Group No. 2*) *a larger dose ;* and where we then find that in *Group No. 1* the figures taken, when those for the uninoculated become equally good, are inferior to those afterwards reached ; and that in *Group No. 2* the figures, taken again when those for the uninoculated become equally good, are not afterwards improved upon—we may legitimately infer that in *Group No. 1* prophylactic inoculation left something undone which was afterwards achieved by self-immunisation ; and that in *Group No. 2* prophylactic inoculation accomplished, so far as appears, all that self-immunisation was capable of achieving.

It will be realised that this amounts to saying that *the optimum dose, or series of doses, of vaccine is that which produces an immunising response which is incapable of any further reinforcement ;* and that we ought to aim at effecting by prophylactic inoculation all that living in the presence of infection, and having actual dealings with infection, is capable of achieving.

(4) Comparative statistics furnish only a very incomplete account of the profit earned by any successful prophylactic inoculation. It has among statisticians been too much the habit to give credit only for the profit shown upon comparative statistics, and to disallow credit for maintained advantage unless proof is produced that the incidence- and death-rates of the disease have been, as the ordinary statistician would wish, maintained in the uninoculated section of the population.

Examples of this method of auditing profits are furnished by the fact that when, in connexion with small-pox vaccination, the statistical records for France, which is very incompletely, and Germany, which is very completely vaccinated, are contrasted ; or when, in connexion with typhoid inoculation, the condition in the Army in India before typhoid inoculation, is contrasted with its condition to-day, when the large bulk of the British troops are inoculated ; or when, in connexion with that same process, a comparison is made between the statistics for the Jacksonville Camp in the Spanish-American War, where out of a strength of nearly 11,000 U.S. troops, all being uninoculated, at least 1729 contracted typhoid, and the statistics for the San Antonio Camp on the infected Mexican frontier where nearly 13,000 U.S. troops, who were all inoculated, were massed with only one case of typhoid [1] ;

[1] Russell, United States Army Medical Department, Bulletin No. 2, January, 1913, p. 11.

the comparison is in each case disallowed on the ground that there were not sufficient uninoculated controls interspersed among the inoculated, or that the contrasted populations were not quite homologous, or that the conditions to which they were exposed were not exactly alike.

The statistical auditor, who refuses to give any credit upon vouchers which have been left in some point incomplete, is, of course, keeping himself well within the law. But he is leaving quite out of regard the equities.

When the question as to how we should evaluate the results of an effective inoculation process (and we may here deal specifically with pneumococcus inoculation) is regarded from this point of view—in other words, when we take a broad view of the probabilities—it will become clear that it would be altogether proper to claim credit:—*in connexion with the inoculated:* (*a*) for any diminished incidence- and any diminished death-rate from pneumonia which is attested by comparative statistics for inoculated and uninoculated; (*b*) for any diminished incidence- and death-rate from other pneumococcic infections (whether diagnosed as such, or undiagnosed) which comes upon the record; and (*c*) for any diminished incidence- and death-rate from infections which were not due to the pneumococcus, but were directly consequent upon pneumococcic infection.

Further, *in connexion with the uninoculated* section of the community, credit may equitably be claimed (*d*) for any diminution in the incidence-rate and the case-mortality of pneumococcic infections—for the former, because a reduction in the number of pneumococcic cases in the inoculated would diminish the general volume of infection; for the latter, because a reduction in the virulence of the infection might reasonably be expected from the diminished 'passaging' (i.e., transference from case to case) of the infective microbe.

It is to be noted that it is only the establishment of the general principle that we may anticipate profits under each of these headings, and that these profits cannot equitably be left out of account, which is here vital. The matter of real concern in connexion with any effective inoculation procedure is that by it we are transported out of a vicious circle—a *circulus vitiosus* of infection and non-resistance, into a 'propitious circle'—a *circulus felix* of increased resistance and diminished infection.

In comparison with the establishment of this, it is a small matter that, when fairly entered upon a propitious circle, it becomes impossible to tell how much of the sum-total of effects is due to inoculation, and how much to other causes.

With these preliminary explanations and reserves, we may here subjoin a further table which has reference to the mass-experiment on the Premier Mine.

TABLE XI.—*Showing for the whole Native Population of the Premier Mine the Incidence- and Death-rate for Pneumonia; the Incidence- and Death-rate for 'Other Diseases'; and also the number of Working Days lost through illness; for the months February to May,[1] in 1911, 1912, and 1913 respectively.*

	1911	1912	1913
Population (daily average strength)	10,426	12,549	15,284
Proportion of the population inoculated	0	About 50 per cent.	About 92 per cent.
Incidence-rate of pneumonia	4 per cent.	1·28 per cent.[2]	0·74 per cent.[2]
Death-rate from pneumonia	0·97 per cent.	0·31 per cent.	0·14 per cent.
Incidence-rate of other diseases	31 per cent.	20·7 per cent.	14·4 per cent.
Death-rate from other diseases	0·51 per cent.	0·38 per cent.	0·34 per cent.
Number of working days lost per hundred native labourers	275	177	131

[1] We have been furnished with data for this comparison only up to May, 1913.

[2] In 1912 the incidence rate was 0·86 per cent. for the inoculated and 1·7 per cent. for the uninoculated. In 1913 it was 0·6 per cent. for the inoculated and 3 per cent. for the controls.

APPENDIX

TABLE XII

Return showing the Incidence- and Death-rate from Pneumonia and other Medical Diseases on the Premier Mine for the period January 1st, 1908, to December 31st, 1913.

(To be read in association with the History of the Preventive Inoculations against Pneumonia on the Premier Mine, pp. 101–102 and 104–110 *supra*.)

Year	Daily average strength of native labourers	Incidence-rate per thousand		Death-rate per thousand		Case mortality per cent.	
		Pneumonia	Other medical diseases	Pneumonia	Other medical diseases	Pneumonia	Other medical diseases
1908	7,906	70·71	442·95	14·17	7·84	20·04	1·77
1909	7,790	104·88	603·21	17·97	5·26	17·14	0·87
1910	11,287	153·54	658·10	28·97	13·20	18·87	2·01
1911	10,800	128·98	888·15	26·94	13·80	20·89	1·55
1912	14,125	67·68	665·27	19·96	13·38	29·50	2·01
1913	14,172	31·75	480·74	4·80	8·82	15·11	1·83

Table XIII

Return showing the Death-rate and Case Mortality from Pneumonia and other Medical Diseases on the Premier Mine for the four months, January to April inclusive, during the years 1908 to 1914.

Year	Daily average strength	Death-rate per annum per thousand of population		Case mortality per cent	
		Pneumonia	Other medical diseases	Pneumonia	Other medical diseases
1908	7,856	12·22	11·07	34·41	4·01
1909	7,708	5·84	3·11	19·23	0·69
1910	11,915	19·64	12·34	18·66	2·24
1911	10,375	32·96	19·66	21·15	1·98
1912	11,257	6·93	10·39	16·76	1·42
1913	14,912	3·82	8·45	15·57	1·79
1914	13,201	2·27	15·00 [1]	15·63	3·41

[1] The increased sickness amongst the Natives (other Medical Diseases) in January, 1914, is accounted for by an epidemic of Typhoid, which broke out in the early part of the month.

SUR LA PRODUCTION DE SUBSTANCES BACTÉRICIDES NON SPÉCIFIQUES AU MOYEN DES VACCINS ANTISTAPHYLOCOCCIQUES ET ANTISTREPTOCOCCIQUES *IN VIVO* ET *IN VITRO*.

BY THE AUTHOR

(*Reprinted from 'Comptes Rendus, Académie des Sciences', 1918*)

Il a été établi, par les expériences déjà publiées de l'auteur, que les microbes de la plaie infectée se laissent classifier en *séro-saprophytes* et *sérophytes*. Les microbes appartenant à la première de ces catégories (et ce sont la grande majorité des espèces microbiennes), tout en trouvant dans les écoulements corrompus de la plaie un milieu de culture approprié, ne peuvent se cultiver dans les liquides sanguins normaux. Le sérum et la lymphe normaux exercent sur cette catégorie de microbes un effet empêchant ou bactéricide. Les microbes de la deuxième catégorie (et il s'agit ici en première ligne du staphylocoque et du streptocoque) se cultivent au contraire facilement dans le sérum normal. Au lieu d'exercer sur ces microbes un effet bactéricide, ou sensiblement empêchant, ce sérum constitue pour eux un excellent milieu nutritif et nous fournit pour leur culture un milieu électif précieux.

Il s'ensuit que, pour tuer le staphylocoque ou le streptocoque au moyen du sang normal, il n'y a d'autre ressource que de recourir aux leucocytes. Et l'on peut, en effet, en recourant à ces éléments, arriver à une destruction du staphylocoque et du streptocoque *in vitro*. Mais il y a pour cela des dispositions spéciales à prendre. Il est à conseiller d'employer des leucocytes émigrés et il est essentiel d'éloigner tout surplus de sérum qui fournirait pour ces microbes un milieu de culture favorable.

On peut, pour tuer les microbes *in vitro*, agir de deux différentes manières : on peut employer ou bien des leucocytes retenant entre eux un reste de sérum (la destruction des microbes se fait alors par la phagocytose), ou bien on peut éloigner les dernières traces de sérum (la destruction des microbes se fait alors avec la même intensité). Et dans ce cas il s'agit, suivant toute apparence, d'une libération de substances bactéricides non spécifiques venant des leucocytes vivants mis en rapport avec des microbes.

Tel est le point de départ des présentes études. Il s'agissait surtout de voir si l'on pourrait, par le moyen des vaccins staphylococciques et streptococciques, aboutir à une production de substances bactéricides non spécifiques.

C'est donc cette question que j'ai étudiée avec l'aide de mon élève, le capitaine L. Colebrook. Voici les méthodes que nous avons employées et les résultats les plus importants de ces expériences. Nous avons injecté nos vaccins à des lapins dans la veine de l'oreille. Préalablement, nous avions isolé une des veines jugulaires, fermant soigneusement tous les affluents latéraux et posant au-dessous de la veine des ligatures d'attente dans la partie supérieure et inférieure du cou. De cette façon, nous étions à même de serrer les nœuds sur la veine après l'injection du vaccin et d'isoler une portion de sang mélangé au vaccin dans l'intérieur du corps dans un

récipient à parois imperméables. Nous avons en même temps, avec toutes précautions aseptiques, introduit aux flancs de l'animal, dans le tissu sousentané, des morceaux de lint stérilisé destinés à recueillir le suc des tissus. Ceci fait, nous faisions une petite prise de sang dans la veine de l'oreille et, immédiatement après, l'injection du vaccin dans la veine du côté opposé. Celle-ci était suivie à peu près directement d'une seconde prise de sang et, aussitôt après, on opérait la clôture de la jugulaire.

D'autres petites prises du sang circulant ont été faites de demi-heure en demi-heure et, au moment de la sixième prise, 2 heures et demie après l'injection du vaccin, nous avons retiré le sang qui était resté isolé dans la jugulaire et en même temps un des fragments du lint placé sous la peau. Les autres ont été retirés ensuite, un à un et de jour en jour, pour en exprimer le suc.

Voici compendieusement les résultats de ces expériences. Déjà presque aussitôt après l'injection d'une dose appropriée de vaccin (il s'agit ici d'une dose bactérienne à plusieurs millions de staphylocoques morts), un pouvoir bactéricide non spécifique commence à se manifester dans le sérum, et ce pouvoir va en augmentant au moins pendant les premières heures après l'injection du vaccin. Ce pouvoir se manifeste non seulement dans le sang circulant, mais aussi et d'une façon beaucoup plus intense dans le sang isolé dans la jugulaire.

Prenons comme nouveau point de départ, pour nos raisonnements et nos expériences ultérieures, ce fait de la production d'une substance bactéricide non spécifique dans la veine contenant un sang isolé additionné d'un vaccin staphylococcique ou streptococcique. Cette production serait évidemment à rapporter ou bien à l'endothélium ou bien au sang même. Il est clair que c'est à ce dernier que nous avons dû provisoirement la rapporter. De sorte que nous étions évidemment contraints de répéter sur le sang *in vitro* les expériences réussies *in vivo* et, d'autre part, de répéter *in vivo* toutes expériences supplémentaires qui réussiraient sur le sang *in vitro*.

Nous avons tout d'abord repris les expériences ci-dessus décrites sur le lapin en y apportant seulement la légère modification de prélever à chaque prise de sang, au lieu d'un seul, deux échantillons dont l'un des deux resterait à froid pendant que l'autre serait mis à l'étuve. Il ressortit des comparaisons instituées entre ces deux séries d'échantillons qu'il se produisait à l'étuve une élaboration de substances bactéricides à peu près comparable à celle qui se produisait dans le sang isolé dans la jugulaire.

Ces données obtenues, il ne manquait que l'expérience cruciale, à savoir : de mélanger le vaccin directement *in vitro* au sang retiré de l'organisme.

Je présente ici en forme de Tableaux des échantillons des résultats obtenus dans ces expériences.

Expérience I

Sang de l'auteur mélangé *in vitro* dans la proportion de 9^{vol} de sang d'une part à 1^{vol} d'eau physiologique et, d'autre part, à 1^{vol} de vaccin staphylococcique plus ou moins riche en corps microbiens.

Après 2 heures et demie à l'étuve, le sérum de chaque portion de sang est

éprouvé en l'ensemençant avec des dilutions progressives d'un mélange de staphylocoques et streptocoques. 12 à 14 heures après, contrôle microscopique et cultural du résultat.

TABLEAU I.—*Dilutions progressives du mélange bactérien ensemencé*

		1	2	3	4	5	6	7	8	9	10	11	12	13
A.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	0	0
	Str	Str	Str	Str	0	0	0	0	0	0	0	0	0	0
B.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0	0	0
	Str	Str	Str	Str	0	0	0	0	0	0	0	0	0	0
C.	Sta	Sta	Sta	0	0	0	0	0	0	0	0	0	0	0
	Str	Str	Str	Str	Str	0	0	0	0	0	0	0	0	0
D.	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0	0	0	0	0
	Str	Str	Str	Str	0	0	0	0	0	0	0	0	0	0

A = Sérum du sang de contrôle.
B = Sérum du sang traité par 10,000 staphylocoques morts par centimètre cube.
C = Sérum du sang traité par 3300 staphylocoques morts par centimètre cube.
D = Sérum du sang traité par 1000 staphylocoques morts par centimètre cube.

Expérience II

Sang de l'auteur mélangé *in vitro* à un vaccin streptococcique plus ou moins riche en corps microbiens.

Mêmes dispositions et même épreuve. 18 heures à l'étuve.

TABLEAU II.—*Dilutions progressives du mélange bactérien ensemencé*

		1	2	3	4	5	6	7	8	9	10	11	12
A.	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0	0	0
	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str
B.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0
	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str
C.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	0	0	0	0
	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str
D.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0
	Str	Str	Str	Str	Str	Str	Str	0	0	0	0	0
E.	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0	0	0
	Str	Str	Str	0	0	0	0	0	0	0	0	0

A et B = Sérum du sang de contrôle (A, portion I ; B, portion II).
C = Sérum du sang traité par 500 streptocoques morts par centimètre cube.
D = Sérum du sang traité par 60 streptocoques morts par centimètre cube.
E = Sérum du sang traité par 30 streptocoques morts par centimètre cube.

Expérience III

Inoculation du sang de l'auteur *in vitro* avec un dixième d'un vaccin staphylococcique contenant 25,000 staphylocoques par centimètre cube.

Mêmes dispositions et même épreuve. 3 heures à l'étuve.

TABLEAU III.—*Dilutions progressives du mélange bactérien ensemencé*

	1	2	3	4	5	6	7	8	9	10	11	12
A.	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0	0	0
	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	0	0
B.	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0	0	0
	Str	Str	Str	Str	Str	0	0	0	0	0	0	0
C.	Sta	Sta	Sta	Sta	0	0	0	0	0	0	0	0
	Str	Str	0	0	0	0	0	0	0	0	0	0

A = Sérum du sang de contrôle.
B et C = Sérum du sang immunisé (B, portion I ; C, portion II).

Expérience IV

Inoculation du sang de l'auteur *in vitro* avec un dixième d'un vaccin staphylococcique contenant 40,000 staphylocoques par centimètre cube.

Mêmes dispositions et même épreuve. 2 heures et demie à l'étuve.

TABLEAU IV.—*Dilutions progressives du mélange bactérien ensemencé*

	1	2	3	4	5	6	7	8	9	10	11	12
A.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta
	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	0
B.	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0	0	0
	Str	Str	Str	Str	Str	0	0	0	0	0	0	0
C.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0
	Str	Str	Str	Str	Str	Str	Str	0	0	0	0	0

A = Sérum du sang de contrôle.
B et C = Sérum du sang immunisé (B, portion I ; C, portion II).

Expérience V

Inoculation *in vitro* du sang d'un homme normal qui devait, le jour suivant, fournir son sang pour la transfusion d'un blessé avec grave infection streptococcique.

Mêmes dispositions et même épreuve. 3 heures à l'étuve.

TABLEAU V.—*Dilutions progressives du mélange bactérien ensemencé*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	0	0
	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	0
B.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	0
	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str	Str
C.	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	Sta	0	0	0	0	0
	Str	Str	Str	Str	Str	Str	0	0	0	0	0	0	0	0

A et B = Sérum du sang de contrôle (A, portion I; B, portion II).
C = Sérum du sang traité par 1000 staphylocoques par centimètre cube.

Il ressort très clairement de ces expériences qu'on peut, avec une addition directe de vaccin au sang extravasculaire, arriver à une production de substances bactéricides non spécifiques. Il ressort aussi que cette production dépend en première ligne de la dose de vaccin employée. Il se voit, par exemple, dans le Tableau II que nous avons, avec une addition de 30 streptocoques par centimètre cube de sang, une réaction fructueuse ; avec 60 une réaction un peu moins fructueuse et avec 500 streptocoques par centimètre cube de sang, à ce qu'il paraît, au lieu d'une réaction immunisante, une phase négative. On aboutit au même avec des doses excessives du vaccin staphylococcique. Les résultats quant au dosage sont influencés aussi par la durée de l'incubation.

Tous ces phénomènes étudiés *in vitro* se retrouvent *in vivo*. Quant au dosage dans les inoculations *in vivo* (il n'est question ici que des inoculations interveineuses) nous avons placé des nœuds ouverts sur les deux jugulaires, et alors fait deux inoculations successives de vaccin dans la veine de l'oreille. Après la première injection ne comportant qu'une très petite dose nous avons serré les nœuds sur la jugulaire du côté opposé, et après la deuxième injection fait de même sur la jugulaire correspondant à la veine auriculaire réceptrice. De cette façon nous étions à même de faire la comparaison entre l'effet produit sur le sang *in vivo* par la dose de choix et l'effet produit par une dose excessive. Les résultats étaient tout à fait pareils à ceux obtenus dans les prises de sang correspondantes placées à l'étuve et, d'autre part, à ceux obtenus par l'addition directe du vaccin *in vitro*.

Quant à la sérosité qui imprègne les fragments de lint placés sous la peau, elle possédait, après 24 et 48 heures, un pouvoir bactéricide non spécifique supérieur à tout ce que nous avons observé pour le sang. Alors que le deuxième jour la propriété bactéricide avait disparu du sang, elle n'était pas diminuée dans la sérosité retirée avec les morceaux de lint.

Il semble tout indiqué d'essayer, sur les malades infectés, la transfusion non pas avec du sang ordinaire, mais avec du sang auquel on aurait conféré le pouvoir bactéricide par le contact préalable avec une dose convenable de vaccin.

Au lieu d'injecter dans un but soit préventif, soit curatif, une dose arbitraire de vaccin, on doit rechercher s'il ne conviendrait pas de déterminer au préalable, par une expérience *in vitro*, quelle est la quantité de vaccin qui, mélangée à une quantité donnée de sang, produit le plus haut pouvoir bactéricide.

Quand il s'agit d'obtenir un pouvoir bactéricide non spécifique, il n'est pas indispensable d'employer le microbe causant l'infection ; un vaccin qui donnerait un pouvoir bactéricide supérieur serait préférable (Tableau V).

L'apparition du pouvoir bactéricide dans un sang contenant des microbes et placé à l'étuve, pourrait expliquer l'échec des hémocultures, même quand il s'agit de bactéries sérophytes.

A LECTURE ON THE LESSONS OF THE WAR AND ON SOME NEW PROSPECTS IN THE FIELD OF THERAPEUTIC IMMUNISATION.

BY THE AUTHOR, EMBODYING RESEARCH WORK DONE IN CONJUNCTION WITH DR. LEONARD COLEBROOK

(Excerpt from a Lecture delivered before the Royal Society of Medicine, 25th February, 1919) [1]

(Reprinted from the 'Lancet', 29th March, 1919)

Anti-microbic Powers of Serum.

You have by this time very clearly appreciated where lies the weak point in our defence against micro-organisms. The weak point in our armour lies in this that the normal blood fluids provide a culture medium in which serophytic microbes can grow and multiply.

Is Nature then—so we asked ourselves—incompetent to strengthen that weak place in her defences? It does not come into consideration here that an increase in the opsonic power of the blood is obtained in response to inoculations and auto-inoculations of staphylococcus and streptococcus. For that could be useful only in the case of the leucocytes having access to the microbes. But it does come under consideration that the body responds to all wound infections (I drew attention to this early in the war), to many other infections, and also, so far as we know, to inoculations, by the development of increased anti-tryptic power in the serum. That renders the serum a less favourable culture medium for serophytes. It remained, however, to be determined whether the blood fluids ever acquire a positive power of killing serophytic microbes. If such power is ever acquired we should expect to find it in patients suffering from septic infection with severe auto-inoculations.

We accordingly examined the serum of such patients, employing the wash and after-wash implantation into serum which I have already described [2] in connexion with making cultures from pus.

Here I employed as my implanting fluid instead of pus a 20-fold diluted 24-hour broth culture of streptococcus to which was added what I call $\frac{1}{4}$ wash of one day old broth culture of staphylococcus. The serial cultures obtained from such plantings into the sera of septic patients were, after remaining over-night in the incubator, examined both microscopically and by subculturing on agar. There was

[1] *The first part of this Lecture which had to do with the Treatment of War Wounds was reprinted in Vol. I of these 'Researches', pp.* 113–128. *The second part dealing with Immunising Reactions was reserved for republication here.*

[2] *Vide* these *Collected Researches*, Vol. I, pp. 38 and 133.

obtained in this way evidence that the serum in septic infections does acquire a power of killing serophytic organisms.

The bactericidal effect in question affects the staphylococcus and the streptococcus without distinction.

You appreciate what a large issue this last-mentioned fact opens up. It brings up the issue of non-specific immunisation.

To make any further advance it was obviously incumbent to inoculate animals. We chose rabbits for our experiments and we planned to test the results of inoculation upon samples of the circulating blood ; on blood isolated in a vein, and also on samples of lymph obtained from the subcutaneous tissues—these samples being taken before, and at intervals after, the intravenous inoculation of vaccine.

In order to obtain blood isolated in the vein immediately after the injection of the vaccine, we cut down in advance upon the jugular vein, tied off all its tributaries, and then passed ligatures under the upper and lower ends of the vein, so as to be able to tie off the enclosed blood as soon as the vaccine, which was inserted in another vein, had been carried round by the circulation.

To collect lymph from the subcutaneous tissue we introduced pieces of lint into it aseptically and when these had remained in position for the desired period we removed them, and pressed out the contained fluid, aseptically. The pieces of lint were inserted either sometime before the inoculation was given, or immediately after, or later—according to the particular stage at which we wanted to begin the collection of lymph.

The animal received in each case either a dose of staphylococcus or streptococcus vaccine ; and the procedures employed in testing the bactericidal power of sera or lymphs were identical with those employed in testing the sera of the septicaemic patients.

Bactericidal Powers of Serum and Lymph after Vaccination.

The results obtained in an experiment of this kind conducted with a dose of staphylococcus vaccine containing $2\frac{1}{2}$ million microbes are shown in the drawing (Fig. 1). We have here agar slopes divided up horizontally into compartments and implanted, in each case proceeding from above downwards, with material from a series of wash and after-wash sero- or lymph-cultures made in a capillary pipette.

On the *left-hand side of the figure* we have cultures made from (A) sero- and (B) lymph-cultures, the serum and lymph in question having been obtained from the animal immediately before intravenous injection of the vaccine. We have here in each case a growth of both streptococcus and staphylococcus in the whole 13 compartments of the agar—these corresponding to the 13 successive wash and after-wash volumes of the serum and lymph.

In the *centre of the figure* we have cultures derived from samples of serum and lymph procured two hours after inoculation and implanted with the same mixture of microbes used for implanting the first serum and lymph. We have at C a tube implanted with the series of sero-cultures from the circulating blood showing growth only in 7 out of the 13 compartments corresponding to growth in 7 out of 13 successive wash and after-wash samples of serum and lymph ; and at D a tube implanted

with the sero-cultures from the blood isolated in the jugular vein, with growth only in four compartments ; and at E a tube implanted with a series of lymph cultures from the subcutaneous tissue with growth in all 13 compartments.

The result obtained in tube C shows that the serum of the circulating blood has developed bactericidal properties.[1]

Tube D shows that the bactericidal properties are developed also in the serum of the blood which was tied off in the vein.

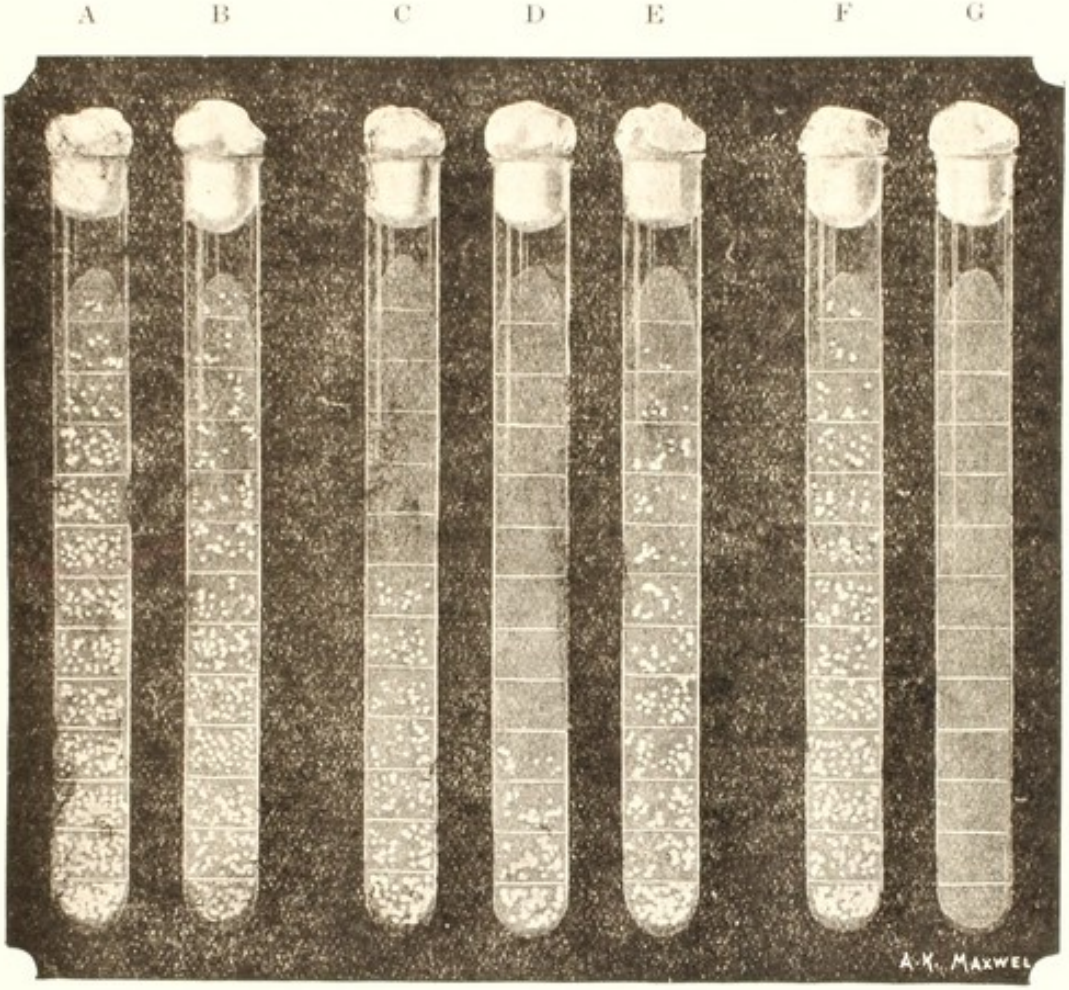


Fig. 1.

Sero- and lymph-cultures from a rabbit inoculated with staphylococcus. Description in text.

The results obtained in tube E show that bactericidal properties have not been developed in the lymph of the subcutaneous tissue, and have not passed out into this from the blood.

On the *right of the figure* are the cultural results obtained with samples of serum from the circulating blood and lymph from the subcutaneous tissues taken 48 hours afterwards and implanted with a mixture of microbes similar to that employed before. It will be seen that the circulating blood has, so far as can be judged,

[1] The blood of the rabbit here in question had begun to manifest bactericidal power already within two minutes after the injection of the vaccine.

returned to its original condition. In other words we have a culture of staphylococcus and streptococcus in all the 13 compartments of the agar.

The subcutaneous lymph, on the other hand, has killed all the implanted microbes.

The results of these experiments may be summarised as follows :[1]

(1) Bactericidins were, in the animal which was inoculated with staphylococcus vaccine, fabricated by, or set free from, the leucocytes in the circulating blood ; while none was formed in the subcutaneous tissue.

(2) Bactericidins (in apparently even larger quantities) were developed in the blood which was isolated in the vein. (This of course is inconsistent with Aschoff's theory of the derivation of antigenic substances.)

(3) Staphylococcus vaccine, instead of exerting upon the animal a specific bactericidal effect on the staphylococcus, made the animal's serum and the lymph bactericidal to both staphylococcus and streptococcus.

(4) Further points of interest are (*a*) that the bactericidins of the blood-fluids do not immediately pass out into the lymph, and (*b*) that when these substances have appeared there they are still to be found there after they have disappeared from the circulating blood.

I want before going further to bring out two further points.

The first is that we are not yet sufficiently masters of the conditions to obtain with constancy results like those here shown. In particular, when we employ two large doses of vaccine we get instead of an epi-phylactic an apo-phylactic result.

The second point is that my fellow-worker, Dr. Leonard Colebrook, has made a series of experiments in which there was inserted into the subcutaneous tissue on the one side of the rabbits lint impregnated with bacterial vaccines, and on the other side, as a control, lint moistened with normal salt solution—the pieces of lint being left in position for several days. Here the fluid which was expressed from the control lint was found to have as much bactericidal power as the impregnated lint —this result being no doubt due to the fact that both pieces of lint were infiltrated with leucocytes which set free bactericidins.

Immunisation *in vitro*.

Let me before I pass on to deal with this explain to you the lines of thought which led us to look for such immunisation *in vitro*. The results obtained with blood confined in the jugular vein immediately invited us to ask : Seeing that bactericidal power is developed in blood which is after inoculation isolated and incubated *in vivo*, should not the same result be obtained when after inoculation we withdraw blood and incubate *in vitro* ? And if it should prove that bactericidal power is also under these conditions obtained, should it not also be obtained when we work entirely *in vitro* ?

In both these directions expectation has been borne out by experiment. When we have inoculated *in vivo* we get our result whether we confine the blood in the jugular and incubate *in vivo* ; or draw off a sample of blood and incubate it *in vitro*. And again we get our result just as well when we draw off the blood and then vac-

[2] The paragraphs which here immediately follow have been rewritten.

cinate and incubate *in vitro*. That is, we get when we employ large doses of vaccine an apophylactic change which renders the blood fluids a better cultivation medium for serophytes; or we get, but less regularly (for all the factors are not known to us), an epi-phylactic change in the form of a development of bactericidal power in the serum.

The experiments which are here tabulated (those cited are, of course, selected experiments) supply exemplification of apo-phylactic and epi-phylactic changes—i.e., positive and negative phases produced by the inoculation of vaccines in blood *in vitro*.

The technique employed was as follows. A series of dilutions of the vaccine were made in physiological salt solution. Blood was then obtained [1] by a puncture made into a sterilised finger, or it was drawn off from a vein. In the former case it was collected on a paraffin-coated slide. Then nine volumes of blood and one volume of the first dilution of vaccine were mixed and aspirated into a pipette and sealed up. And so on throughout the series until finally (for the purposes of control) 9 volumes of blood were mixed with 1 volume of physiological salt solution. The bloods were then placed in the incubator for periods of 2 to 3 hours (Experiments 1, 3, 4, and 6), or 6 hours (Experiment 9), or 18 to 24 hours (Experiments 2, 5, 7, 8, 10, 11, and 12). They were then centrifuged and the supernatant serum drawn off. This was implanted by the wash and after wash method with a dilution of a staphylococcus culture; or with a mixture of staphylococcus and streptococcus (a 16–24-fold dilution of a 24-hours broth culture of streptococcus with $\frac{1}{4}$–$\frac{1}{2}$ wash of staphylococcus); or in one instance with a mixture of staphylococcus and pneumococcus. The capillary pipettes containing the implantations into serum were then incubated overnight, and the contents blown out in separate drops on to sterilised slides. Preparations were then made for microscopic examination, and subcultures were made either upon agar slants divided up as shown in Fig. 1, or upon agar poured into Petri dishes divided up after the manner of a dial (Figs. 2 and 3).

Non-specific Immunisation.

Let us just glance at the prospects which are here opened up.

In the foreground stands the question of non-specific immunisation. That immunisation is always strictly specific counts as an article of faith; and it passes as axiomatic that microbic infections can be warded off only by working with homologous vaccines; and that we must in every case before employing a vaccine therapeutically, make sure that the patient is harbouring the corresponding microbes.

I confess to having shared the conviction that immunisation is always strictly specific. Twenty years ago, when it was alleged, by Haffkine, before the Indian Plague Commission, that antiplague inoculation had cured eczema, gonorrhoea, and other miscellaneous infections, I—as a member of that Commission—thought the matter undeserving of examination. I took the same view when it was reported to me in connexion with antityphoid inoculation that it rendered the patients much less susceptible to malaria. Again, seven years ago, when applying pneumococcus

[1] In Experiments 1 to 8 the blood was obtained from A. E. W.; in Experiments 9 to 11 from other laboratory workers, and in Experiment 12 from a patient.

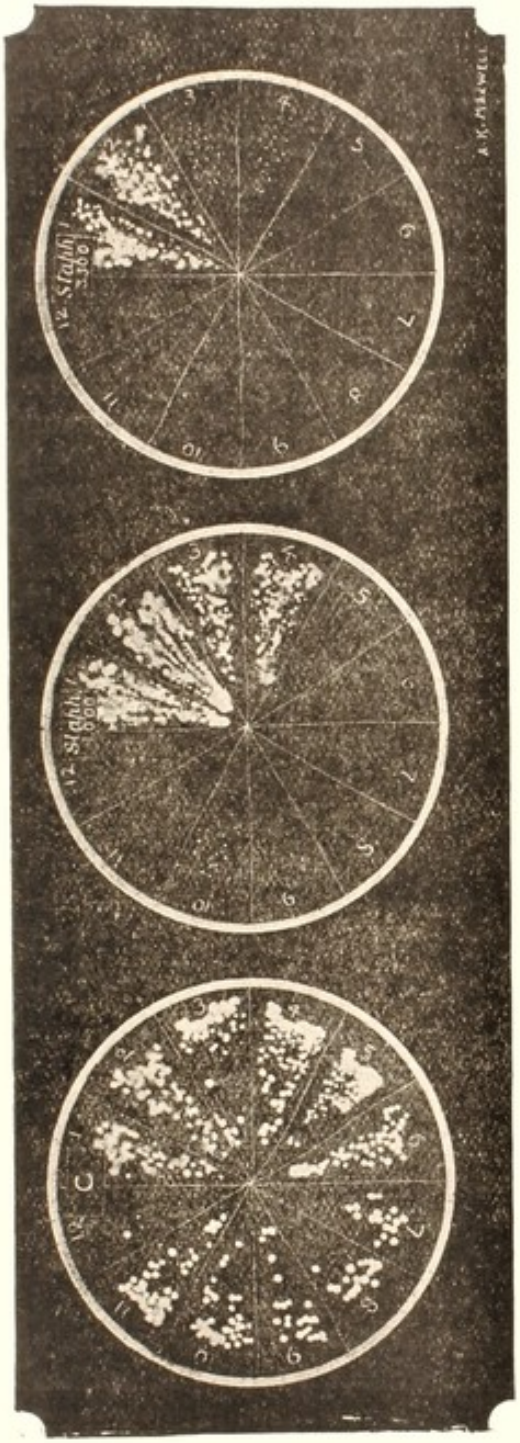


FIG. 2.—STAPHYLOCOCCUS INOCULATION OF BLOOD *in vitro*: Experiment 1 in the Table.—On the *left* hand are the results obtained with the serum of the control blood; in the *middle* those obtained with the serum of the blood inoculated *in vitro* with 1000 staphylococci per c.cm.; and on the *right* those obtained with the serum of the blood inoculated with 3300 staphylococci per c.cm.

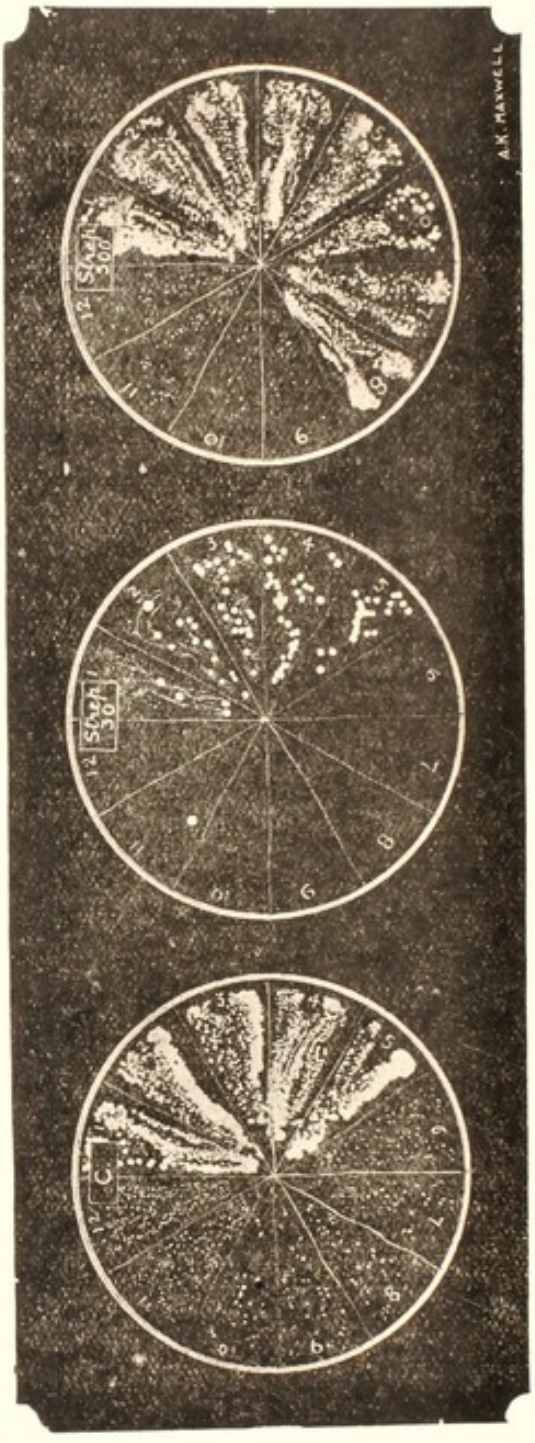


FIG. 3.—STREPTOCOCCUS INOCULATION OF BLOOD *in vitro*: Experiment 2 in the Table.—On the *left* are the results obtained with the serum of the control blood; in the *middle* those obtained with the serum of a blood inoculated *in vitro* with 30 streptococci per c.cm.; on the *right* those obtained with the serum of blood inoculated with an overdose of vaccine—500 streptococci per c.cm.

Table of Wash and After-wash Experiments showing that the Serum Acquires Non-specific Bactericidal Powers when the Blood is Inoculated in vitro *with a Variety of Vaccines.*

Experiment I. (*vide Fig. 2*)

Cont. Bl. = Control Blood. Vac. Bl. = Vaccinated Blood.

Serum derived from	Number of microbes added per c.cm. of blood to evoke epiphylactic response	Microbes employed for testing the serum implantation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cont. Bl.	—	Staphylococcus (A) and streptococcus (E)	A	A	A	A	A	A	A	A	A	A	A	0	0	0	—	—
			E	E	E	0	0	0	0	0	0	0	0	0	0	0	—	—
,,	—		—	—	—	—	A	A	A	A	A	A	A	0	0	0	—	—
			—	—	—	—	0	0	0	0	0	0	0	0	0	0	—	—
Vac. Bl.	10,000 staph.		A	A	A	A	A	A	0	0	0	0	0	0	0	0	—	—
			E	E	E	0	0	0	0	0	0	0	0	0	0	0	—	—
,,	5,000 staph.		A	A	A	A	A	A	0	0	0	0	0	0	0	0	—	—
			E	E	E	0	E	0	0	0	0	0	0	0	0	0	—	—
,,	3,300 staph.		A	A	0	0	0	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	0	0	0	0	0	0	0	0	0	0	—	—
,,	1,000 staph.		A	A	A	A	0	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	0	0	0	0	0	0	0	0	0	0	0	—	—

Experiment II. (*vide Fig. 3*)

Cont. Bl.	—	Staphylococcus (A) and streptococcus (E)	A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	E	E	E	E	E	—	—
,,	—		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	E	E	E	E	E	—	—
Vac. Bl.	500 strep.		A	A	A	A	A	A	A	A	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	E	E	E	E	E	—	—
,,	60 strep.		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	0	0	0	0	0	0	0	—	—
,,	30 strep.		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	0	0	0	0	0	0	0	0	0	0	0	—	—

Experiment III

Cont. Bl.	—	Staphylococcus (A) and streptococcus (E)	A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	E	0	0	0	0	—	—
Vac. Bl.	2,500 staph.		A	A	A	A	A	A	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	0	0	0	0	0	0	0	0	—	—
,,	2,500 staph.		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	0	0	0	0	0	0	0	0	0	0	0	—	—

Experiment IV

Cont. Bl.	—	Staphylococcus (A) and streptococcus (E)	A	A	A	A	A	A	A	A	A	A	A	A	0	0	—	—
			E	E	E	E	E	E	E	E	E	E	E	0	0	0	—	—
Vac. Bl.	4,000 staph.		A	A	A	A	A	A	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	0	0	0	0	0	0	0	0	—	—
,,	4,000 staph.		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	0	0	0	0	0	0	0	—	—

Experiment V

Cont. Bl. = Control Blood. Vac. Bl. = Vaccinated Blood.

Serum derived from—	Number of microbes added per c.cm. of blood to evoke epiphylactic response	Microbes employed for testing the serum implantation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cont. Bl.	—	Staphylococcus (A) and streptococcus (E)	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	0 E	0 E
,,	—		A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	0 E	0 —
Vac. Bl.	500 staph.		A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	0 0	0 0
,,	1,000 staph.		A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A 0	A 0	A 0	A 0	0 0	0 0
,,	2,000 staph.		A E	A E	A E	A E	A E	A E	A E	A E	A E	0 E	0 E	0 0	0 0	0 0	0 0	0 0
,,	3,300 staph.		0 E	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
,,	5,000 staph.		A E	A E	A E	A E	A E	A E	0 E	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
,,	10,000 staph.		A E	A E	0 E	0 E	A E	A E	A E	0 E	0 E	0 E	0 0	0 0	0 0	0 0	0 0	0 0
,,	100,000 staph.		A E	A E	A E	A E	A E	A E	A E	A E	A E	0 E	0 E	0 E	0 E	0 0	0 0	0 0

Experiment VI

Serum derived from—	Number of microbes added per c.cm. of blood to evoke epiphylactic response	Microbes employed for testing the serum implantation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cont. Bl.	—	Staphylococcus (A) and streptococcus (E)	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	0 E	0 0	— —	— —
,,	—		A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	A E	0 E	— —	— —
Vac. Bl.	1,000 staph.		A E	A E	A E	A E	A E	A E	A 0	A 0	0 0	0 0	0 0	0 0	0 0	0 0	— —	— —
,,	5,000 staph.		A E	A E	A E	A E	A E	A E	0 E	0 E	0 E	0 0	0 0	0 E	0 E	0 0	— —	— —

Experiment VII

Serum derived from—	Number of microbes added per c.cm. of blood to evoke epiphylactic response	Microbes employed for testing the serum implantation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cont. Bl.	—	Staphylococcus (A) and pneumococcus (P)	— —	— —	A P	A P	A P	A P	0 P	0 P	0 P	0 P	0 P	0 P	0 P	0 P	— —	— —
,,	—		— —	— —	A P	A P	0 P	0 P	0 P	0 P	0 P	0 P	0 P	0 P	0 P	0 P	— —	— —
Vac. Bl.	50 pneum.		A P	A P	A P	A P	A P	A P	0 0	0 P	0 P	0 P	0 P	0 P	0 P	0 P	— —	— —
,,	100 pneum.		A P	A P	A P	A P	A P	A P	0 P	0 P	0 P	0 P	0 0	0 0	0 0	0 0	— —	— —
,,	500 pneum.		A P	A P	A P	A P	0 P	0 P	0 P	0 0	0 0	0 0	0 0	0 0	0 0	0 0	— —	— —
,,	8,000 pneum.		A P	A P	A P	0 P	0 P	0 P	0 0	0 P	0 0	0 0	0 0	0 0	0 0	0 0	— —	— —
,,	10,000 pneum.		A P	A P	A P	A P	A P	A P	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	— —	— —

Experiment VIII [1]

Cont. Bl. = Control Blood. Vac. Bl. = Vaccinated Blood.

Serum derived from	Volume of vaccine added per 1 c.cm. of blood to evoke epiphylactic response	Microbes employed for testing the serum implantation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cont. Bl.	—	Staphylococcus (A) and Streptococcus (E)	A	A	A	A	A	A	A	A	A	A	A	A	A	A	—	—
			E	E	E	E	E	E	E	E	E	E	E	E	E	E	—	—
,,	—		A	A	A	A	A	A	A	A	A	A	A	A	A	0	—	—
			E	E	E	E	E	E	E	0	0	0	0	0	0	0	—	—
,,	—		A	A	A	A	A	A	A	A	A	A	A	0	0	0	—	—
			E	E	E	E	E	E	E	E	0	0	E	E	E	E	—	—
Vac. Bl.	$\frac{1}{2000000}$th vol. of influenza vaccine		A	A	A	A	A	A	A	A	A	A	A	0	A	0	—	—
			E	E	E	E	E	E	E	E	0	0	0	E	E	0	—	—
,,	$\frac{1}{1000000}$th vol. of influenza vaccine		A	A	A	A	A	A	A	A	A	0	0	0	0	0	—	—
			E	E	E	E	E	E	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{500000}$th vol. of influenza vaccine		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{300000}$th vol. of influenza vaccine		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	0	0	0	0	0	0	0	0	0	0
,,	$\frac{1}{100000}$th vol. of influenza vaccine		A	A	A	A	0	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	0	0	0	0	0	0	0	0	—	—
Vac. Bl.	$\frac{1}{2500000}$th vol. of mixed cold vaccine	Staphylococcus (A) and Streptococcus (E)	A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	0	0	0	0	0	0	—	—
,,	$\frac{1}{250000}$th vol. of mixed cold vaccine		A	0	0	0	0	0	0	0	0	0	0	0	0	0	—	—
			E	E	0	0	0	0	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{50000}$th vol. of mixed cold vaccine		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{25000}$th vol. of mixed cold vaccine		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	E	E	0	0	0	—	—
,,	$\frac{1}{10000}$th vol. of mixed cold vaccine		A	A	A	A	A	A	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	E	E	E	E	E	—	—

[1] The 'influenza vaccine' here employed was that made up to the 'Army formula'. The 'mixed cold vaccine' was that prepared at the Inoculation Department of St. Mary's Hospital.

The formulae of the vaccines were as follows:—*Influenza vaccine*: Pneumococcus, 200; streptococcus, 80; Pfeiffer's bacillus, 60 millions per c.cm. *Mixed cold vaccine*: Pneumococcus, 30; streptococcus, 12; Pfeiffer's bacillus, 300; staphylococcus, 300; Friedländer's bacillus, 30; bacillus septus, 50; micrococcus catarrhalis, 30 millions per c.cm.

Experiment IX

Cont. Bl. = Control Blood. Vac. Bl. = Vaccinated Blood.

Serum derived from—	Number of microbes added per c.cm. of blood to evoke epiphylactic response	Microbes employed for testing the serum implantation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cont. Bl.	—	Staphylococcus (A) and streptococcus (E)	A	A	A	A	A	A	A	A	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	0	0	0	0	0	—	—
,,	—		A	A	A	A	A	A	A	A	0	A	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	0	0	0	0	0	—	—
Vac. Bl.	5,000 typhoid bacilli		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	0	0	0	0	0	0	0	0	0	—	—
,,	25,000 typhoid bacilli		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	0	0	0	0	0	0	0	0	0	—	—
,,	50,000 typhoid bacilli		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	0	0	0	0	0	0	0	0	—	—
,,	100,000 typhoid bacilli		A	A	A	A	A	A	0	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	0	E	0	0	0	0	0	0	—	—
,,	200,000 typhoid bacilli		A	A	A	A	A	A	A	A	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	0	0	0	0	0	0	0	—	—

Experiment X

Cont. Bl.	—	Staphylococcus (A) and streptococcus (E)	A	A	A	A	A	A	A	A	A	A	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	0	0	0	0	0	—	—
,,	—		A	A	A	A	A	A	A	A	A	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	0	0	0	0	0	0	—	—
Vac. Bl.	5,000 typhoid bacilli		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	0	0	0	0	0	0	0	—	—
,,	25,000 typhoid bacilli		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	0	0	0	0	0	0	0	—	—
,,	50,000 typhoid bacilli		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	0	0	0	0	0	0	0	—	—
,,	100,000 typhoid bacilli		A	A	A	A	A	A	A	A	0	0	0	0	0	0	—	—
			E	E	E	E	E	E	E	E	E	E	0	0	0	0	—	—
,,	200,000 typhoid bacilli		A	A	A	A	A	A	A	A	A	0	A	A	A	A	—	—
			E	E	E	E	E	E	E	E	E	E	E	E	E	E	—	—

Experiment XI [1]

Cont. Bl.	—	Staphylococcus (A)	A	A	A	A	A	A	A	A	A	A	A	A	A	A	—	—
Vac. Bl.	1,000,000 gonococci		A	A	A	A	A	A	A	A	A	0	0	0	0	0	—	—
,,	50,000 gonococci		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
,,	5,000 gonococci		A	A	A	A	A	A	A	A	A	A	0	0	0	0	—	—
,,	500 gonococci		A	A	A	A	A	A	A	A	A	A	A	0	0	0	—	—
,,	50 gonococci		A	A	A	A	A	A	A	A	A	0	0	0	0	0	—	—
,,	5 gonococci		A	A	A	A	A	A	A	A	A	A	0	0	0	0	—	—

[1] I am indebted to my fellow-worker, Lieutenant-Colonel F. J. Clemenger, U.S.M.S., for this experiment.

Experiment XII [1]

Cont. Bl. = Control Blood. Vac. Bl. = Vaccinated Blood.

Serum derived from—	Weight of bacterial substance added per c.cm. of blood to evoke epiphylactic response	Microbes employed for testing the serum implantation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cont. Bl.	—	Staphylococcus (A)	A	A	A	A	A	A	A	A	A	A	A	A	0	0	—	—
,,	—		A	A	A	A	A	A	A	A	A	A	A	A	0	0	—	—
Vac. Bl.	$\frac{1}{10000000}$th mg. tuberculin B.E.		A	A	A	A	0	0	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{20000000}$th mg. tuberculin B.E.		A	A	A	A	0	0	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{30000000}$th mg. tuberculin B.E.		A	A	A	A	0	0	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{40000000}$th mg. tuberculin B.E.		A	A	0	0	0	0	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{50000000}$th mg. tuberculin B.E.		A	A	A	A	A	A	A	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{60000000}$th mg. tuberculin B.E.		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{70000000}$th mg. tuberculin B.E.		A	A	A	A	A	A	A	A	A	A	A	A	0	0	—	—
,,	$\frac{1}{80000000}$th mg. tuberculin B.E.		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—
,,	$\frac{1}{90000000}$th mg. tuberculin B.E.		A	A	A	A	A	A	A	A	0	0	0	0	0	0	—	—
,,	$\frac{1}{100000000}$th mg. tuberculin B.E.		A	A	A	A	A	0	0	0	0	0	0	0	0	0	—	—

[1] I am indebted to Colonel Clemenger for this experiment.

inoculations as a preventive against pneumonia in the Transvaal mines, I nourished exactly the same prejudices.

But as the critical review of the statistical results obtained in the Premier Mine[1] by pneumococcus inoculation has shown, the pneumococcus inoculations there undertaken not only brought down the mortality from pneumonia by 83 per cent. (Table XII), but also reduced the mortality from 'other diseases' by 34 per cent.

From that on we had to take up into our categories the fact that inoculation produces in addition to 'direct' also 'collateral' immunisation. This once recognised, presumptive evidence of collateral immunisation began gradually to filter into my mind.

Among, I suppose, many thousands of patients whom I have treated by vaccine therapy in private and in hospital, it happened every now and then that a patient was treated with a vaccine which did not correspond with his infection, and that that patient indubitably benefited. Again, it was not an uncommon experience for

[1] *Vide supra*., pp. 98 and 99, Tables XI–XIII.

the subjects of a very chronic infection (such as pyorrhoea) who were treated first by a stock vaccine, and afterwards with an auto-vaccine, to assert that they derived more benefit from, and to ask to be put back upon treatment by, the stock vaccine.

From such cases hints are conveyed to us that there may exist a useful sphere of application for collateral immunisation ; and that such sphere may, perhaps, be found in those cases where the infection is of very long standing, and where the patient has become very sensitive to, and has probably come very near the end of his tether in the matter of immunising response to, the particular species or strain of microbe with which he is infected.

We are, however, here considering primarily the question of principle ; and in connexion with this what is of fundamental importance is that we should discard the confident dogmatic belief that immunisation must be strictly specific, and that what is required in every case of failure is to make our immunisation more and more strictly specific. We should instead proceed upon the principle that the best vaccine to employ will always be the vaccine which gives, when this comes to be tested, the best immunising response against the microbe we propose to combat.

I would point out that this would almost certainly not involve any revolutionary change in the accepted practice either in serum therapy or in prophylactic or ordinary therapeutic inoculation. But it would mean taking into account in cases which proved intractable to treatment with the homologous vaccine the possibility of seeking for collateral immunisation by inoculating a microbe or mixture of microbes other than that with which the patient is infected. The trial of this procedure might perhaps recommend itself where from the outset there is very little immunising response to the homologous vaccine, and also where, as in very long-standing cases of tubercle or streptococcus infection, the power of direct immunising response to the corresponding vaccines may be becoming exhausted.

Practical Applications of the *in vitro* Method.

I spoke above of the vaccine which gives, when it comes to be tested, the best immunising response against the microbe we want to combat. What was in my mind was immunising response *in vitro*, and I have it in view that it may become a practicable routine measure to test the response of the patient's blood *in vitro* by experiments such as those which are incorporated in the foregoing Table of experiments. You will appreciate that as soon as we shall have learned all the determining factors which here come into play, we shall be in a position not only to test the efficacy of vaccines on normal blood *in vitro* (as was done, for instance, in Experiment VIII), but also in a position to determine upon the patient's blood *in vitro*—thus avoiding the necessity of tentative experiments *in vivo*—what vaccine, and what dose of that vaccine, will give us the desired effect.

I did this in a case of which I shall presently have to tell you. But let us note in the present connexion that in choosing the dose of vaccine for addition to the blood *in vitro* we shall have to take a submultiple of the dose we should tentatively employ upon the patient. If we were operating upon 1 c.cm. of blood we should obviously have to consider that we were dealing with something like 1 in 5000 to 1 in 6000 of the patient's blood volume, and since the fluid in the tissues must also

come into consideration we should probably have to take into our calculations the relation which our 1 c.cm. of blood bears to the patient's body-weight.

There is still one other therapeutic prospect which I want you to consider. And again the practicability of exploiting this method will depend upon our obtaining constant immunising response to vaccine added to blood *in vitro*.

When we have to deal with a general infection, and when surgical operations undertaken to abolish the ec-phylactic focus have failed, and where immunising responses cannot be obtained, there still remains, as a last resource—transfusion. But simple transfusion—and the method has been extensively tried in this war—has in these cases proved unavailing. It has even been held to do positive harm.

That should not be a matter for wonder when we reflect that the normal blood fluids provide for serophytic microbes an excellent cultivation medium—generally a more favourable medium than the blood fluids of the patient. And from another point of view also the failure of simple transfusion should not surprise us, for though leucocytes, as I have shown you, do effective bactericidal work outside the blood, the conditions where the leucocytes are suspended in blood fluids are, as we have learned, unfavourable to their bactericidal operations.[1]

Appreciating these points you will see that the outlook for the patient would be much more favourable if we could take the donor's blood and immunise it *in vitro*, and so render the plasma bactericidal for the microbe with which the patient is infected.

Immuno-transfusion.

I had not very long before coming home an opportunity of putting this plan of campaign into execution.

The case I have here in view was that of a patient who was the subject of streptococcal wound infection with extensive involvement of the sacrum and ilium, and who was suffering from a continuous high temperature, which had reduced him to such a condition that his life was despaired of. A secondary very radical operation with chiselling of bone having under these conditions been undertaken without any improvement in his condition, it was determined to try a transfusion of blood which should be immunised *in vitro* against the patient's streptococcus. With a view to ascertaining whether such a blood could be obtained for the patient a syringeful of blood was taken from the appointed donor on the day before that fixed for the operation, and different portions of this blood were digested for three hours *in vitro* with a series of graduated dilutions of a staphylococcus and also of a streptococcus vaccine. The centrifuged sera with controls were then implanted by the wash and after-wash method with a mixture of staphylococcus and the patient's own streptococcus. Of all the sera thus obtained that of the blood portion which had been digested with 1000 staphylococci per c.cm. gave the best result. While the serum from the control blood grew staphylococcus up to the thirteenth and streptococcus up to the fourteenth dilution, the serum from the blood which had been digested with 1000 staphylococci per c.cm. grew the staphylococcus up to the ninth and streptococcus only up to the sixth dilution. In view

[1] *Vide Collected Researches*, Vol. I, p. 41.

of this result we added to the litre of blood, which was drawn off from the donor into a paraffin-coated receptacle, a quantum of vaccine which contained 1,000,000 staphylococci. After the transfusion of this blood a very striking change came over the patient's condition. His temperature promptly fell and he rapidly became apyrexial. The wound also rapidly healed ; and his serum, which before provided for the streptococcus a much better culture medium than our normal sera, was found after transfusion to inhibit the growth of this organism.

The therapeutic method here employed is, as you see, a combined method of serum therapy and transfusion. We may perhaps call it ' immuno-transfusion '. Over the ordinary methods of serum therapy which have been tried for septicaemic cases it has, of course, the advantage that we are dealing with compatible human blood, and can therefore, if we succeed in obtaining protective substances, incorporate these in indefinitely large quantities.

NEW PRINCIPLES IN THERAPEUTIC INOCULATION

BEING AN EXPANSION OF A LECTURE DELIVERED AT A SPECIAL MEETING OF THE ROYAL SOCIETY OF MEDICINE ON NOV. 30TH, 1923

BY THE AUTHOR (BASED ON RESEARCHES CARRIED OUT IN CONJUNCTION WITH L. COLEBROOK, M.B., B.S.Lond., AND E. J. STORER, M.R.C.S.Eng., L.R.C.P.Lond.)

(*Reprinted from 'The Lancet', 24th Feb., 3rd and 10th March, 1923*)

PART I

Pasteur was the first to think out the principles involved in Jennerian vaccination, to show how these could be applied to bacterial infections, and to conceive the idea of prophylactic inoculation against all infective diseases.

The tenets he laid down—let me in view of what is to follow speak of them as Code No. 1 or the original Pasteurian Code—may be set out somewhat as follows :—

Code No. 1.—The Original Pasteurian Code.

(1) The essential preliminary to any prophylactic procedure is to possess ourselves of the pathogenetic organism, or if this is as yet undiscovered of the 'virus' that contains it, and to manufacture a vaccine from this.

(2) The vaccine must consist of living germs ; but these must, with a view to the risk that would attach to the employment of virulent material, be attenuated.

(3) When an appropriately attenuated vaccine—that is, a vaccine which can be warranted to produce only a moderate clinical reaction—has been obtained, the exact quantum implanted will not be of material importance.

(4) Bacterial vaccines should be inoculated subcutaneously.

(5) Vaccination is applicable only to the uninfected.

(6) The protection conferred by the vaccine is always specific—in other words, protection is obtained only against the species of pathogenetic agent of which the vaccine consists.

(7) Protection is obtained only after the lapse of ten or more days from the date of inoculation.

By putting into practice these principles—and they relate, as you see, only to prophylaxis—great and notable successes were achieved. In particular, Pasteur succeeded in protecting sheep and cattle against anthrax. Later Ferran and after him Haffkine—the latter employing a much more exact technique—applied the Pasteurian methods to man in their anti-cholera inoculations.

In the meanwhile, impelled to do so by that 'pain in the mind' which is felt when one is appealed to and is powerless, Pasteur had addressed himself to another

problem in connexion with immunisation—the problem as to whether it would, by any application of vaccination, be possible to arrest the development of rabies in a patient bitten by a rabid animal. Here, of course, his principle that vaccination was applicable only to the uninfected suggested that nothing could be done. But emotional tension is intolerant of any intellectual impasse, and its special function in such circumstances is to insist on a critical probing of subsisting rules, on a definition of all undefined or loosely-defined terms, and on a sharper focusing of all nebulous elements of thought. Here, therefore, it was bound inevitably to lead to a critical review of *the principle that vaccination is applicable only to the uninfected*, and to a general or sharper focusing of the terms ' infected ' and ' uninfected '. And then it was discerned by Pasteur—and afterwards, of course, by all the world—that a patient in whom the virus of rabies has been implanted should, pending the development of events, be reckoned as uninfected ; and, further, that if it takes for a virus like that of rabies much more than ten days, and for protection after the inoculation of a vaccine only some ten days, to develop, the patient is within that period a perfectly suitable subject for preventive inoculation.

This forward step in thought, in which Pasteur broke away from what had been handed down and also from what he himself had formulated, bestowed upon us the preventive treatment of rabies. It was, as we shall see, destined to lead also to therapeutic inoculation.

That brings to a close the great opening chapter of scientific immunisation—the chapter contributed by Pasteur.

The Next Advance in Immunisation—Anti-typhoid Inoculation.

We have seen in the foregoing that emotional tension conducts to a closer scrutiny and sharper focusing of ideas, and that it is by the clarification which results from such closer focusing that problems are resolved. This holds true over the entire domain of knowledge, and is again exemplified in connexion with the next two advances in the field of immunisation—those made in connexion with anti-typhoid inoculation. These improvements were (*a*) the substitution of dead for living vaccines, and (*b*) the preliminary gauging of the efficacy of prophylactic procedure by measurement of the anti-bacterial elements produced in the blood. Of these forward steps, the first was taken because of hesitation to risk inoculation with living typhoid bacilli ; the second, from unwillingness to launch out with a vaccine of dead microbes without first obtaining proof of its efficacy.

The general progress of knowledge had already furnished certain facts and general inferences which bore on these issues.

To begin with the conception that the antigens required were the metabolic products elaborated by *microbes growing in the organism* was becoming discredited, and was being gradually replaced by the conception that the antigens required were elements derived from the bacterial protoplasm. And that, of course, opened the way to the employment of vaccines made from killed cultures.

Again, the general principle—enunciated by Ehrlich in the apophthegm *corpora non agunt nisi soluta* was silently pointing to the conclusion that in vaccines

the living or dead germs, as such, could not be the actual antigens; that these must be dissolved elements; and that the microbes in vaccines, whether they were dead or alive, could in reality be only mother substances of antigens.

Lastly came the important fact that Pfeiffer had, by the incorporation of dead typhoid microbes into man, obtained a production of agglutinating substances.

In consequence attention in the first anti-typhoid inoculations was focused upon the production of agglutinins.[1] But the measurement of these showed very little more than that an immunisation response was obtained by the incorporation of dead microbes.

Measurements of the bactericidal power of the blood[2] were then undertaken; and it was these that brought illumination. They established that when quanta of typhoid vaccine which produce moderately severe constitutional disturbance are incorporated, inoculation is followed first by a negative phase, in which the bactericidal power of the blood is reduced, and then by a positive phase in which the bactericidal power may be increased as much as 1000-fold; further, that when doses which produce very severe constitutional disturbance are employed the negative phase is protracted, and in some cases perhaps indefinitely protracted; and lastly, that when doses which produce only trifling constitutional disturbance are employed a positive phase is obtained without the intervention of any negative phase, and the bactericidal power of the blood is already after an interval of 24 hours very considerably increased.

These facts supply the principles which everywhere regulate—or perhaps it would be safer to say should everywhere regulate—anti-typhoid, anti-cholera, anti-pneumonia, anti-plague, and all other prophylactic inoculations.

Again, the observation that protective substances can be made to appear in the blood within 24 hours from the incorporation of a vaccine was one which carried consequences. It was deducible from this that the procedure of inoculation in the incubation period cannot be applicable only to rabies with its average incubation period of many months, but must, in theory, be applicable to typhoid and Malta fever with their incubation periods of a couple of weeks, and even—as contended by Haffkine—to plague with its incubation period of only a couple of days.

But the full scope of application of the facts brought to light in connexion with anti-typhoid inoculation did not appear until the concept of an incubation period had been sharply focused, and until this had been seen to import a period when the microbe is cultivating itself in the body, not broadcast but confined in one region. Once that fact is regarded, there is, as you will see, no getting away from the conclusion that the successful event of the Pasteurian preventive treatment in rabies, and, it may be added, the occasional successful event of anti-plague inoculation administered in the incubation period of plague, must be due to immunising responses successfully evoked by the vaccine in the still uninfected regions of the body. That idea—the idea that the uninfected and still inactive regions of the

[1] Wright: *The Lancet*, Sept., 19th, 1896; Wright and Semple: 'Vaccination against Typhoid Fever', *B. M. J.*, Jan., 30th, 1897.

[2] Wright: 'Changes Effected by Anti-typhoid Inoculation in the Bactericidal Power of the Blood, with Remarks on the Probable Significance of these Changes', *The Lancet*, Sept., 14th, 1901.

body can, by applying the stimulus of a vaccine, be made to bring succour to the infected regions was, as you know, the mother idea of vaccine-therapy.

Now let me—for I shall want this as a point of departure for setting out my new matter—formulate in the form of a code the principles we have just been considering. The code now in question—for convenience of reference I call it Code No. 2—has been gradually elaborated in the Inoculation Department of St. Mary's Hospital. You will observe that here only the first, fourth, and seventh tenets have been taken over practically unmodified from Pasteur.

Code No. 2.—Founded upon a Study of the Bactericidal and Opsonic Changes Produced in the Blood by the Inoculation of Vaccines into the Patient.

(1) The essential preliminary to all immunisation procedures is to possess ourselves of the microbe of the disease, or failing that of its virus, and to employ this as material for the manufacture of the vaccine. And here it may be parenthetically pointed out that inasmuch as in prophylactic inoculation the vaccines are stock vaccines and give good results it cannot in any form of inoculation be theoretically essential to employ vaccines made directly from the patient.

(2) Vaccines should in all cases where the microbes can be cultivated outside the body consist of sterilised cultures.

(3) Vaccines may be turned to account in a variety of different ways. They may be employed not only for prophylaxis but also for preventive treatment in the incubation period of general infections. Again, they may be therapeutically employed in all localised infections other than those complicated by pyrexia and heavy and frequent auto-inoculations. In this last class of infections, and also in those septicaemic processes in which bacterial toxins in large quantity are circulating in the blood, vaccines are contra-indicated.

(4) Bacterial vaccines should be incorporated hypodermically.

(5) The quantum of vaccine incorporated is of supreme importance ; it affects both the kind of response and the rate at which protective substances appear. With only small doses of vaccine, or comparatively light auto-inoculations, a positive phase—or as it may be better called, an epiphylactic or immunising effect—may be registered in less than 24 hours after the incorporation of the vaccine. A similar but evanescent increase, which I have called ' the false rise ', may be registered within a couple of hours after larger doses of vaccine and heavier auto-inoculations. These larger inoculations of vaccine and heavier auto-inoculations produce after that very fleeting positive phase a negative phase or—for these are better terms—an apophylactic or de-immunising effect. And this effect is greater and lasts longer the larger the quantum of antigen carried into the blood.

(6) In correspondence with the above the following rules of dosage may be laid down. In prophylactic operations undertaken in uninfected surroundings the dose should be that which evokes the optimum epiphylactic response, and it is for the attainment of that end permissible to employ doses which produce a temporary negative phase. When inoculating prophylactically in the presence of an epidemic, or in the incubation period of a general infection, and generally in the treatment of localised infections, reduced doses should be employed in order to avoid the

constitutional disturbance and temporary aggravation of symptoms and dispersal of microbes in the organism. Reduced doses should also be employed where the chief matter of concern is to obtain with promptitude some clinical improvement.

All these rules can be summarised into one general principle—that the dose of vaccine incorporated should depend on the patient's being infected or uninfected, and that where he is infected the dose should stand in inverse relation to the volume of his infection.

(7) The anti-bacterial substances elaborated in response to inoculation operate specifically upon the variety of microbe which has furnished the vaccine, but it is possible that in addition some collateral immunisation is achieved.

I cannot here attempt to give even a brief summary of what has been achieved by the following out of this newer code. To do so I should have to chronicle all that has in the last 20 years been achieved by prophylactic and therapeutic inoculation in connexion with bacterial disease. Instead of attempting that, let me fix your attention on the fact that, except in a few isolated instances, therapeutic inoculation has proved unavailing in pyrexial phthisis and in septicaemia. And I may again point out that it is in the nature of things that the failures here in question should evoke in some of those who have had over and over again to witness them, the same emotional tension and the same closer focusing of ideas which led to Jennerian vaccination, to Pasteur's thinking out the principles of immunisation, to the extension of prophylactic treatment to human diseases, to the Pasteurian preventive treatment of rabies, to the treatment of diphtheria by antitoxins, to vaccine-therapy, and to therapeutic advances along other lines. All this is, as you have discerned, only a prelude to setting out to you the outcome of an inquisition into those universally accepted principles which have hitherto guided vaccine-therapy, and to telling you of certain new principles which have in the last few years gradually emerged.

I have here to choose between two different modes of exposition. I could start by describing the new technical procedures which have been devised, could then, in connexion with each method, tell you the new facts it has furnished, and could then leave it to you to make at the end your own generalisations. That would be like leading you along a number of new paths, showing you hurriedly a portion of what could be seen from each, and leaving it to you to build up from these glimpses a general map of the country. The alternative, and proper, method will be for me, who have been every day engaged in exploring these paths, to give you at the start the general scheme of the landscape and then afterwards to describe to you the methods of exploration and tell what each of these has yielded.

The new principles which have emerged will best be set out in the form of yet another code. This will be Code No. 3, and I would wish you, when taken aback at its subversive character, to observe that there is here a going back, not upon anything directly established by experiment, but only upon dogmata built upon a foundation of uncoercive inferences. I have in view here not only the tenets of the Pasteurian code, but also the doctrine of phagocytosis with its implication that leucocytes can kill microbes only by phagocytosis. I wish you also to bear in mind that the code here brought forward rests almost exclusively on experiments with

the staphylococcus and streptococcus, that up to the present it has been put into actual operation almost exclusively in the treatment of infections by those microbes, and that the question of its application to prophylactic procedures is yet quite unstudied.

Code No. 3.—Founded upon a More Detailed Study of the Changes Produced in the Blood by Inoculation of Vaccines *in Vivo* and *in Vitro*.

(1) While the nature of the infecting microbe should in every case be ascertained, it is not theoretically necessary that the vaccine employed in treatment or arrest of the incubating infection should be derived from the species of microbe which causes the disease. Sufficient justification for recourse to a particular vaccine is afforded when that vaccine has been shown to increase the antibacterial substances which operate upon the infecting microbe.

(2) When vaccines in appropriate doses are added to the blood, whether *in vivo* or *in vitro*, instantaneous epiphylactic response is evoked, and the maximal response may be expected after only very short delay.

(3) The epiphylactic response here in question consists in an extrusion of opsonic and bactericidal elements from the leucocytes. And it is mainly by this ectocytic chemical action, and only to an insignificant extent by phagocytosis and internal digestion, that the bactericidal action of the leucocytes is exerted.

(4) The antibacterial substances here in question are polytropic—in other words, they operate not only upon homologous but also upon quite unrelated species of microbes.

(5) Where the effective dose of vaccine for intravenous application has been ascertained, this method of administration is from considerations of certainty and rapidity of therapeutic action to be preferred to subcutaneous inoculation.

(6) In septicaemias and other heavy bacterial infections the patient's leucocytes lose their power of responding to vaccines. In such cases it is essential before inoculating to satisfy one's self that the patient's blood still retains its power of epiphylactic response.

(7) Where by reason of the poisoning of leucocytes, active immunisation by means of vaccines is ruled out, the method of immuno-transfusion should be resorted to—in other words, healthy human blood which has made proper epiphylactic response should be incorporated.

Having now formulated the new principles arrived at, I must next show the data upon which these are founded. Those data were, as you will understand, obtained by adding measured quanta of vaccines or, as the case may be, living microbes, to the blood as a whole or to its separate elements. Let me begin with a conspectus of the methods of blood examination which have here come into application.

Conspectus of Methods of Blood Testing.

I. *Whole Blood.*

(1) Measurement of haemo-bactericidal power. (Bactericidal effect of the leucocytes and serum acting in conjunction.)
- Implanting and explanting.
- Implanting and inculturing.

(2) Measurement of phagocytic power.
- With defibrinated blood.
- With washed leucocytes and serum (reconstituted blood).

II. *Serum.*

(1) Measurement of sero-bactericidal power.
- Implanting and explanting.
- Implanting and inculturing.

(2) Measurement of opsonic power.

III. *Leucocytes.*

(1) Measurement of phagocytic efficiency.
(2) Measurement of spontaneous emigration and chemotactic reactions.
(3) Measurement of ectocyto-bactericidal power (i.e., power of killing extracellularly).
(4) Measurement of opsonecphoric power (i.e., power of furnishing opsonins).

IV. Combined measurement of—

Phagocytic Power, Opsonic Power, and Phagocytic Efficiency of the Leucocytes.—*Chiastic procedure.*

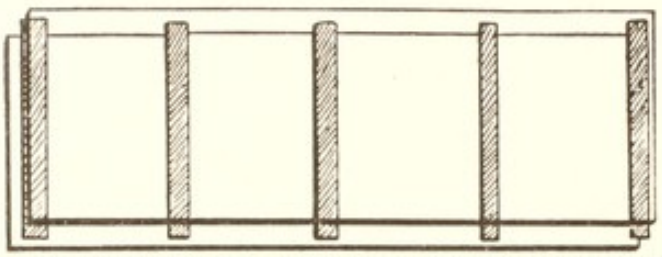
A few words of general introduction and, in the case of those methods which have been newly devised, a brief description will be required. Let me deal first with the measurement of the bactericidal power of the whole blood.

Methods of Measuring the Haemobactericidal Action.—This can be measured (1) by the *Implanting and Explanting Procedure*, i.e., by bringing together measured volumes of defibrinated blood and graduated dilutions of a bacterial culture, and then after a fixed interval diluting with nutrient broth, and then either growing the surviving microbes in the broth-diluted blood, or explanting on to an agar surface. An alternative method of measuring the bactericidal power of the whole blood—available only when dealing with serophytic microbes—is (2) the *Implanting and Endo- or In-culturing Procedure.* The principle of the latter method is to implant graduated quanta of microbes into the blood; to introduce the implanted blood into very fine capillary tubes, or, better, into 'slide cells'; and to incubate, so as to let such microbes as survive grow out into colonies.

Slide Cells.—These are made from ordinary microscopic slides. We take a sterilised slide, place it with its convex side uppermost on the bench, and then provide confining walls for four (or five) compartments by taking strips of paper dipped

in very hot vaseline, and laying these down at both ends and at equal intermediate distances along the slide. We then take another slide, hold it in the flame, concave side downwards, and then immediately press it down upon the vaselined strips. The paper employed for these strips must be of standard thickness so as to give blood films of appropriate and uniform depth. If the blood film is too thick the growing colonies are concealed, and if it is too thin the leucocytes are too remote from the implanted microbes—moreover, there is not a sufficient depth of red corpuscles to

FIG. 1.



Slide cell ready for the reception of the implanted blood.

show up the colonies to advantage. A convenient depth of film is obtained by using for our strips a paper about 1/300 inch (1/12th mm.) in thickness. The detail of the procedure for measuring the haemo-bactericidal power in slide cells is as follows: We begin by making a series of progressive dilutions of our bacterial culture. Then we measure out a series of 50 c.mm. volumes of defibrinated blood and introduce into each of these (from a calibrated capillary pipette drawn out at the extremity into a fine point) 2·5 c.mm. of one of the dilutions of our culture. Then after thorough mixing we introduce the implanted blood into one of the compartments of our slide cell. In preparation for this, the edges of the slide cell are re-sterilised by passing them through the flame, and the upper slide is pushed back a little so as to leave a projecting lower lip upon which to rest the point of the pipette which purveys the blood. When the compartments of the slide cell have been filled in with the allocated volumes of blood, the upper slide is brought back into position and the ends and sides are carefully sealed up by brushing them over with very hot melted paraffin wax mixed with an equal volume of vaseline. After 24 hours' incubation the staphylococcus colonies show up against the scarlet background of the blood by a surrounding dark purple discoloration. Then after a few more days this purple fades out and each colony is represented by a small bleached patch. When instead of implanting staphylococcus into defibrinated blood we implant it into a blood which coagulates in the slide cell, an additional feature arrests the attention. Each colony is encompassed by a ring. It is just as if we had taken an agar plate upon which staphylococcus colonies are growing, and a cork borer of much greater diameter than these, and had then, centring upon the colonies, cut into the medium and then withdrawn our cutting implement. It may be taken that the rings which encircle the staphylococcus colonies in the blood delimit the regions in which the fibrinous network has been digested by tryptic ferment generated either by the microbes or by the leucocytes which have gathered round them.

Reverting to the more general consideration of the measurement of haemo-bactericidal power, it will be well before passing on to appreciate that the oppor-

tunity for phagocytosis afforded in the quiescent blood *in vitro* is certainly much greater than that afforded in the circulation. In that swift torrent the leucocytes have as little opportunity for ingestion as would fish if, instead of facing upstream, or swimming down faster than the current, they were only passively swept along side by side with all other flotsam. In the tranquil conditions provided *in vitro* the leucocytes are able to congregate round their bacterial prey.

Methods of Measuring the Phagocytic Power of the Blood.—We may here add one volume of the microbic suspension to two volumes of defibrinated blood [1]—or, following out the opsonic technique more closely, we may take one volume of washed corpuscles from the blood that is to be tested, one volume of serum, and add one volume of the microbic suspension.

Methods of Measuring the Sero-bactericidal Power.—Here, as in connexion with the whole blood, we have two methods at our disposal. We can, using the procedures described above in connexion with blood, implant and then explant. When dealing with serophytic microbes, in particular with the staphylococcus, we can also implant and inculture, filling our serum into fine capillary tubes and incubating in these.[2] (Fig. 2.) By this method we obtain, as is seen in the figure, quite compact and separate colonies. The number of these will, of course, correspond, in the case of a non-bactericidal serum, to the number of microbes implanted ; and in the case of a bactericidal serum to the number of microbes left alive. We can accordingly by this procedure compare the bactericidal potency of two sera, and we can for further control in such measurements count also the colonies which develop in nutrient broth. But in the case of broth we must, because of the flatness of the colonies of non-agglutinated staphylococcus, limit ourselves to implantations of less than 100 microbes per 50 c.mm. volume, and must employ capillary tubes of such fineness that a 50 c.mm. volume may occupy a length of not less than 25 to 50 cm. ; and above all, we must very carefully avoid all mechanical disturbances.

Fig. 2.—Measurement of sero-bacterial power. Staphylococci implanted into serum growing out into discrete colonies of agglutinated microbes.

Experiments: Graduated Implantations of Living Microbes into Blood *in Vitro*.

Inculturing Experiments.—We may now, leaving till later what requires to be said about the remaining methods of blood testing, study what happens when we make graduated implantations of living microbes into blood. And it will here, as in all experimenta-

[1] Wright and Colebrook : *Technique of the Teat and Capillary Tube*, second edition, Constable, 1921.

[2] The method of culture in capillary tubes was employed nearly 50 years ago by Salomonsen for the isolation of microbes from putrid blood.

tion, be well to visualise and formulate quite clearly in advance what we expect will happen; for then both such results which conform exactly, and those which are at variance with expectation, will engrave themselves more deeply upon the mind.

Here, if anyone were required to say *a priori* what would follow upon making graduated implantations of living microbes into blood, he would assuredly say that as fewer and fewer microbes were added to blood a larger and larger percentage, and after a certain point a full 100 per cent. of the added microbes would be killed. Similarly he would predict of such graduated implantations that as more microbes were added the percentage of survivors would go up in a perfectly regular manner.

The next step will be to see what does actually happen. Here a distinction has to be made between blood and blood. And I would ask you to let me tell you first what happens when I take my own blood and implant into it graduated quanta of microbes. I begin with my own blood because it so happens that this, by virtue of its having a subnormal number of leucocytes, and serum which has very little bactericidal action upon the staphylococcus, furnishes a specially favourable object for the experiments here in view. One of many dozens of quite conformable experiments made with the blood is set out in tabular form (Table I). This particular experiment was carried out in duplicate by separate observers. The results of both workers are given.

It will be seen that we have here, with implantations of 10,000 to 150 staphylococci per c.cm., an average killing of 78·8 and 76·3 per cent. respectively; and with implantations below 150 staphylococci per c.cm. no killing at all—in other words with moderate implantations an average survival of 22 per cent., and with quite small implantations a survival of a full 100 per cent.

These figures, I think, tell their tale quite plainly. Their import, as I read it, is that we have in the blood an apparatus for primary and an apparatus for secondary defence—a phylactic and an epiphylactic machinery. The former—making use, we may take it, of phagocytic action, and where the serum is naturally bactericidal, also of sero-bactericidal action—would deal (according to circumstances more or less effectively) with small bacterial implantations and septicaemic subinfections. The machinery for epiphylactic defence—making use, as we shall see, of anti-bacterial elements given out by the leucocytes when these are incited by antigen—would deal with larger bacterial implantations and graver blood infections.

Interpreting now in the light furnished by this conception, the results set out in Table I, we see that in this blood the machinery of primary defence is quite singularly inefficient, while that of secondary defence is far more efficient. The blood, implanted with 33 microbes per c.cm., cannot kill one; when implanted with 10,000 per c.cm. it can kill 2000. This increased efficiency plainly indicates that an *epiphylactic response* has been evoked; and whenever we speak of the bactericidal action of the blood we must distinguish sharply between what the blood does where epiphylactic response is evoked and what it does where such a response is not evoked; and we should, when dealing with microbic implantations and infections, distinguish between a sub-vaccinating, a vaccinating, and a critical or optimum vaccinating dose.

But to interpret and generalise safely, we must regard not only bloods which

TABLE I.—*Implantation of graduated dilutions of staphylococcus culture into 40 c.mm. volumes of A. E. W.'s defibrinated blood. The figures represent the number of staphylococcus colonies which developed after 24 hours' incubation in slide cells.*

Observer, A. E. W.				Observer, L. C.			
Implantation per c.cm. of blood	Number of microbes implanted (I.) and colonies found (F.) in 40 c.mm. of blood		Percentage of survivors	Implantation per c.cm. of blood	Number of microbes implanted (I.) and colonies found (F.) in 40 c.mm. of blood		Percentage of survivors
	I.	F.			I.	F.	
8400	336	64	19	9600	384	74	19
4200	168	28	17	4800	192	44	23
2100	84	24	28	2400	96	27	28
1050	42	7·5	18 } 21·2	1200	48	13	27 } 23·7
525	21	11·5	55	600	24	15	63
262	10½	5	50	300	12	6	50
131	5	5		150	6	5	
66	3	7	100	75	3	5	100
33	1·5	1·5		38	1·5	2	
—	671	153	22·8	—	766	191	24·9

It may be added here that precisely similar results are obtained when instead of, as in the tabulated experiments, implanting progressive dilutions of a bacterial suspension into separate samples of blood, we modify the procedure either (*a*) by implanting heavily into a volume of blood and then progressively diluting down this implanted blood with unimplanted, sampling it after each dilution ; or (*b*) by making repeated small implantations into one and the same volume of blood, sampling it after each successive implantation.

have a feeble, but also those which have a powerful machinery of phylactic defence. When we take a blood which has a full complement of leucocytes, and a serum which has an appreciable bactericidal power, and make into this graduated implantations of staphylococcus, quanta up to 1000 and more per c.cm. are killed without residue ; and with heavier implantations we have incomplete destruction —with (for this would seem to be the rule) first an increasing, then a diminishing, and after that again an increasing percentage of survivors. With implantations of 10,000 to 20,000 staphylococci (these would, in case of powerfully bactericidal bloods, seem to be optimum implantations) we may have as few as 1 to 2 per cent. of survivors.

It will, on reflection upon the technical aspect of the question, be realised that with slide cells clear demonstration of epiphylactic response will, in the case of these powerfully bactericidal bloods, be difficult. A preliminary difficulty is created by the circumstance that with the heavier implantations which are here necessary accurate enumeration of the colonies becomes (owing to their crowding) impossible. A second difficulty is created by the fact that when we have instead of a nearly zero base line, a high base line of phylactic power, epiphylactic response can no

longer show up clearly. And yet a third difficulty is created by the fact that instead of obtaining, as before, with vaccinating implantations, a bactericidal effect due wholly to epiphylactic response, we obtain, where the primary apparatus of defence is efficient, a result which is due in part to the factor of phylactic and in part to the factor of epiphylactic action.

These difficulties can be diminished by reducing the phagocytic power of these powerfully bactericidal bloods. Instead of working with the normal proportion of serum to corpuscles which we have in defibrinated blood, we can by diluting down the blood with additional serum, work with an increased proportion of serum and a diminished proportion of leucocytes. Or, again, we can, by incubating our defibrinated blood for half an hour in fine capillary tubes, deplete it of leucocytes. Or finally, by using a combination of these methods we can obtain a blood which is both impoverished in leucocytes and diluted with serum. Such a blood will still, in that its serum exerts a bactericidal action on staphylococcus, differ from a blood of feeble phylactic power. But it will, in despite of that, behave very similarly. Small implantations will no longer be killed off without residue, and we obtain with geometrically diminishing implantations, a characteristically prolonged cauda of survivors.

Fixing our attention upon this cauda, let me explain to you how we can, where we have before us a table which sets out the results obtained from geometrically diminishing implantations of microbes, tell whether epiphylactic response has or has not been evoked. Here I would beg you—for this will greatly facilitate my exposition—to let me call the first figure in any column of implantations or results the *caput*, and all the figures which follow, the *cauda*. You will now on turning to Table I and looking at the column of geometrically diminishing implantations see that the number of microbes in the caput must always exceed by a fraction (a fraction which corresponds to the number of dilutions) the total of microbes in the cauda. If now in the column of results we find the number of colonies in the caput sensibly the same as that in the cauda, the conclusion will clearly be that the bactericidal efficiency of the blood does not vary with the magnitude of the implantation. If the colonies in the caput much out-number those in the cauda, the inference is that the bactericidal efficiency of the blood increases as the implantation is reduced. (Let it be remarked that it would inevitably do so if the serum exerts a bactericidal action.) But if the colonies in the caput of the results column are definitely outnumbered by those in the cauda we may—provided always that the microbes in the first blood sample are not so numerous as to interfere with each other's development—confidently conclude that the bactericidal efficacy of the blood increases as the implantation is increased ; in other words, that epiphylactic response has been evoked. And by going down the column of results in a methodical manner, comparing the number of colonies in each successive blood sample with the total for the two or three samples next in series, it will be possible to detect the point at which epiphylactic response comes in.

Employing these criteria in connexion with the results obtained from a powerfully bactericidal blood diluted down with serum, we see that evidence of epiphylactic response is here obtained. (Table II.)

TABLE II.—*Experiment showing that when geometrically diminishing numbers of microbes are implanted into a powerfully bactericidal blood, and into the same blood diluted with serum, the serum-diluted bloods give a longer cauda of colonies, and that in the diluted bloods the cauda furnishes more colonies than the caput.*

Number of living staphylococci implanted into the 50 c.mm. samples of blood	Number of colonies of staphylococcus which developed in—			
	Undiluted blood	Blood diluted—		
		With half its bulk of serum	With its own bulk of serum	With double its bulk of serum
100	1	3	4	3
50	0	1	3 or 2	5
25	0	1	1	5
12	1	0	1	1
6	0	0	0	0

Having now seen that bloods of powerful phylactic power when diluted respond, after the manner of undiluted bloods of feeble phylactic power, to larger bacterial implantations by increased bactericidal action (and all our other methods of testing epiphylactic response will bring confirmation of that) we shall, perhaps, do well before proceeding further to make a general survey of the field of bacterial infections, and see whether the doctrine of phylactic and epiphylactic defence enunciated above accords with the events which come every day under clinical observation.

Three types of cases should be called to mind :—

(1) The type of infection (exemplified by streptococcic endocarditis) with for a long time only very few microbes in the blood, with only moderate pyrexia, comparatively little constitutional disturbance, and a protracted and almost always fatal course. These cases would well be explained by assuming that the machinery of first defence has failed to do its office and that the epiphylactic machinery has never been called into action.

(2) The type of case (exemplified by croupous pneumonia) with from the outset an intense infection, high temperature, and heavy bacterial intoxication, and a course which very rapidly terminates either in death or in recovery by crisis. The cases which recover would be well explained by assuming that the epiphylactic machinery is here effectively, though tardily, brought into operation ; those cases which terminate fatally, by supposing the epiphylactic machinery to have been put out of action by overwhelming bacterial intoxication.

(3) The type of infection where we have either a regular hectic temperature, as in phthisis and locked-up suppuration, or else such steep ascents and descents of temperature as accompany the rigors of acute streptococcus septicaemias. Here we may assume—and such opsonic and bactericidal observations as are to hand are in accord with this—that whenever bacterial poisons in sufficiency are conveyed into, or generated in the blood, the machinery of epiphylactic defence is, with results that are for the nonce satisfactory, called into action.

When once we survey the phenomena of infection from the point of outlook here suggested, the question arises in the mind whether in all these cases it might not be possible to bring the machinery of epiphylactic defence into effective operation by a direct introduction of vaccine into the blood. The proper dose to inoculate here would, of course, be that quantum (I might, perhaps, call it the critical dose) which would, taken together with the bacterial elements already in circulation, evoke in the patient's blood a maximum response. If the epiphylactic response to infection could in this way be expedited and reinforced, three advantages would be gained. First, the poisoning of the leucocytes—poisoning which upsets the machinery of both phagocytic and epiphylactic defence—would be avoided. Secondly, the patient would be spared that intense systematic poisoning which may bring him near to death before the life and death struggle with his infection even begins. And thirdly, the patient would be spared all that grave damage which is inflicted on his tissues at the site of infection.

Implantations of Dead and Living Microbes conjointly into the Blood *in Vitro*.

Inculturing Experiments.—Coming back to our experiments, we may consider next the effect of introducing into the blood instead of only living microbes a proportion of dead and a proportion of living microbes : (1) The dead may be implanted into the blood first and afterwards the living. That will be comparable to prophylactic inoculation undertaken with a vaccine of dead microbes, and followed by either a test implantation of living microbes or exposure to infection. (2) Or again the dead and the living microbes may be implanted into the blood simultaneously. (3) Or lastly—and this will be analogous to inoculation in the incubation period and to vaccine-therapy—the living microbes may be implanted first and afterwards the dead.

Let me put off for a moment telling you of the results obtained. For we must first clarify our ideas and see whether there is really a definite line of division between vaccinating and assaying, and ask ourselves whether we have a perfectly clear idea as to what we mean by a vaccinating and an assaying or test dose. These will probably seem to you to be perfectly idle questionings, and you will, perhaps, think that they could be settled straight off, by defining the *vaccinating dose* as that which comes first in order of time, which normally consists of dead microbes, and which evokes the epiphylactic response ; and the *assaying dose* as that which follows after, which consists of living microbes, and which finds out what epiphylactic effect has been obtained. In reality, however, these definitions do not go down to the root of things, and they regard only prophylactic immunisation.

When we pass to the case of inoculation in the incubation period and to vaccine-therapy, the terms vaccinating dose and test dose must, it will be allowed, be interpreted somewhat differently. The living microbes which have effected a lodgment in the body here officiate as the test dose, and the dead microbes which are subsequently inoculated, as the vaccinating dose. But here it may still be maintained that vaccinating and assaying are operations apart, and that here only the order of the doses has been changed. When, however, we come to the experiments in which an epiphylactic response is evoked by importing living microbes into the

blood, it becomes plain that the distinction between vaccinating and assaying can no longer be sustained. For we have here not two separate and independent operations, but a single and indivisible vaccinating and assaying operation. And, of course, the same holds good of septicaemic infections. There the infecting microbes are the agents which produce, as the case may be, epi- or apo-phylactic response, and they officiate at the same time as indicators of the changes effected.

There is still, in connexion with this subject, one further point that requires to be considered, and it is one which has already been adverted to. We saw in connexion with the discussion of dead and living vaccines that it is not the dead or living microbes as such, but soluble elements extracted from the bacteria which officiate as the antigen, and we further saw that such soluble elements are extracted irrespectively of the bacteria being living or dead. It follows that where (1) dead and living bacteria are implanted simultaneously or in succession into the blood, or (2) living microbes (in the form of abortive infections or test doses) are introduced into an organism already vaccinated, or (3) dead microbes are inoculated into an already infected organism, all the microbes, irrespectively of their being dead or alive, will make their contribution to the final result. And especially in connexion with therapeutic inoculation it will be important to observe that the really operative dose—the dose which, as the case may be, produces an epiphylactic or apophylactic effect—will in each case be the amount of antigen introduced in the vaccine, supplemented by that contributed by the infecting microbes.

This is the rationale of well-established rules—of the rule that in prophylactic operations we may, because here the active principle supplied from without will not be supplemented from within, employ a considerable dose of vaccine ; and of the rule of therapeutic inoculation, that we must carefully consider the volume of infection, so as to introduce, as a larger quotum is supplied from within, a smaller quotum in the form of vaccine ; and again of the rule that we should abstain from inoculation where a hypervaccinating dose is already circulating in the blood.

But though it is important to realise that when it is merely a question of furnishing antigen, dead microbes can take the place of living and vice versa, we must not allow ourselves to forget that, except only in the matter of furnishing antigens, the dead microbes which are introduced in vaccines stand on an absolutely different footing to the living microbes which produce the infection.

The principles are in reality very simple. The dead microbes provide our therapeutic agent, but the living decide the issue. Upon the dose of vaccine administered will depend the magnitude of the vaccinating response, but the outcome will, both in prophylactic and therapeutic procedures, depend upon the number of living microbes that have to be dealt with. We shall see that precisely the same holds also *in vitro*. It is essential to regulate the total of antigen which is brought into operation. But more important still is it to keep within certain limits the number of living microbes we implant in the assaying dose.

Three other points in connexion with experiments in which dead and living microbes are implanted into blood must also be kept in mind in connexion with every one of the experiments which I have cited and am about to cite. The *first* is that in what are designated living cultures there is always a variable and therefore

unknown proportion of dead microbes. The *second* is that from the point of view of the evocation of physiological effects we must not range in the same category of efficacy all kinds of dead microbes or, as the case may be, all sorts of living microbes. To take the case of dead microbes, some like typhoid bacilli which have been killed at 56° C. readily dissolve in serum, and to a less extent in watery media. Others like typhoid bacilli, which have been heated above 72° C., are comparatively insoluble, and furnish vaccinating elements which are not only less in quantity, but also presumably less efficacious. And the *third* point to be borne in mind is that in vaccinating we are dealing, not as in ordinary chemical operations with reagents which are fully dissolved, but with substances which may sometimes fail to pass into solution, and may at other times pass into solution very slowly.

It is in conformity with this that a dose of vaccine should sometimes give only a positive result, and at other times, especially when inoculated intravenously, first a negative and then a positive phase. Let me now show you the results of some experiments in which dead and living microbes were implanted into defibrinated blood *in vitro*.

Effect of Implanting Dead and Living Microbes into Blood

Here the dead and living microbes were implanted simultaneously into 25 c.mm. volumes of A. E. W.'s blood and the implanted blood was afterwards filled into and incubated in fine capillary tubes.

Experiment 1

Test dose [1]	Vaccinating dose [2]	Aggregated implantation	Negative or positive result of culture
Living staph. per c.cm.	Dead staph. per c.cm.		
Circ. 2000	Circ. 32,000	Circ. 34,000	+
,,	,,	,,	+
,,	16,000	18,000	+
,,	,,	,,	+
,,	8,000	10,000	+
,,	,,	,,	+
,,	5,000	7,000	0
,,	,,	,,	0
,,	4,000	6,000	+
,,	,,	,,	+
,,	2,000	4,000	+
,,	,,	,,	+
,,	Nil	2,000	+
,,	,,	,,	+
,,	,,	,,	+

Staph. = staphylococci.

[1] 1 wash of a dilute suspension of living staphylococcus.

[2] 1 wash or a multiple or submultiple of a sterilised 8-fold stronger staphylococcus suspension.

Details of the Bacterial Enumerations upon which the Figures obtained in Experiment 1 are based.—The staphylococcus suspension which furnished the test doses was further diluted 10-fold, and of this a wash or a submultiple of a wash was implanted and incultured in 25 c.mm. volumes of my serum.

1 wash gave 5 colonies.
1 ,, ,, 5 ,,
$\frac{1}{2}$,, ,, 3 ,,
$\frac{1}{4}$,, ,, 1 colony.

There being thus in 1 wash of the diluted culture used for this enumeration an average of 5 microbes, there would be in 1 wash of the suspension which furnished the test dose 50 microbes. And this (seeing that 25 c.mm. of blood were in each case employed) works out as an implantation of 2000 (50 × 40) staphylococci per c.cm.

Further Experiments showing that Increased Haemo-bactericidal Effect is Obtained by Vaccinating Blood *in Vitro* with Dead Microbes.

Experiment 2

Here again the author's blood was employed ; and the technique was the same as in Experiment 1.

Test dose	Vaccinating dose	Aggregate implantation per c.cm.	Number of survivors per c.cm.
Living staph. per c.cm.	Dead staph. per. c.cm.		
Circ. 1200	Circ. 12,000	Circ. 13,200	363
,,	,,	,,	
,,	6,000	7,200	48
,,	,,	,,	
,,	4,000	5,200	180
,,	,,	,,	
,,	3,000	4,200	462
,,	,,	,,	
,,	Nil.	1,200	200
,,	,,	,,	
,,	,,	,,	

Experiment 3

Here the vaccine of dead staphylococci was implanted *in vitro* into a powerfully bactericidal defibrinated blood. The vaccinated blood was kept on the bench and then after three-quarters of an hour graduated dilutions of living staphylococci were implanted—the volume of staphylococcus suspension being always 2·5 c.mm. in 50 c.mm. of blood. After implantation the blood was filled into slide cells and incubated.

Variety of blood into which living microbes were implanted	Number of microbes per cent. which developed from an implantation of—							
	20,000	10,000	5,000	2,500	1,250	600	300	150
	living staph. per c.cm.							
Unvaccinated blood	7·4	5·2	6	5	16	6·6	0	0
Blood vaccinated with—								
12,000 dead staph. per c.cm.	1·5	1·4	0·8	1·6	1·6	3·2	0	0
6000 dead staph. per c.cm.	0·6	0·4	0·8	0·0	1·6	3·3	7	0
3000 dead staph. per c.cm.	6·1	1·6	5·2	8	1·6	3·2	0	0
1500 dead staph. per c.cm.	5	5·4	5·6	6	1·6	3·2	0	0

Details of the Bacterial Enumeration upon which the Figures in the Table are founded.— Here in each case 2·5 c.mm. of the 32-, 64-, and 128-fold dilution of the bacterial suspension used for the strongest implantation was incorporated into 50 c.mm. of serum, and then incultured. The number of colonies which developed in the serum implanted with the 32-, 64-, and 128-fold dilutions were respectively (the counting operations being conducted in duplicate) 31 and 30, 18 and 19, and 6 and 7. This (taking the first figures) works out as (30 × 20), an implantation of approximately 600 living staphylococci per c.cm. in column 6 of the table.

In studying the above experiment attention may be directed to three points :—

(1) All the vaccinated bloods kill here all along the line more microbes than the unvaccinated blood, the best results being in this particular case obtained with blood vaccinated with 6000 dead staphylococci. This blood in the case where 20,000 living staphylococci were implanted killed off per c.cm. 1300 more staphylococci than the unvaccinated blood ; and it killed off without residue 2500 as compared with 300 staphylococci.

(2) The second point is one of general theoretical importance. When comparable numbers of microbes are employed, these being, in the one case, all derived from a living culture, and, in the other case, in part from a living and in part from a sterilised culture, better results are in every case obtained with the blend. Thus, for example, we have in column 1 with an aggregate implantation of 20,000 living staphylococci a percentage survival of 7·4 and in column 2 with an aggregate implantation of 22,000 staphylococci, consisting of 12,000 dead and 10,000 living, a percentage survival of 1·4; and again, we have in column 2, with an aggregate implantation of 10,000 living staphylococci a percentage survival of 5·2, and in column 3, with an aggregate of 11,000 staphylococci, consisting of 6000 dead and 5000 living, a percentage survival of 0·8. It would thus seem—but one must be cautious in inference, because there are no cultures which contain only living microbes — that dead microbes are, micro-organism for micro-organism, more efficacious than living ; and that vaccines of sterilised cultures should be preferred to living vaccines, not only because their use is unassociated with risk of infection, but also because they are superior in quality.

(3) The third point is also of fundamental interest. And it is a point that has

already been considered. When in our assaying operations we introduce microbes into the whole blood or into a serum or pus fluid which contains leucocytes, or even, as in the opsonic technique, into an artificial mixture of washed leucocytes and serum, the microbes we implant may, despite of our having implanted them solely for the purpose of testing, evoke (this will depend on their number) a secondary epiphylactic response which will give increased anti-bacterial power such as is presumably achieved when slighter infections supervene upon a prophylactic procedure. Or again, the microbes we implant for the purpose of testing may, like graver infections supervening upon a prophylactic procedure, get the better of the blood resistance, and eat up, or more than eat up, the surplus of anti-bacterial power furnished by the original vaccinating operation. It will, in view of these superadded effects, be very difficult to assess the actual achievement of the prior vaccinating operation. Apparently the best that can be done in the way of assessment will be to get the leucocytes out of the way before we undertake our tests, and content ourselves with measuring the bactericidal and opsonising power of the serum. Consideration of the effect produced by the intravenous inoculation of vaccines upon the haemo-bactericidal power may with advantage be postponed until the argument of this lecture has been a little further developed.

Implanting and Explanting Experiments.

Up to the present we have been studying the results obtained by implanting living microbes separately or associated with dead microbes into the blood and inculturing. This method measures the destructive power of leucocytes and blood fluids operating in conjunction. But while it tells us what number of microbes are killed it tells us nothing about the pace of destruction, and nothing even indirectly about the agency by which the microbes are destroyed. And again the inculturing method has a definitely limited range of utility. For when over, let us say, 60 colonies develop in 50 c.mm. of blood accurate enumeration [1] begins to become difficult. The implanting and explanting method supplies what is wanted in these respects. It will be proper first of all to satisfy ourselves that the general results obtained by this method are conformable to those obtained by inculturing. And, inasmuch as we are here specially concerned with the verification of the proposition that epiphylactic response can be evoked in the blood *in vitro*, and is evoked only when we implant sufficiently heavily, it will be well to first study my blood whose feeble phylactic power makes it specially well adapted for the institution of experiments on epiphylactic response.

In these implanting and explanting experiments implantations of 2·5 c.mm. volumes of graduated dilutions of a suspension of living staphylococci were made into 50 c.mm. volumes of A. E. W.'s defibrinated blood. Then samples were taken from each implanted blood, and these were first diluted 25-fold and then spread out over the surface of agar plates. The first samples were explanted instantaneously —instantaneously being here understood to mean instantaneously after the blood and microbes had been duly mixed—and the other samples after longer intervals.

[1] This refers to the technique which was at the time available.

The implanted bloods were in each case kept not in the incubator but at the temperature of the air.

Experiment A

Blood vol.	Number of living staphylococci implanted—		Number of colonies which developed from that volume explanted after the stated interval				Number of staphylococci implanted into the samples		Number of colonies which developed out of those samples	
	per c.cm. of blood	per 2 c.mm. of blood	0′	30′	60′	120′				
1	16,500	33	26	20	27	23	132		96	
2	11,000	11	12	6	11	—	66	264	29	148
3	5,500	22	8	2	3	5	44		18	
4	2,750	5·5	2	1	1	1	22		5	
5	920	1·9	1	2	2	1·5	7·6		6·5	
6	460	·9	1	0·5	0	1·5	3·6	11·65	3	10
7	230	·45	0	0	0	0·5	·45		0·5	

Experiment B. (The same technique was employed)

Blood vol.	Number of living staphylococci implanted per c.cm. of blood	Bactericidal effect in sample explanted immediately			Bactericidal effect in the four samples explanted within three hours			
1	12,000	22	reduced to	8	88		15	
2	8,000	15	,,	10	60	182	15	42
3	4,000	7·5	,,	0	22·5	reduced to	5	
4	2,000	3·75	becomes	5	11·5		7	
5	800	2·25	,,	2	7·75		8	
6	546	1·5	,,	2	4·5	14·5 becomes	5	16
7	270	0·75	,,	0	2·25		3	

Experiment C. (Again the same technique was employed)

1	5400	10·8 reduced to 5	43	57·5	18	25
2	1800	3·6 ,, 1	14·5	reduced to	7	
3	360	0·75 becomes 1	3	4	3	3
4	170	0·25 ,, 0	1	becomes	0	

It will be seen that the conclusions which were reached by the inculturing method are here all confirmed. We again arrive at the result that the particular type of blood here in question exerts no bactericidal effect when only small numbers of staphylococci (1000 per c.cm. and fewer) are implanted, and that it exerts a very conspicuous effect when larger numbers of staphylococci are introduced. In the

three experiments here set out there is with the smaller implantations no killing whatever, and with the larger implantations an average killing of 60 per cent.

Experiment D

Here 7·5 c.mm. of an enumerated staphylococcus culture was implanted into 300 c.mm. of A. E. W.'s defibrinated blood and immediately afterwards a 30 c.mm. sample was explanted on to an agar plate. That done another 7·5 c.mm. of the staphylococcus suspension was implanted, and immediately another 30 c.mm. sample was explanted—the same procedure being followed throughout.

Number of staphylococci implanted per c.cm. of blood	Number implanted in the 30 c.mm. of blood used for explantation	Number of colonies which developed from that 30 c.mm. of blood	Percentage of survivors
400	12	11	99
840	25	10	40
1,310	40	—	—
1,850	56	18	32
2,450	75	26	34
3,125	100	17	17
4,625	140	48	29
6,300	190	69	31
8,150	245	108	44
10,250	310	148	48

The figures for the implantations were obtained by enumerating the staphylococcus suspension in 50 c.mm. volumes of serum. An implantation of 2·5 c.mm. gave 44 colonies, one of 7·5 c.mm. 140 colonies, and one of 10 c.mm. 135 colonies, giving a total of 319 colonies in the 20 c.mm. of suspension implanted. This works out as 16 staphylococci in 1 c.mm., and 12 in 0·75 c.mm., the volume of the bacterial suspension in the 30 c.mm. of blood used for the first explantation.

Further, these experiments bring out—and this is their new contribution—that of the total of microbes killed by the blood the larger proportion (here two-thirds of those killed) are killed instantaneously.

Let me in connexion with this make clear the following points : (1) Subitaneous killing of microbes is obtained practically invariably. Where it fails to occur that may perhaps depend upon the antigen not being brought into solution. (2) The fact that microbes can be killed instantaneously by the evoking of epiphylactic response has, as we shall presently see, very important practical applications in connexion with the treatment of bacterial disease. (3) The fact that microbes are killed instantaneously and at ordinary temperatures throws important light on the mode of bacterial destruction and on the nature of epiphylactic response. If we had had to depend here only upon the inculturing method we might perhaps have supposed that the implanting of a sufficient quantum of microbes led to an activation of the machinery of phagocytosis. But when we learn, as we do from these experiments, and also from the heating experiments reported on p. 92 *infra*, that

microbes are killed instantaneously at room-temperature at which phagocytosis begins only after very appreciable delay, we are compelled to conclude that subitaneous killing cannot be due to phagocytosis. It must therefore perforce be attributed to a bactericidal action of the serum.

The generalisation that subitaneous killing of microbes in the blood is due to a bactericidal action exerted by the serum furnishes in its turn a new articulating point upon which a further framework of generalisations can be assembled. From the fact that the blood can kill microbes instantaneously it can be inferred that a bactericidal serum also must be competent to kill instanteously. Further, when we have realised that the serum receives, when we vaccinate the blood, a quite sudden access of bactericidal power, and when we reflect that where massed leucocytes washed free from serum are imposed upon agar implanted with staphylococci or streptococci, these [1] last are killed extracellularly and by chemical agency, we are almost inevitably conducted to the conclusion that the bactericidal elements found in vaccinated bloods are derived from the leucocytes, and that leucocytes can, under the influence of a vaccinating dose of microbes, instantaneously export their anti-bacterial elements into the surrounding blood fluids.

We shall, as we proceed, see all of these inductions confirmed by actual experiment. But here another issue must take precedence. I have already elsewhere [2] shown that when epiphylactic response is evoked by adding vaccine to blood the serum becomes bactericidal, or as the case may be, more bactericidal, not only to the species of microbe which has furnished the vaccine, but also to many non-homologous microbes. This does not stand by itself. It would seem that all the anti-bacterial elements that are brought forth in the blood—and it will presently appear that opsonic substances also are generated—are polytropic.

It is unnecessary to say much in the way of general comment on the fact that the anti-bacterial substances here in question are polytropic. For while established notions prejudice us against the idea of vaccination conferring a non-specific protection, reflection puts out of court all alternative suggestions. In the first place, where the blood kills microbes instantaneously there is time for delivery but not for manufacture of anti-bacterial elements. And again, it is much more difficult to conceive that the leucocytes should have stored up in their protoplasm special monotropic bactericidal substances and opsonins for each and every description of microbe than to conceive of the leucocytes being furnished with a common store of polytropic anti-bacterial elements which could be brought into operation upon all microbic invaders without distinction of species.

And in this connexion something further may be added. When once we have pictured to ourselves that non-specific anti-bacterial substances can, under the stimulus of any antigen, be exported from leucocytes, it does not perhaps involve a much more adventurous flight of imagination to conceive that an effective vaccinating stimulus might be supplied to the leucocyte by chemical agents other than

[1] Wright, Fleming, and Colebrook: 'Sterilisation of Wounds by Physiological Agency', *vide Collected Researches*, Vol. I, pp. 93–113; Wright and Colebrook: *Technique of the Teat and Capillary Tube*, pp. 279–283.

[2] See *supra*, pp. 50–54 and 57–65.

bacterial antigens. Certain clinical and also experimentally established facts would very well harmonise with such a conception. Here would come the fact that emetin, though not directly nocuous to the *Amoeba histolytica*, can rid the intestine of that infection. To this might be added the fact that salvarsan can in syphilis, complicated with a heavy streptococcic infection, very rapidly rid the body of the latter —a fact which should be brought into the same visual field with the observations of S. R. Douglas and L. Colebrook, which establish that after salvarsan and neosalvarsan injections the blood becomes for a period of a few hours bactericidal to streptococcus and staphylococcus. And again, there might be added the observations of Walbum,[1] which show that the yield of diphtheria antitoxin and of agglutinins to the *Bacillus coli* can be very appreciably increased by the exhibition of manganese chloride and other metallic salts.

PART II

We are now in a position to examine in more detail the nature of epiphylactic response. Certain very simple reconnoitring experiments give us our bearings. The experiments in question consist in killing the leucocytes, in some cases before, in other cases after, implanting microbes into the blood and seeing how far this killing of leucocytes[2] affects bacterial destruction. An illustrative experiment is subjoined. Here before the microbes were implanted, the blood was heated to a temperature of 46° C.–48° C., this being a temperature which kills the leucocytes and leaves the serum, and if not kept up unduly long, also the microbes unaffected.

Here graduated implantations of staphylococcus were made by the ' wash and after-wash ' method into a series of volumes of A. E. W.'s blood, these volumes being drawn up one after the other into a long-stemmed capillary tube.

—	Number of colonies which developed in vols.															Total in the last 12 vols.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
A (1)	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
A (2)	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B (1)	+	+	+	3	3	2	1	1	0	1	0	1	1	0	0	13
B (2)	+	+	+	5	5	2	0	0	0	0	1	0	0	0	0	13

A = Unheated blood. B = Blood pre-heated to 48° C.

The sign + is employed to indicate that the colonies were too closely packed to allow of accurate enumeration.

It will be clear from this quite typical experiment that the killing of leucocytes makes a very profound difference. The pre-heating of the blood abolishes or nearly abolishes, in two several ways, the bactericidal power of the blood for staphylococci

[1] *Communications de l'Institut Sero-thérapique de l'État Danois*, Tome xii, 1922.

[2] I had before this paper was published shown that the filtering out of leucocytes from the blood removes all its staphylo-bactericidal power, and Fleming had extended these observations, and had suggested a better way of filtering blood.

and streptococci. First, it puts an end to phagocytic action (in the case of my own blood that would apparently not count for much); and further (and this in all bloods counts for a great deal more) it abolishes also the power of epiphylactic response, the blood being then left with only such microbicidal powers as it possessed *ab origine*. With respect to the experiments in which the blood, after microbes have been implanted, is heated sufficiently to kill the leucocytes and not sufficiently to affect the serum or injure the implanted microbes, these experiments give, as you will expect to hear, results which are in absolute agreement with those obtained in implanting and explanting experiments.

When we implant into a blood and then immediately apply a temperature of 46°–48° C. we obtain very much less growth than in an ante-heated blood. When the application of heat is postponed for a minute, appreciably more microbes are killed; and when the implanted blood is allowed to lie upon the bench for an hour or so before it is heated the cultural results are practically the same as in a blood left unheated.

Experiments which show that when Epiphylactic Response is Evoked in the Blood the Bactericidal Power of the Serum is Increased.

Such experiments as those cited in which the blood is heated either before or after the implantation of microbes are, it must be remembered, only reconnoitring experiments. They do not make it absolutely certain that it is by the action of the leucocytes or almost exclusively by their action that staphylococci and streptococci are killed in the blood. And again, these heating experiments—and the same applies, of course, to all experiments made with the whole blood—tell us nothing except by way of indirect inference with regard to the manner in which the killing of the microbes is accomplished. To arrive at certain knowledge we must experiment with each of the elements of the blood separately.

We may advantageously begin by satisfying ourselves that epiphylactic reponse goes hand in hand with a development of increased bactericidal power in the serum. As I have already elsewhere [1] shown that this holds true of human blood vaccinated *in vitro*, I may profitably here cite experiments of a different kind—experiments in which vaccines of dead microbes are inoculated intravenously into animals and man, and experiments in which the sera are tested immediately and at different time-intervals after inoculation.

Experiments on Rabbits.

Here a vaccine of dead microbes was inoculated intravenously, samples of blood were withdrawn before and at intervals after inoculation, and then similar implantations of living staphylococci were by the wash and after-wash method made into a sequence of 10 c.mm. volumes of each serum. The figures in the table show the number of staphylococci which grew out into colonies in the different sequences of 10 c.mm. volumes—the numbers being in each case expressed in terms of microbes per c.cm. of serum.

[1] See *supra*, p. 90, footnote 2.

	No. of Microbes which formed colonies in the sera of bloods drawn off before and after injection of vaccine							Additional number of microbes killed by the serum after injection of vaccine
	Before	1 min. after	15 min. after	30 min. after	1 hr. after	3 hrs. after	6 hrs. after	
Rabbit 1. Inoculated with 20,000 staph. per c.cm. of blood	5000	100	—	100	—	0	0	At least 5000 per c.cm.
Rabbit 2. Inoculated with the same dose of staph. as Rabbit 1	2200	1200	200	—	—	400	0	At least 2200 per c.cm.
Rabbit 3. Inoculated with 14,000 streptococcus pyogenes per c.cm. of blood	5000	500	0	—	0	—	0	At least 5000 per c.cm.

Staph. = Staphylococci.

Experiments on Man.

1. Here 250,000 dead streptococci—i.e., about 40 streptococci per c.cm.—were injected intravenously into A. E. W.'s blood, samples of blood were drawn off before and after the injection, and the bactericidal powers of the sera were tested by implanting by the wash and after-wash method graduated doses of living staphylococci into a sequence of equal volumes of serum, filled in each case into a long capillary tube.

—	Positive or negative result of culture in successive unit-vols. of serum															Number of vols. which showed growth	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1. (*a*)	+	+	+	+	+	+	+	+	+	+	?	?	+	+	+	13	14
1. (*b*)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15	
2. (*a*)	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	7	8
2. (*b*)	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	9	
3. (*a*)	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	6	$6\frac{1}{2}$
3. (*b*)	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	7	

1. Serum immediately before injection. 2. Serum 2 min. after injection. 3. Serum 15 min. after injection.

2. Here a dose of streptococcus vaccine equivalent to some 15 streptococci per c.cm. of circulating blood was inoculated intravenously into a patient suffering from a streptococcus infection. Samples of blood were taken immediately before, immediately after, and 24 hours after the injection. The sera were tested by implanting with living staphylococci by the 'wash and after-wash' method in each case into a sequence of one 20 c.mm. and two 10 c.mm. volumes of serum.

		Number of colonies which developed in—				
		Vol. 1	Vol. 2	Vol. 3	In the 3 vols.	
Serum of blood before inoculation	(*a*)	11	1	1	13	13·5
	(*b*)	13	1	0	14	
Serum of blood immediately after inoculation	(*a*)	8	1	0	9	7
	(*b*)	5	0	0	5	
Serum 24 hours after	(*a*)	15	0	0	15	14
	(*b*)	13	0	0	13	

The difference of 6·5 colonies in 40 c.mm. of serum corresponds to a killing of 162 more staphylococci per c.cm. of serum.

If we take into consideration only the first, more heavily implanted 20 c.mm. volumes we have a difference of 5·5 colonies corresponding to a killing of 275 staphylococci per c.cm.

3. Here a dose of vaccine corresponding to about 15 streptococci per c.cm. of circulating blood was inoculated intravenously into a patient suffering from a heavy cold, followed by a further intravenous injection of 10 dead streptococci per c.cm. of blood 24 hours after, when the cold was decidedly better.

The sera were tested by implanting 5 c.mm. of a staphylococcus suspension into 100 c.mm. volumes of serum.

August 23rd, 1922 : Serum before inoc. furnishes 76 staph. colonies.

Serum immediately after inoc. furnishes 63 staph. colonies.

The difference of 13 colonies per 100 c.mm. corresponds to a killing of 130 more staphylococci per c.cm. of serum.

August 24th, 1922 : The sera were here tested in the same way but, of course, with a different staphylococcus suspension.

Serum immediately before inoc. furnishes 40 colonies.
,, ,, after ,, ,, 30 ,,
,, 24 hours ,, ,, ,, 40 ,,

The difference of 10 colonies in 100 c.mm. corresponds to a killing of 100 more staphylococci per c.cm.

4. Intravenous inoculation of a streptococcus vaccine into a normal man.

May 10th, 1922 : 500,000 dead streptococci were inoculated, and the sera were tested by implanting a 20 c.mm. wash of a staphylococcus suspension in each case into three 20 c.mm. volumes of serum.

The volumes of serum immediately before inoculation furnished—
36, 38, and 30 colonies—average 35.

Those of serum immediately after inoculation furnished—
27, 34, and 29 colonies—average 30.

Those of serum 20 hours after inoculation furnished—
23 and 28 colonies—average 25.

May 15th, 1922: same man; 800,000 dead streptococci were inoculated. The sera were tested as before with streptococcus.

The volumes of serum immediately before inoculation furnished—
13, 16, and 9 colonies—average 13.

The volumes of serum immediately after inoculation furnished—
7 and 8 colonies—average 7·5.

The volumes of serum 24 hours after inoculation furnished—
16 and 19 colonies—average 17·5.

Calculation shows that the blood immediately after the first inoculation killed 250 more staphylococci per c.cm., and 20 hours after 500 more per c.cm.: and that it killed immediately after the second inoculation 275 more per c.cm.

5. Intravenous inoculation of streptococcus vaccine into a normal man.

Dec. 29th, 1922: Sera from bloods drawn off before, 1½ hours after, and 5 hours after the inoculation of 160,000 dead staphylococci; 50 c.mm. volumes of each serum were implanted with 2·5 c.mm. of a 2700-fold dilution of a broth culture of staphylococcus.

Serum before inoculation furnished 47 colonies.
Serum 1½ hours after ,, ,, 21 ,,
Serum 5 hours after ,, ,, 42 ,,

Calculation here shows that the serum 1½ hours after inoculation kills 520 more staphylococci than before.

6. Streptococcus endocarditis.

Sept. 16th, 1922: Sera from bloods drawn off before and immediately after an intravenous inoculation of 25,000 dead staphylococci. The technique employed and the staphylococcus suspensions employed were the same as in the last case.

Serum Before Inoculation.—Implanted with the more dilute suspension of staphylococcus furnishes 35 and 48 colonies (average 40·5); implanted with the more concentrated suspension 71 and 83 colonies (average 77).

Serum Immediately After Inoculation.—Implanted with the more dilute suspension furnishes 32 and 34 colonies (average 33); implanted with the more concentrated suspension 65 and 66 colonies (average 65½).

This, taking again the figures for the heavier implantation, works out as an increased killing of 11·5 per 50 c.mm. and 230 for 1 c.cm. of serum.

Experiments which Show that the Vaccination of Blood may Increase the Opsonic Power of Serum and also the Phagocytic Power of Blood.

Let me now go on to show that when epiphylactic response is evoked in the blood the opsonic power of the serum, and *pari passu* with this, if the phagocytic efficiency of the leucocytes is not reduced, the phagocytic power of the blood is increased.

Reference has already been made to the fact that the inoculation of the blood with bacterial vaccines increases the opsonic power of the serum. This development of opsonic power proceeds on quite similar lines to the development of bactericidal power. It manifests itself both *in vitro* and *in vivo*, and it begins practically immediately.

Experiments in which Vaccines were Inoculated Intravenously in Rabbits.

Rabbit 1.—Inoculated with 10 million of dead staphylococci (about 40,000 per c.cm.). *Opsonic power of serum*—before inoculation, 1 ; 3 minutes after, 2·9 ; 1 hour after, 2·3 ; $1\frac{1}{2}$ hours after, 3·0.

Rabbit 2.—Inoculated with 5 million of dead staphylococci (about 20,000 per c.cm.). Opsonic power of serum—before inoculation, 1 ; 3 minutes after, 1·2 ; $2\frac{1}{4}$ hours after, 1·3.

Experiments in which Vaccines were Inoculated into Human Blood *in Vitro* and *in Vivo*.

Experiment 1.—Intravenous inoculation of about 40 streptococci per c.cm. into A. E. W.'s blood. Blood *1* was drawn immediately before ; Blood *2* immediately after ; and Blood *3* 15 minutes after inoculation. The opsonic power of the sera and the phagocytic efficiency of the leucocytes were then measured—the opsonic power by taking 1 volume of serum, 1 volume of the staphylococcus suspension, and 1 volume of washed leucocytes ; the phagocytic efficiency by comparing the microbic intake obtained when the microbic suspension was used with the same sera and different leucocytes.

—	Staphylo-phago-cytic count	Staphylo-opsonic index	Average ingest with the 3 sera	Phago-cytic efficiency
Washed corps. of Blood *1*				
+ serum 1 + staph.	3·4	1		
+ ,, 2 + ,,	5·5	1·6	5	1
+ ,, 3 + ,,	6	1·8		
Washed corps. of Blood *3*				
+ serum 1 + staph.	3·5	1		
+ ,, 2 + ,,	3·9	1·1	4	0·8
+ ,, 3 + ,,	4·8	1·4		

I want to draw attention here to three points : To the fact that the opsonic index of the blood goes up instantly ; to the fact that the phagocytic efficiency of the leucocytes goes down ; and to the fact that the effect of the increased opsonic power is in this case partially masked by the decreased leucocytic efficiency. This

comes out when we regard serum 3, and note that it gives with the corpuscles of blood 1 an average ingest of 6, and with the corpuscles of blood 3 an ingest of 4·8 only.

We shall presently see that the changes which occur in the blood in acute streptococcic infections follow this pattern except only in the respect that we have there usually a smaller rise in the opsonic index and a much profounder fall in the leucocytic efficiency.

In the next experiment (II) it will be seen there is : (1) A notable increase of strepto-phagocytic power obtained with both varieties of vaccine. (2) Further, in each case when a certain dose is exceeded the phagocytic power begins again to decline. Other results show that this decline is probably due to the increased opsonic power of the serum being now counterbalanced by a poisoning of the leucocytes. (3) In this particular case a greater rise in the streptophagocytic power is obtained with the homologous vaccine. This might, of course, be due to the homologous character of the vaccine, but it might also be due to the dose of streptococcus having been here more fortunately chosen, either from the point of view of its evoking a better opsonic response, or from the point of view of its being less toxic to leucocytes.

The same probably holds true of all the following experiments. In connexion with the sixth experiment of the series, it will, on comparing the phagocytic indices with the opsonic indices—i.e., on comparing the amount of phagocytosis obtained with the leucocytes of the vaccinated blood with that obtained with the leucocytes of the unvaccinated blood—be seen that increase in the opsonic power of the serum is to a large extent masked by the poisoning or exhaustion of the leucocytes.

Experiment 2.—Here, with a view to finding out what would be the better kind and optimum quantum of vaccine to add to a prospective donor's blood for the purposes of immuno-transfusion, graduated additions of typhoid and streptococcus vaccines were made to his defibrinated blood *in vitro*. The samples of blood were incubated with the vaccine for one hour *in vitro*, and were then tested for phagocytic power by adding one volume of streptococcus suspension to two volumes of defibrinated blood.

—	Strepto-phagocytic index	—	Strepto-phagocytic index
Unvaccinated blood	1	Unvaccinated blood	1
Blood vaccinated with typhoid bacilli :		Blood vaccinated with strep-tococci :	
1000 per c.cm.	1·7	20 per c.cm.	3·6
2000 ,,	2	40 ,,	4
4000 ,,	1·35	80 ,,	2
8000 ,,	1·75	200 ,,	2·3

Experiment 3.—Here a number of volumes of A. E. W.'s defibrinated blood were inoculated by the wash and after-wash method with graduated dilutions of

staphylococcus vaccine. After incubation for half an hour at 37° C. their phagocytic power was tested with a staphylococcus suspension containing about 200,000,000 staphylococci per c.cm.

—	Staphylo-phagocytic index	—	Staphylo-phagocytic index
Unvaccinated blood	1	Bloods vaccinated with staph. per c.cm.	
Bloods vaccinated with staph :		Circ. 10,000	1·9
Circ. 2500 per c.cm.	1·3	,, 40,000	2·1
,, 5000 ,,	1·5	,, 80,000	1·55

Experiment 4.—Portions of the same blood were inoculated by the same technique with typhoid vaccine, the phagocytic power being afterwards tested with staphylococcus.

—	Staphylo-phagocytic index	—	Staphylo-phagocytic index
Unvaccinated blood	1	Bloods vaccinated with typhoid bacilli per c.c. :	
Bloods vaccinated with typhoid bacilli :		Circ. 20,000	0·6
Circ. 5,000 per c.cm.	1·4	,, 40,000	0·8
,, 10,000 ,,	1·45		

Experiment 5.—Same blood, same technique, and same typhoid vaccine were employed.

	Staphylo-phagocytic index
Unvaccinated blood	1
Blood vaccinated with :	
Circ. 1,500 per c.cm. typhoid bacilli	1·65
,, 3,000 ,, ,,	1·7
,, 6,000 ,, ,,	1·0
,, 12,000 ,, ,,	0·85

Experiment 6.—Same blood, the same technique, and the same typhoid vaccine were employed.

—	Staphylo-phagocytic index	—	Staphylo-opsonic index
Unvaccinated blood	1	Serum of unvaccinated blood	1
Blood vaccinated with typhoid bacilli :		Serum of blood vaccinated with typhoid bacilli :	
Circ. 4000 per c.cm.	1·25	Circ. 4000 per c.c.	1·95
,, 8000 ,,	1·1	,, 8000 ,,	1·75

Experiment 7.—Here a series of volumes of L. C.'s defibrinated blood were implanted with typhoid vaccine and were then immediately centrifugalised. The serum was then quickly pipetted off, and was tested for opsonic power by adding washed corpuscles and staphylococcus suspension.

—	Staphylo-opsonic index	—	Staphylo-opsonic index
Serum from unvaccinated blood	1	Serum from blood vaccinated *in vitro* with typhoid bacilli:	
Serum from blood vaccinated *in vitro* with typhoid bacilli:		12,500 per c.cm.	1·19
3000 per c.cm.	1·2	25,000 ,,	1·2
6250 ,,	1·38	50,000 ,,	1·0

It will be seen that here *in vitro*, as in Experiment *1*, *in vivo*, opsonic substances are delivered into the blood immediately—that is to say, they are furnished within the time that it takes to separate the serum from the corpuscles.

Derivation of Bactericidal and Opsonic Substances from the Leucocytes.

The leucocytes provide the bactericidal and the opsonic substances that appear in the serum when epiphylactic response is evoked in the blood. To make sure of this we must first isolate the leucocytes from the other elements of the blood, must then make graduated additions of vaccines to serum (or other menstruum), bring this into contact with the leucocytes, and then test its opsonic and bactericidal power. These requirements—and, of course, only the first presents any technical problem—are, as a matter of fact, easily satisfied. The leucocytes can be obtained entirely separate, for they will, if provided with facilities for doing so, emigrate from the blood. All they require is congenial warmth, a meshwork of fibrin to travel along, and a firm surface—such, for example, as the glass floor of 'open emigration cells' or the walls of capillary pipettes—to collect upon.

Where open emigration cells, such as are shown in Fig. 3 A, are employed, these are, as in Fig. 3 B, filled brim full with blood taken direct from the finger.[1] After incubation in a moist chamber for from one-half to three-quarters of an hour the clots are washed off with physiological salt solution. Where capillary pipettes are employed, the blood from the finger is drawn up into the stems, a little normal salt is aspirated after an air bubble into the distal orifice of the pipette, and then a little more salt solution is introduced into the proximal part of the stem. The pipettes are then incubated for from half to three-quarters of an hour. After this the tip of the stem is broken across, a teat is then fitted to the barrel of the pipette, and the clot is slowly evacuated.

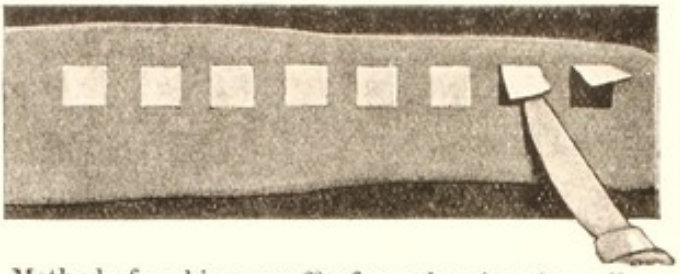
An emigration cell whose floor is coated with emigrated leucocytes may conveniently be called a *leucocyte carpeted cell*, and a capillary tube similarly coated may be called a *leucocyte lined tube*—the terms *uncarpeted* and *unlined* would then serve to denote control cells and capillary tubes with naked floors and walls.

[1] Wright and Colebrook: *Technique of the Teat and Capillary Glass Tube*, Constable, 1921.

The following experiments were carried out :—

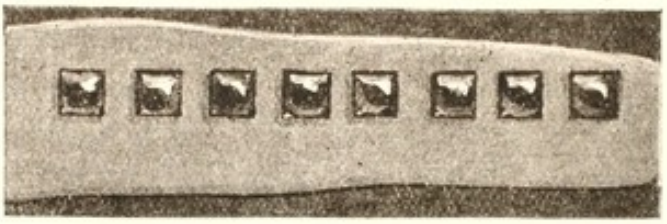
I. Carpeted and uncarpeted emigration cells. A. E. W.'s leucocytes and serum. Here a 70-, a 300-, and a 600-fold dilution were made from a broth culture of staphylococcus. Of the first 5 c.mm. were implanted into 100 c.mm. of serum, and of the second and third 2·5 c.mm. into 50 c.mm. In each case half of the implanted serum

FIG. 3.—A.



Method of making paraffin-framed emigration cells.

B.



Blood imposed on emigration cells.

was imposed on a carpeted and half on an uncarpeted cell. The two portions of serum were then, after a fixed interval, during which they were kept in a moist chamber on the bench, aspirated into fine capillary pipettes. These pipettes were then incubated for 24 hours, and the colonies which developed were counted.

(1) Serum with the heaviest implantation (which works out as 2200 staphylococci per c.cm.).

	Colonies
25 c.mm., placed on an uncarpeted cell and immediately re-aspirated furnished	52 56
25 c.mm., placed on a carpeted cell and re-aspirated after 45 min. furnished	26 15

(2) Serum with the intermediate implantation (which works out as 520 staphylococci per c.cm.).

	Colonies
25 c.mm., placed on an uncarpeted cell and re-aspirated after 45 min. furnished	13
25 c.mm., placed on a carpeted cell and re-aspirated after 45 min. furnished ..	7

(3) Serum with the lightest implantation (which works out as 160 staphylococci per c.cm.

	Colonies
25 c.mm., placed on an uncarpeted cell and re-aspirated after 45 min. furnished	4
25 c.mm., placed on a carpeted cell and re-aspirated after 45 min. furnished ..	2

(4) A supplementary volume of serum with the same implantation as in (2).

	Colonies
25 c.mm., imposed on an uncarpeted cell and re-aspirated after 45 min. furnished	14
25 c.mm., imposed on the carpeted cell employed in (1) and re-aspirated after 45 min. furnished	2

II. Carpeted and uncarpeted emigration cells. I. F.'s leucocytes and serum. Same technique.

Serum implanted with circ. 4800 staphylococci per c.cm. imposed on an *uncarpeted* cell and aspirated after 5 mins. furnished innumerable colonies.
Serum implanted with circ. 4800 staphylococci per c.cm. imposed on a *carpeted* cell and aspirated after 5 mins. furnished 79 colonies.

Serum implanted with circ. 1200 staphylococci per c.cm. imposed on an *uncarpeted* cell for 5 mins. furnished 30 colonies.
Serum implanted with circ. 1200 staphylococci per c.cm. imposed on a *carpeted* cell for 5 mins. furnished 14 colonies.

Serum implanted with circ. 520 staphylococci per c.cm. imposed on an *uncarpeted* cell for 5 mins. furnished 13 colonies.
Serum implanted with circ. 520 staphylococci per c.cm imposed on a *carpeted* cell for 5 mins. furnished 2 colonies.

The possibility that the diminution in the number of microbes in the serum which had been imposed upon carpeted cells might be due to a percentage of the microbes sticking to the leucocytes is negatived by considering the effect of implanting in duplicate into broth progressive dilutions of a staphylococcus culture, and explanting the one series of volumes directly, and the other after imposition upon carpeted cells. An experiment of this kind gave the following result :—

Series of volumes of broth explanted directly on to agar gave 33, 14, 7, 5, and 4 colonies—in all 63 colonies.
A similar series explanted on agar after imposition on a carpeted cell gave 33, 20, 10, 2, and 1 colony—in all 66 colonies.

III. Carpeted and uncarpeted emigration cells, and also lined and unlined capillary pipettes. A. E. W.'s leucocytes and 30 c.mm. volumes of serum. A 120-, a 240-, a 480-, and a 960-fold dilution were made of a broth culture of staphylococcus. 2·5 c.mm. volumes of the last two dilutions were implanted into 50 c.mm. volumes of broth which were then aspirated into very fine capillary tubes. The two heavier implanted volumes furnished 55 and 54 colonies ; (a similarly implanted volume of serum furnished 47). The two more lightly implanted volumes of broth furnished 25 and 27 colonies.

A series of 30 c.mm. volumes of serum were now implanted with 2·5 c.mm. of the different staphylococcus suspensions ; were then imposed in each case for 30

minutes on carpeted emigration cells, and then drawn up into capillary tubes and incubated.

Vol. 1 implanted from the 120-fold dilution of staph. (i.e., with about 220 staph.) furnished	106	
Vol. 2 implanted from the 240-fold dilution of staph. (i.e., with about 110 staph.) furnished	25	
Vol. 3 implanted from the 480-fold dilution of staph. (i.e., with about 55 staph.) furnished	29	28
Vol. 4 implanted from the 480-fold dilution of staph. (i.e., with about 55 staph.) furnished	27	
Vol. 5 implanted from the 960-fold dilution of staph. (i.e., with about 28 staph.) furnished	22	19
Vol. 6 implanted from the 960-fold dilution of staph. (i.e., with about 28 staph.) furnished	16	

Calculation shows that here with an implantation of about 7500 staphylococci per c.cm. we have a killing of 50 per cent., with an implant of about 3600 a killing of 77 per cent., with an implant of 1800 a killing of 50 per cent., and with an implant of 900 a killing of only 27 per cent.

Supplementary Experiment.—Three 50 c.mm. volumes of serum, implanted respectively with 2·5 c.mm. of the 120-, 240-, and 480-fold dilution of the staphylococcus culture, were drawn up into lined capillary tubes. All these volumes remained sterile. This, if we take the figures for the heaviest implantation, works out as equivalent to the killing of 7200 staphylococci per c.cm. by the leucocytes obtained from 1 c.cm. of blood.

IV. Lined and unlined capillary pipettes. A. E. W.'s leucocytes and 40 c.mm. volumes of his serum. A 150-, a 300-, a 600-, a 1200-, a 2400-, and a 4800-fold dilution were made of a staphylococcus broth culture.

2·5 c.mm. of the 4800-fold dilution gave in serum in *unlined* tubes 17 and 18 colonies.

2·5 c.mm. of the 2400-fold dilution gave in serum in *lined* tubes 15 and 17 colonies. In *unlined* tubes in serum 27 and 31 colonies and in an *unlined* tube in broth 31 colonies.

2·5 c.mm. of the 1200-fold dilution gave in serum in *lined* tubes 19 and 15 colonies, as compared with a calculated number of 60 for serum in an *unlined* tube.

2·5 c.mm. of the 600-fold dilution gave in serum in a *lined* tube 21 colonies, as compared with a calculated number of 120 for serum in an *unlined* tube.

2·5 c.mm. of the 300-fold dilution gave in serum in a *lined* tube 15 colonies, as compared with a calculated number of 240 for serum in an *unlined* tube.

2·5 c.mm. of a 150-fold dilution gave in serum in a *lined* tube 10 colonies, as compared with a calculated number of 480 for serum in an *unlined* tube.

In the case of the lined tubes the colonies that grew were in each case confined to the very narrow distal end of the capillary stem where the lining of leucocytes

was probably deficient. Taking the figures for the most heavily implanted serum as they stand they testify to a killing of 63 per cent. with an implantation of 750 staphylococci per c.cm., a killing of 72 per cent. with an implant of 1500 per c.cm., a killing of 82·5 per cent. with an implant of 3000 per c.cm., a killing of 94 per cent. with an implant of 6000 per c.cm., and a killing of 98 per cent. with an implant of 12,000 per c.cm.

V. Lined and unlined capillary pipettes. A. E. W.'s leucocytes and 50 c.mm. volumes of his serum. A 30-, a 150-, a 300-, a 600-, and a 2400-fold dilution were made of a broth culture of staphylococcus.

2·5 c.mm. of the 2400-fold dilution gave in serum in a *lined* tube 0 colonies, in *unlined* tubes 6 and 10 colonies.

2·5 c.mm. of the 600-fold dilution gave in serum in a *lined* tube 6 colonies, as compared with 27 in an *unlined* tube.

2·5 c.mm. of the 300-fold dilution gave in serum in a *lined* tube 7 colonies, as compared with a calculated number of 54 for serum in an *unlined* tube.

2·5 c.mm. of the 150-fold dilution gave in serum in a *lined* tube 0 colonies, as against a calculated number of 108 for serum in an *unlined* tube.

2·5 c.mm. of the 30-fold dilution gave in serum in a *lined* tube 100 colonies, as compared with a calculated number of 500 for serum in an *unlined* tube.

Here again the colonies (except in the heaviest implanted serum where they were distributed over the whole length) were all confined to the distal extremity of the pipette.

Leaving this out of consideration and taking the figures as they stand calculation shows that with an implantation of 10,000 staphylococci per c.cm., 80 per cent. were killed; with an implant of 2000, 100 per cent.; with an implant of 1000, 87 per cent.; and with an implant of 560, 78 per cent.

We thus see that the laws governing epiphylactic response are the same for the leucocytes and serum as for the whole blood. Instead of obtaining with increasing implantations progressively less efficient killing (as we inevitably should if the bactericidal power were not enhanced by epiphylactic response) we obtain with increasing implantations (up to a certain limit) more and more effective killing.

I have now come to the end of what I have to say about the modus operandi of vaccines.

PART III

Résumé of the Conclusions Reached in the Foregoing Inquiry.

We have seen that epiphylactic response can be evoked in the blood by vaccines; that it can be evoked both *in vitro* and *in vivo*; that it is characterised by a sudden increase in the bactericidal and opsonic power of the serum, and that this increase is the result of a sudden evacuation of polytropic bactericidins and opsonins from the leucocyte. We have learned also something about the conditions under which epiphylactic response can be obtained. We have seen that when we vaccinate

a normal blood *in vitro* with dead microbes and then test it with living, and also when we test a patient's blood which has been acted upon by antigens *in vivo*, the event will depend upon the total of antigen which has been brought into operation in the vaccinating procedure or auto-inoculation on the one hand, and the assaying procedure on the other.

With respect to the causative nexus between the application of excessive doses of vaccines and the development of apophylactic effects there is still a very serious lacuna in our knowledge. Seemingly the apophylactic effects produced by staphylococcus vaccine are different in origin and kind from those produced by typhoid and cholera vaccine. Whereas typhoid and cholera exert their apophylactic effect when brought into contact with serum, staphylococcus vaccine operates apophylactically only when brought in contact with the whole blood. This would seem to warrant the inference that when staphylococcus vaccine reduces the anti-bacterial power of the blood it does so not by adsorbing anti-bacterial elements from the serum, but by inciting the leucocytes to excrete into the serum some element which favours the growth of microbes.

Let me—asking you to remember that I cannot do any justice to the subject in the space now left to me—briefly indicate to you to what uses it may be possible to put the new information now in our possession.

Practical Applications.

Preparation of Vaccines and Control of the Efficacy and Dosage.

We can put it to account in the first place in the preparation of bacterial vaccines. It will be clear that every point in connexion with the preparation of vaccines can be practically tested by the procedures that have been described above. For example, it will be possible to set on one side all those statistical inquiries which everyone proposes but nobody really carries out, and to ascertain directly whether one particular strain of microbes gives a better vaccine than another ; whether one particular sterilising temperature is better than another ; and whether it will be justifiable or unjustifiable to employ this or that particular vaccine in combating this or that infection. Further, we have here methods which will enable a comparison to be made between different kinds of vaccines. It will therefore henceforth be possible to call upon those who claim superiority for a particular brand of vaccine to show scientific justification for their claims. Lastly, it will be possible in the case of every vaccine to determine what is the dose which will give optimum response.

Here, however, we are launched upon a question which teems with difficulties and ambiguities. I will just indicate what they are and pass on. Consideration will show that when we speak of the optimum dose of a vaccine we really have in view a particular mode of administration and some approximate body weight (generally that of an average adult), and also a particular condition of the organism—the condition of being uninfected, or, as the case may be, lightly or heavily infected. Ordinarily, in speaking of the optimum dose, we have in view—and this is the particular point I wish here to consider—subcutaneous inoculation, this channel of

administration being chosen, upon the assumption that protective substances are elaborated by the tissues at the site of inoculation. Doubts, however, are cast upon the doctrine of the derivation of the protective substances from the tissues by the following general considerations: (1) When we implant lint impregnated with vaccines into the tissues, and then squeeze out that lint we obtain from it a lymph which has no whit more anti-bacterial potency than that obtained from a companion piece of plain sterile lint implanted elsewhere in the body. (2) The companion pieces of lint here in question are permeated with leucocytes, and the fluids obtained from them are indistinguishable, and contain in each case bactericidal substances which are in their range of action precisely similar to those which have been obtained directly from leucocytes. (3) Bactericidal and opsonic substances can, as has been seen above, be obtained by the operation of vaccines upon the blood *in vitro*—in other words, in conditions where the intervention of the tissues is definitely excluded.

When these facts are regarded hypodermic inoculation stands out under a very different light. Its paramount advantage would appear to lie in the circumstance that the vaccine is under favourable circumstances fed slowly and continuously into the blood. But where a large dose of vaccine is administered and carried into the blood too rapidly a formidable negative phase is bound to supervene. (It will be remembered that when the patient was not kept quiet after typhoid inoculation very severe negative phases used often to follow.) And, again, it will be clear that where a small dose of vaccine is given and convection into the blood takes place very slowly, the quantum of antigen in the blood may never arrive at the concentration required for the production of epiphylactic response. These uncertainties —which would, of course, be exaggerated if the method of oral administration were followed—are avoided by the intravenous administration of a dose of vaccine standardised so as to give the optimum number of microbes per c.cm. of blood. With this system of dosage—which has here been followed throughout—there remain only the minor difficulties of estimating the patient's blood volume, and allowing for a progressive dissolution of microbes, and a consequent delivery of more than the anticipated quantum of antigen into the blood. But these minor difficulties need not be too minutely insisted upon.

Extension of the Range of Therapeutic Inoculation.

We saw at the outset that ordinary vaccine-therapy had proved ineffective in chronic streptococcal septicaemias, in many of the acute septicaemic conditions of local origin, and in phthisis when associated with hectic temperatures. And it might have been added that vaccine-therapy has in pneumonia, in typhoid, and in other similar infections given either uncertain or negative results. For example, the inoculation of pneumococcus in native pneumonia patients in the hospitals of the Johannesburg mines [1] gave in our hands completely negative results. To the unsolved problems of the treatment of these infections we must now return, asking ourselves whether we are still compelled to make choice between a policy of non-

[1] Wright: *On Pharmako-therapy and Preventive Inoculation Applied to Pneumonia in the African Native*, Constable, London, 1914, pp. 83, 85.

intervention—disguised under the name of the expectant treatment—and a policy of purely empirical and ignorant intervention. It will be clear that we are no longer upon the horns of that dilemma.

To begin with—and this in itself is a step forward—we can now by a ' vaccine-response test ' undertaken upon the blood *in vitro* decide whether a patient is or is not capable of making immunising response. Where he is competent to make such response we can—and this is a further advance—ascertain to what dose of vaccine, intravenously inoculated, he will be able to make optimum response. Lastly, in the alternative of his not being able to make immunising response to a vaccine, we can straight off—for such cases do not admit delay—evoke epiphylactic response in a healthy human blood and resort to what I have called *immuno-transfusion*.

In addition to these therapeutic suggestions, which apply, as will be seen, to cases where life and death may already hang in the balance, two other proposals of perhaps lesser import may be brought up for consideration. The first relates to inhibitory inoculation in the incubation period of all zymotic fevers of bacterial or non-bacterial origin ; the second to the possibility of arresting even after they have declared themselves, those minor but incapacitating infections grouped together under the heading of common colds. When we regard the fact that a non-specific epiphylactic response can be evoked instantaneously by the intravenous inoculation of appropriate doses of vaccine, it is not too venturesome to suggest that these desirable objects might possibly be achieved.

Only a small part of the domain which has here been rapidly reconnoitred has yet been explored, so it will perhaps be better to defer for a further communication any detailed account of successes achieved and disappointments experienced. But it will be indispensable to explain the method of going to work upon cases of septicaemia and acute infection, and to show how the methods of blood examination which have been described above are to be used in connexion with these cases.

Procedure to be Followed in Dealing with Cases of Septicaemia and Acute Infection.

For the scientific treatment of any grave infection there is required before all an equipment of principles. But that does not suffice. One requires in addition specific information with regard to the patient's condition. We require to know how he stands in the matter of the anti-bacterial power of his blood, and in the matter of its capacity for making immunising response to vaccines. And it is also essential to ascertain by direct means, with regard to each therapeutic intervention in its turn, whether it has been useful or ineffectual or hurtful. The laboratory methods described above can furnish us with this information. The first point to consider is how the anti-bacterial power of the patient's blood is to be gauged. Here the slide-cell inculturing procedure, and another technique which I shall have to describe to you, furnish the required data.

Slide-cell Inculturing Procedure for Measurement of Haemo-bactericidal Power.

The following examples give an idea of what can be learned from the slide-cell procedure. It may be explained that in each case graduated dilutions of a broth culture of staphylococcus were implanted into 50 or 100 c.cm. volumes of defibrinated blood (*vide supra*, pp. 76 and 77, Figs. 1 and 2), and that the number of microbes implanted was enumerated by introducing similar quanta of suspension into serum and enumerating the colonies which developed in this after incubation in capillary tubes or slide-cells.

Observation 1.—Patient suffering from Puerperal Streptococcal Septicaemia

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted respectively in volume—							
	1	2	3	4	5	6	7	8
	1000	500	250	125	62	31	16	8
Patient's blood	182	88	43	27	BD	BD	BD	BD
Control blood	—	36	10	2	0	1	1	0

BD = Diffuse blue discoloration indicative of scattered bacterial growth

Observation 2.—Patient suffering from Coryza

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted respectively in volume—							
	1	2	3	4	5	6	7	8
	1120	560	280	140	70	35	17	8
Patient's blood	48	31	31	4	4	1	0	0
Control blood	24	15	10	2	0	4	0	0

Observation 3.—Patient suffering from Puerperal Septicaemia

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted respectively in volume—			
	1	2	3	4
	600	200	67	22
Patient's blood	51	22	7	4
Control blood	8	3	1	0

Observation 4.—Patient suffering from Streptococcal Endocarditis[1]

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted in volume—							
	1	2	3	4	5	6	7	8
	320	160	80	40	20	10	5	3
Patient's blood	30	7	3	2	2	2	2	2
Control blood	1	1	0	0	0	0	0	0

Observation 5.—Patient suffering from Puerperal Streptococcal Septicaemia

Number of staphylococcal colonies which developed in—	Number of staphylococci implanted in volume—							
	1	2	3	4	5	6	7	8
	2000	1000	496	248	124	52	26	13
Patient's blood	55	33	11	9	5	4	3	0
Control blood	11	3	0	3	1	0	0	0

Chiastic Procedure for Measurement of Phagocytic Power of Blood, Opsonic Power of Serum, and Phagocytic Efficiency of Leucocytes.

What the slide-cell method supplies is, as has already been appreciated, a measurement of the haemo-bactericidal power obtained with different additions of living microbes. It does not tell us anything about the different factors upon which haemo-bactericidal power depends; nor does it, where a blood has a diminished bactericidal power, disclose to us the nature and reason of the default. A good deal of what is left untold by ordinary phagocytic methods is supplied by the *chiastic method*. The procedure consists in preparing defibrinated blood from the patient and a normal man; separating the serum from each of the bloods; preparing from each washed corpuscles; and then making by what has come to be called 'the opsonic technique' a series of four phagocytic mixtures, constituted as follows:—

(1) One volume of the patient's serum, 1 volume of his washed corpuscles, and 1 volume of any suitable bacterial suspension.

(2) One volume of the normal serum, 1 volume of the washed normal corpuscles, and 1 volume of the same bacterial suspension.

(3) One volume of the patient's serum, 1 volume of the normal corpuscles, and 1 volume of the bacterial suspension; and

(4) One volume of the normal serum, 1 volume of the patient's corpuscles, and 1 volume of the bacterial suspension.

[1] *Vide infra*, p. 118, Example 9.

It is, of course, from the interchange and crossing of the corpuscles and sera in phagocytic mixtures 3 and 4 that the appellation *chiastic* is derived. The four phagocytic mixtures having been incubated, and films having been made from them, we counted them in the accepted manner, and collated and marshalled the results in the following fashion :—

(*a*) By dividing the phagocytic count (i.e., the average microbic intake) of preparation 1 by that of preparation 2 we obtain the *phagocytic index of the patient's blood.*

(*b*) By dividing the phagocytic count of preparation 3 by that of preparation 2 we obtain the '*Opsonic* (i.e., *Phago-incitor*) *Index*' *of the patient's serum.*

(*c*) By dividing the phagocytic count of preparation 4 by that of preparation 2 we obtain the *Phagocytic Efficiency of the patient's leucocytes.*

A few examples will show clearly what we learn from this triad of phagocytic functions.

Example 1.—Surgical nurse[1] with Acute Streptococcal Infection originating from puncture in finger—temperature 105° F.

						Phagocytic count
Patient's serum	+ Patient's washed corpuscles	+	streptococci	..	..	0·57
Normal ,,	+ normal ,, ,,	+	,,	..	..	3·9
Patient's ,,	+ ,, ,, ,,	+	,,	..	..	2·6
Normal ,,	+ Patient's ,, ,,	+	,,	..	..	1·36

Phagocytic Index of Patient's blood, 0·15 ; 'Opsonic Index' of her serum, 0·65; Phagocytic Efficiency of her leucocytes, 0·34.

Example 2.—Child,[2] aged 3 years, with Acute Streptococcal Infection and abscesses in joints and subcutaneous tissues.

						Phagocytic count
Patient's serum	+ Patient's washed corpuscles	+	streptococci	..	..	0·2
A. E. W.'s ,,	+ A. E. W.'s ,, ,,	+	,,	..	..	1·3
Patient's ,,	+ ,, ,, ,,	+	,,	..	..	1·75
A. E. W.'s ,,	+ Patient's ,, ,,	+	,,	..	..	0·18

Phagocytic Index of Patient's blood, 0·16 ; 'Opsonic Index' of its serum, 1·3 ; Phagocytic Efficiency of its Leucocytes, 0·14.

Example 3.—Woman,[3] aged 55 years, with Streptococcal endocarditis.

						Phagocytic count
Patient's serum	+ Patient's washed corpuscles	+	streptococci	..	..	0·82
A. E. W.'s ,,	+ A. E. W.'s ,, ,,	+	,,	..	..	4·05
Patient's ,,	+ ,, ,, ,,	+	,,	..	..	0·44
A. E. W.'s ,,	+ Patient's ,, ,,	+	,,	..	..	0·12

Phagocytic Index of Patient's blood, 0·2; 'Opsonic Index' of her serum, 0·1; Phagocytic Efficiency of her Leucocytes, 0·03.

Results such as these can be registered in all acute septic infections. The premonitory signs of a grave infection would seem to be a reduction of the leucocytic efficiency, coupled with a rise in the opsonic index, and, as a result of some counter-

[1] *Vide* further, p. 110, 112–113. [2] *Vide* further, p. 111, 113–114. [3] *Vide* further, p. 111, 113–114.

balancing of the two factors just mentioned, a moderate fall in the phagocytic index. That type of result comes quite regularly under observation when we add to the blood *in vitro* excessive quanta of vaccine. We have already seen examples, and shall see others when we deal with the vaccine-response test. In the later graver stages of an infection the opsonic power is also reduced, and then the whole triad of phagocytic functions is prejudicially affected until they all, as in the last example, stand very little above the zero point.

It may here be pointed out, with regard to the leucocytic efficiency, that it appears to be reduced with regard to all microbes indiscriminately. Further, it may be noted that the leucocytes can recover from this heavily-poisoned condition. They can, as will be seen in a case presently to be cited, recover *in vivo* when the blood fluids which bathe them are rendered more wholesome ; and they can recover *in vitro* when transported into, and allowed to stand in, wholesome serum. What has been said above of the blood changes associated with acute sepsis may be completed by saying that the sero-bactericidal power is not even in grave infections sensibly reduced.

Investigation of the Patient's Power of Immunising Response to Vaccine.

When the anti-bacterial power of the blood has been investigated and been found wanting, the next thing to do is to find out whether the patient has any capacity of making immunising response, and with what vaccine and what dose he can make his optimum immunising response. This can be done by introducing graduated quanta of vaccine into a series of volumes of the patient's blood, and seeing whether there is any improvement in the haemo-bactericidal or phagocytic power of the vaccinated bloods, or in the sero-bactericidal or opsonic power of their sera. The information being wanted almost immediately, and there being no time for culture, investigations of the bactericidal power are ruled out. We are therefore restricted to the measurement of the phagocytic power of the inoculated bloods and the opsonic power of their sera.

Let me show you in a series of examples what we can learn from phagocytic vaccine-response tests. I may with advantage consider, first, an example which comes from the bulletin of treatment of the child whose blood furnished a moment ago an illustration of the working of the chiastic procedure.

Vaccine-response Tests

Example 1.—Surgical Nurse with acute streptococcus infection spreading from a punctured wound and a temperature of 105° F. [1]

The results recorded in the first column were obtained with phagocytic mixtures in which the sera and corpuscles of the unvaccinated and vaccinated bloods respectively were mixed with the streptococcus suspension ; the results in the second column were obtained with phagocytic mixtures in which the sera of the unvac-

[1] *Vide supra*, p. 109, Example 1; and *infra*, pp. 112–113.

cinated and vaccinated bloods respectively were employed in combination with the streptococcus suspension and the corpuscles of normal blood.

—	Strepto-phagocytic counts	
	Blood	Serum
Blood unvaccinated	1·81	1·5
Blood vaccinated with 20 stc. per c.cm.	1·9	2·0
Blood vaccinated with 40 ,, ,,	1·6	4·7
Blood vaccinated with 80 ,, ,,	1·4	1·7
Blood vaccinated with 160 ,, ,,	3·2	2·7

These results show (and the contrary might have perhaps been expected from the event of the chiastic test) that the patient's blood still possessed a considerable capacity for immunising response, and that vaccination with 160 streptococci per c.cm. gave the best response. The patient was accordingly immediately inoculated intravenously with this dose of vaccine ; in other words, with 700,000 streptococci.

Example 2.—Blood of a child suffering from a generalised streptococcus infection [1] and blood of a normal man who was about to furnish blood for immuno-transfusion into the child. March 7th, 1922, vaccine-reponse test conducted with defibrinated bloods.

—	Strepto-phagocytic indices	
	Patient	Proposed donor
Blood unvaccinated	0·3	1·0
Blood vaccinated with 20 stc. per c.cm.	0·3	0·8
Blood vaccinated with 40 ,, ,,	0·4	0·9
Blood vaccinated with 80 ,, ,,	0·3	2·3
Blood vaccinated with 160 ,, ,,	0·2	0·9

Stc. = streptococci.

Example 3.—Blood of a patient suffering from acute streptococcus infection with a mastoid suppuration [2] and A. E. W.'s blood. Feb. 9th, 1922.

—	Strepto-phagocytic indices	
	Patient	A. E. W.
Blood unvaccinated	0·27	1·0
Blood vaccinated with 30 stc. per c.cm.	0·1	1·7
Blood vaccinated with 60 ,, ,,	0·09	1·5
Blood vaccinated with 90 ,, ,,	0·03	1·45
Blood vaccinated with 600 ,, ,,	0·15	0·9

It is clear that when a patient's blood gives such results as these, intravenous inoculations of vaccine are bound to do harm instead of good. These are cases for immuno-transfusion.

[1] *Vide* Example 2 *supra*, p. 109. [2] *Vide infra*, p. 114, Example 3*a* and 3*b*.

Example 4.—Patient with chronic streptococcic septicaemia and endocarditis.[1]

—	Staphylo-opsonic index
Blood unvaccinated	1·0
Blood vaccinated with 20 strep. per c.cm.	1·12
,, ,, ,, 50 ,, ,,	1·18
,, ,, ,, 100 ,, ,,	1·6
,, ,, ,, 200 ,, ,,	1·4
,, ,, ,, 1000 ,, ,,	2·35
,, ,, ,, 4000 ,, ,,	1·26

This patient was obviously a suitable subject for intravenous inoculation with streptococcus vaccine.

Verification by Laboratory Methods of Improvement Obtained by Intravenous Inoculation of Vaccine and Immuno-transfusion in Desperate Bacterial Infections.

In grave bacterial infection only three kinds of therapeutic intervention appear to hold out any promise. (1) First among these would come—but the procedures in question apply only where there is localised infection—the evacuation of collections of infected pus, the effective draining of the tissues which encase a putrid wound, and the ablation of foci of infection. Such procedures, of course, abolish that continuous and immoderate auto-inoculation which poisons the leucocytes and interferes with immunising response. Where mechanical procedures for the reduction of the volume of infection are inapplicable our choice would appear to lie between (2) inoculations—by preference intravenous inoculations—of vaccine, and (3) immuno-transfusion. But our scientific task is not completed when we have applied the particular form of treatment selected. *A priori* inference is in medicine an untrustworthy guide. And when verification is in question, the guarantee furnished by quantitative laboratory methods—though this cannot compete with the psychological impression produced upon eye-witnesses by sudden and dramatic clinical improvement—is that which finally counts. In view of this I have set out below examples which show in connexion with each of the three aforementioned kinds of therapeutic intervention evidence of the benefit obtained from it. The examples have here been chosen to furnish illustration of points not previously brought out, and in particular to exhibit the increased anti-bacterial power obtained.

Example 1.—Increased haemo-bactericidal and sero-bactericidal power obtained by the intravenous inoculation of 700,000 dead streptococci (160 per c.cm. of patient's blood).

Surgical nurse suffering from an acute streptococcus infection originating in a punctured wound of the finger.[1] Temperature 105° F. Measurement of haemo-

[1] *Vide supra*, p. 109, Example 1; also pp. 110–111, Example 1; also *infra*, p. 116.

bactericidal power by implanting staphylococcus by the wash and after-wash procedure into a sequence of 10 c.mm. volumes of blood in a long capillary tube.

—	Number of colonies which developed in volumes—					Total in—	
	1	2	3	4	5	40 c.mm.	1 c.cm.
							circ.
10 P.M.: Blood immediately before the intravenous inoculation	+	6	5	2	2	21·5	540
	+	16	8	4	0		
Blood 3 min. after the inoculation	+	6	0	0	0	7	175
	+	6	2	0	0		

The sign + indicates that the colonies were too numerous for accurate enumeration.

Sero-Bactericidal Power (same technique)

—	Number of colonies which developed in volumes—					Total in—		
	1	2	3	4	5	40 c.mm.		1 c.cm.
								circ.
3 P.M.: Serum from blood drawn 7 hours before intravenous inoculation	+	20	12	5	1	38	37	1000
	+	17	14	3	1	35		
	+	19	12	5	2	38		
10 P.M.: Serum from blood drawn immediately after inoculation	+	12	9	0	0	21	22·5	550
	+	17	4	3	0	24		
12.30 A.M.: Serum from blood drawn 2½ hours after inoculation	+	10	5	2	0	17	14·5	360
	+	9	2	1	0	12		

The patient's temperature by next morning had come down from 105° to 99°, and she was quite out of danger. The finger afterwards began to suppurate and was incised, and the patient made a rapid recovery.

Example 2. [1]*—Showing the improved phagocytic and opsonic power and increased vaccine response and leucocytic efficiency obtained by evacuating an abscess.*

Child with generalised streptococcus infection and an unopened abscess in the elbow joint who had been treated by two successive immuno-transfusions, each with 25 c.cm. of blood, on March 21st and 24th, 1922.

[1] *Vide* p. 109, Example 2.

March 27th—*Chiastic Test* : Phagocytic index of blood, 0·07 ; opsonic index of serum, 0·8 ; phagocytic efficiency of leucocytes, 0·1. Abscess was now opened.

March 28th—*Chiastic Test* : Phagocytic index of blood, 1·02 ; opsonic index of serum, 1·4 ; phagocytic efficiency of leucocytes, 0·9.

Vaccine-Response Test.

	Strepto-phagocytic index
Unvaccinated blood	1·0
Blood vaccinated with 20 strep. per c.cm.	1·3
,, ,, ,, 40 ,, ,,	1·6
,, ,, ,, 80 ,, ,,	1·0

To appreciate the improvement, the data of this vaccine response test should be compared with those registered with this child's blood before treatment was begun (*vide* Example 1 *supra*, p. 111). The child's night temperature, which had for three weeks before the first immuno-transfusion ranged up to between 103° and 105°, and which had after the first immuno-transfusion come down to below 102°, and after the second to under 101° F., came down after the opening of the abscess to between 99° and 100° and soon after became normal.

Example 3a.—Showing increased phagocytic power obtained by immuno-transfusion.

Patient with grave streptococcal infection and mastoid suppuration. Feb. 7th, immediately before immuno-transfusion, strepto-phagocytic index 0·3, immediately after 1·2. In this latter case the patient, who had three hours before the immuno-transfusion had a rigor with a temperature of 106° F., and was lying cyanotic and semi-comatose, revived at once after the immuno-transfusion, the colour improving, the mental condition becoming normal, the patient remarking that she felt wonderfully better, eating ravenously, and sleeping quietly at night without requiring any administration of oxygen.

The same patient, after two further high evening temperatures. Feb. 9th, 1922, 24 hours before the second immuno-transfusion, strepto-phagocytic index 0·27 ; on the 10th, immediately before the immuno-transfusion, 0·0. Transfusion of blood vaccinated *in vitro* with 100 streptococci per c.cm. On the 11th, one day after, 0.7 ; on the 12th, two days after, 1·1. On the 14th, four days after, 1·3 ; on the 15th, five days after, 1·4.

Vaccine-Response Test. (This result is to be compared with that recorded in Example 3 *supra*, p. 111.)—On the 12th, unvaccinated blood per c.cm., strepto-phagocytic index 1·1 ; blood vaccinated with 20 streptococci per c.cm., 1·0 ; with 40, 1·3 ; with 80, 1·0.

In connexion with this immuno-transfusion the clinical report says : 'The result of the transfusion was again magnificent, the oxygen inhalation being discontinued, while the patient's colour improved, and she took nourishment well and passed a very good night.' After very slight rises of temperature treated by an inoculation of streptococcus vaccine the patient made a rapid recovery.

Example 3b.—Showing the increased haemo-bactericidal power obtained in the case of donor by the intravenous inoculation of 160,000 streptococci, and in the recipient by the immuno-transfusion of 500 c.cm. of the donor's blood drawn off half an hour after he had been inoculated intravenously.

Patient suffering from necrosis of frontal bone and generalised infection originating in an operation on the nasal sinuses. The slide-cell inculturing procedure was employed, graduated doses of staphylococci being implanted into 50 c.mm. volumes of blood.

Donor's blood				Patient's blood			
Before inoculation		Half-hour after inoculation		Before immuno-transfusion		24 hours after immuno-transfusion	
Imp.	Surv.	Imp.	Surv.	Imp.	Surv.	Imp.	Surv.
206	11	496	10	264	42	164	3
121	6	145	1	137	20	115	1
48	0	72	0	67	6	50	0
23	1	32	0	49	5	23	0
9	0	17	0	22	3	10	0
10	0	9	0	13	0	0	0
1	0	4	0	3	2	2	0
0	0	0	0	2	0	2	0

Imp. = Implanted. Surv. = Survived.

Synopsis of Results

(1) Into the donor's blood taken before inoculation in all 418 staphylococci were implanted, and 4·3 per cent. developed into colonies.

(2) Into the donor's blood taken half an hour after inoculation in all 775 staphylococci were implanted and 1·3 per cent. developed into colonies.

(3) Into the patient's blood taken immediately before immuno-transfusion in all 557 staphylococci were implanted and 14 per cent. developed into colonies.

(4) Into the patient's blood taken 24 hours after immuno-transfusion in all 366 staphylococci were implanted and 1·1 per cent. developed into colonies.

Comment

In connexion with these and all other data furnished by the slide-cell inculturing procedure, it is perhaps advisable to return to a matter which has already been discussed, and to make clear the exact import of the figures. It must be kept in view that the assaying procedure employed is not an assaying procedure pure and simple, but also incidentally a supplementary vaccinating procedure. It follows that the improvement shown in the case of the donor is really the resultant of two vaccinations—a vaccination *in vivo* and a superadded vaccination *in vitro*—and in the same way the improvement shown in the case of the patient is the resultant of an immuno-transfusion *in vivo* and a superadded vaccination *in vitro*. That being so, we are not entitled to claim for the intravenous inoculation and the immuno-

transfusion that it did all that the figures in the tables might at first sight suggest. But we shall have made the proper abatement from that claim if we say this : The bloods of the donor and patient respectively have been so improved by our operations that they would, if they afterwards encountered an infection equivalent to an implantation of in the one case 10,000 (496 × 20) or less staphylococci per c.cm., and in the other case to 3200 (164 × 20) or less staphylococci per c.cm., have shown an improvement equivalent to those set out in the tables in connexion with these figures.

Example 4.—Increased haemo-bactericidal power obtained by intravenous inoculation of 30,000 streptococci. [1]

Patient suffering from streptococcal septicaemia and endocarditis. Slide-cell inculturing method.

Number of staphylococci implanted	Number of colonies which developed in blood drawn off—	
	Before intravenous inoculation	20 mins. after intravenous inoculation
160	31	12
80	16	9
40	7	7
20	3	3
10	4	0
5	0	0
315	61	31

Example 5.—Showing increased opsonic power obtained by immuno-transfusion.

Patient with puerperal septicaemia. March 6th, 1922, immediately before immuno-transfusion, strepto-opsonic index 1·2, immediately after 2.

Example 6.—Showing increased sero-bactericidal power obtained by immuno-transfusion of 500 c.cm. of defibrinated blood obtained from a donor who had four hours before been inoculated intravenously with 150,000 streptococci.

Patient suffering from a chronic hectic temperature supervening upon a staphylococcal infection of both breasts which had led to their amputation.

		Number of staphylococcus colonies which developed in 50 c.mm. volumes of serum.
Serum of blood drawn off	immediately before immuno-transfusion	36
	5 minutes after immuno-transfusion	28
	1 hour after immuno-transfusion	24

This is equivalent to an increased killing power of 240 staphylococci per c.cm. Consideration will show that this method of assay excludes the possibility of any supplementary epiphylactic response *in vitro*.

[1] *Vide supra*, p. 112, Example 4.

Example 7.—Showing increased sero-bactericidal power to staphylococcus obtained by the immuno-transfusion of 500 c.cm. of blood vaccinated in vitro *with 30 streptococci per c.cm.*

Patient with a chronic pyrexia following on a series of local streptococcal infections.

	Number of staphylococcus colonies which developed in 20 c.mm. volumes of serum.
Serum from blood drawn 24 hours before immuno-transfusion ..	{36 / 31} $33\frac{1}{2}$
Serum from blood drawn immediately before	{44 / 36} 40
Serum from blood drawn 5 minutes after immuno-transfusion ..	{24 / 28} 26
Serum from blood drawn 24 hours after immuno-transfusion ..	{24 / 20} 22

Calculation shows that here the serum after immuno-transfusion killed 700 more and the serum 24 hours after 900 more staphylococci per c.cm. than before.

Example 8.—Demonstration by the implanting and explanting method of increased haemo-bactericidal power obtained by immuno-transfusion of 500 c.cm. of defibrinated blood from a donor who had half an hour before been inoculated intravenously with 170,000 streptococci.

Patient with puerperal streptococcic septicaemia.

Number of staphylococci implanted per 5 c.mm. of blood	Number of colonies which developed on agar when 5 c.mm. of blood drawn before immuno-transfusion was explanted		Number of colonies which developed on agar when 5 c.mm. of blood drawn immediately after immuno-transfusion was explanted	
	Immediately	After $\frac{1}{2}$ hour	Immediately	After $\frac{1}{2}$ hour
55	32	94	38	48
29	10	53	15	11
10	6	16	10	7
7	5	6	8	1
$4\frac{1}{2}$	3	2	5	2
105	56	171	76	69

We have here the results of two separate assays. When assayed by implanting and explanting immediately (the comparison is here between columns 2 and 4), the blood drawn off immediately after immuno-transfusion showed a decline rather than an improvement. When assayed by implanting and explanting after half an

hour (the comparison is here between columns 3 and 5), the blood drawn off immediately after immuno-transfusion shows great improvement. While in the blood drawn off before immuno-transfusion 105 microbes had, in the course of half an hour's haemo-incubation, increased to 171, the 105 had, in the blood drawn off after that half-hour of haemo-incubation, decreased to 76.

I may just advert to one further point of theoretical interest in connexion with immuno-transfusion. This is that when the donor, or his blood, has been inoculated with too large a quantum of vaccine, or where insufficient time has intervened between inoculation and transfusion, instead of a positive, a negative phase, such as that which would have been produced by a direct inoculation of vaccine, will supervene.

I may now pass from the illustration of the effects of immuno-transfusion to give further examples of the intravenous inoculation of vaccines into patients whose blood has after examination by the chiastic or vaccine-response test, or on general clinical grounds, been adjudged to be capable of making immunising response. It will—for we are here dealing with blood conditions which are, of course, fundamentally the same as those obtaining in health—suffice here to adduce a couple of examples.

Example 9.—Increased sero-bactericidal power obtained by intravenous inoculation of 125,000 dead staphylococci (50 per c.cm. of patient's blood).

Child with streptococcal endocarditis (M.F.). June 19th, 1922, sera from bloods drawn off before and at intervals after inoculation. Measurement of sero-bactericidal power by implantation of 2·5 c.mm. of staphylococcus suspension into 50 c.mm. samples of serum.

	Number of samples tested	Average number of colonies
Serum before inoculation	6	67·5
,, ½ hour after inoculation ..	3	41·5
,, 2 hours ,, ,, ..	3	49·5

Calculation shows that here the serum half an hour after inoculation killed per c.cm. 520 more, and two hours after inoculation 360 more staphylococci than before.

Example 10.—Increased sero-bactericidal power obtained by intravenous inoculation of 130,000 dead streptococci (55 per c.cm. of patient's blood).

Same patient and the same procedure.

Two volumes of serum *immediately before inoculation* furnished 82 and 91 colonies, average 86·5.

Two volumes of serum *half an hour after inoculation* furnished 82 and 99 colonies, average 90·5.

Two volumes of serum *two hours after inoculation* furnished 78 and 88 colonies, average 83.

One volume of serum *four hours after inoculation* furnished 32 colonies.

Calculation shows that the serum four hours after inoculation killed 1090 more staphylococci per c.cm. than before.

Example 11.—Increased haemo-bactericidal power to staphylococcus obtained by the intravenous inoculation of 200,000 typhoid bacilli (80 per c.cm. of patient's blood).

The same patient. Measurement of haemo-bactericidal power by implantation of 2·5 c.mm. of progressive dilutions of staphylococcus culture into 50 c.mm. samples of blood and inculturing these in slide-cells.

—	Number of colonies of staphylococcus which developed in volumes—								
	1	2	3	4	5	6	7	8	Total
Blood drawn immediately before inoculation	32	16	9	4	5	1	0	0	67
Blood after inoculation	23	7	3	1	0	1	1	1	37

The number of staphylococci implanted was the same for the two bloods.

Example 12.—Increased haemo-bactericidal power obtained by the intravenous inoculation of 40,000,000 staphylococci (8000 per c.cm. of patient's blood).

Patient with chronic hectic temperature following upon staphylococcal infection of the breasts. Procedure as in Example 11.

—	Number of colonies of staphylococci which developed in volumes—								
	1	2	3	4	5	6	7	8	Total
Blood drawn immediately before inoculation	31	13	7	3	2	0	1	1	58
Blood one hour after inoculation ..	6	2	1	1	0	0	0	0	10

The number of staphylococci implanted was the same for the two bloods.

Conclusion.

I have now finished with the setting out of the data of individual experiments, and would, in conclusion, set out two considerations of a more general order. The first has reference to the question of the therapeutic prospects here opened up. The experiments which have been set out have brought into prominence the fact that in immunisation quantitative considerations dominate the situation. When we want to evoke immunising response in the blood we must employ one particular range of doses. And when we want to ascertain what has been achieved we must

again employ one particular range of doses. Once that principle has been accepted, and we realise that in each case only a definite measure of additional anti-bacterial power is engendered, and that with it only such and such an additional number of microbes can be killed, it becomes possible to get down to figures.

In the experiments incorporated in this paper the increased anti-bacterial power engendered runs, as calculation shows, generally into the killing of several additional hundreds or exceptionally several additional thousands of staphylococci per c.cm. of blood or serum. It never gives us an additional killing of tens or hundreds of thousands. These figures, then, give us our upper and lower limits. Within that range there is, as reflection will show, much useful therapeutic work to be accomplished. But we must not close our eyes to the fact that, just as there is an assaying dose of living microbes which no immunised blood can contend against, so there must be a very definite limit to the volume of infection which intravenous inoculation of vaccines, or immuno-transfusion can cope with. And in computing that volume of infection we must take into reckoning not only the microbes actually circulating in the blood (those even in very severe cases of streptococcal septicaemia do not amount to more than a very few hundreds of microbes per c.cm.), but the total bacterial population in the internal organs, which may run into indefinite millions.

The second point — which comes in appropriately at the end of this paper, which began by showing how always a new code of principles comes and supersedes or modifies its predecessor—is that it behoves us to realise that in every code of principles there are tenets based only upon inference as well as tenets based upon direct experiment. But while we regard that, we should also keep before us that in every succeeding code those elements which rest on inference grow less and less, and those which rest directly upon fact—those which are established by laboratory methods—grow more and more. So that in the end, in every science, enduring principles will be reached.

NEW METHODS FOR THE STUDY OF THE PATHOLOGY AND TREATMENT OF TUBERCULOUS DISEASE

BY THE AUTHOR

(*Reprinted from 'The Lancet', February 2nd, 1924*)

Before any substantial advance can be made in the treatment of tuberculous infection two things must be done. We must extend our immunological knowledge so as to discern what is lacking in the patient, and what requires to be done for him. And when we have in the light of such knowledge selected our treatment we must use sure means for discovering whether our therapeutic procedures fulfil their purpose. We must part company with those who overleap by a *saltus empiricus* intermediate events, and undertake to adjudicate by far-off indirect and often ambiguous clinical results; and we must set ourselves instead to follow out step by step, using laboratory methods, the train of effects produced by treatment —adhering doggedly to the *processus scientificus tutus*.

Here, as in all other infections, the proper way to begin will be to find out whether the infective agent can cultivate itself in the blood, in the blood fluids, and in the blood fluids in the presence of leucocytes. In the foregoing papers [1] dealing with staphylococcic and streptococcic infections a general technique for these purposes has been described. That technique with certain changes can, as will be seen, be employed also in researches on the tubercle bacillus.

Haemoculture in Slide Cells and Capillary Tubes.

The method consists in implanting graduated dilutions of a bacterial culture into the blood, and then filling this implanted blood into capillary tubes, or into slide cells. These last (*vide loc. cit.*) are shallow cells made from ordinary microscopic slides held apart and subdivided into compartments by strips of paper of standard thickness. Where staphylococci are implanted into human blood the survivors grow out into colonies which show up as bleached foci encircled by broad purple rings. In the blood of rabbits each staphylococcus colony is surrounded by a broad halo of haemolysis. Similarly, in human blood colonies of haemolytic streptococci may be ringed round with areas of haemolysis. When tubercle bacilli are incubated in human blood the haemoglobin of the red corpuscles is unaffected. We cannot, therefore, in this case tell by simple inspection whether a culture has been obtained.

To decide that we must dissolve out the haemoglobin from the clot, and then stain and examine under the microscope. In the case of slide cells we prise the slides asunder under water, add a little acetic acid or saponin, and then, when

[1] *Vide supra*, p. 75 *et seq.*

haemolysis is complete, transfer our thin slabs of clot, which now look exactly like microscopic sections, to clean slides. We then fix them to the glass by drying in an incubator, and stain with carbol fuchsin, counter-staining with methylene-blue. Blood-clots from capillary tubes—we may conveniently call these *capillary clots*—are treated in exactly the same way. When tubercle-implanted blood-clots thus prepared are microscopically examined—incubated being compared with unincubated specimens—it is immediately apparent that the tubercle bacillus has grown out in the blood. Even after so short a time as 24 hours the single microbes which were implanted and which persist as such in the unincubated clots have in incubated clots grown out into groups of 2 to 5. After 48 hours the clumps of tubercle bacilli are sufficiently large to be easily visible under a low-power objective (magnification of approximately 1 × 100). An idea as to how vigorously the tubercle bacillus grows in blood can be obtained from Figs. 1 and 2. Fig. 1 represents a portion from the edge of a slide cell clot—a portion to which (owing to the containing compartment having here been only incompletely filled with blood) oxygen had access. Fig. 2 represents a portion taken near the end of a capillary blood-clot where, of course, similar conditions would prevail.

The leucocytic reactions and the histological changes which supervene in tubercle implanted clots are interesting. Polynuclear leucocytes are first attracted to the tubercle bacilli and phagocytosis follows. The tubercle bacilli now rapidly bring about the destruction of the ingesting polynuclear leucocytes, and then large

Descriptions of Illustrations on Coloured Plate

FIG. I.—Normal blood implanted with a heavily centrifuged suspension of tubercle bacilli and incubated for nine days on a slide-cell. Marginal portion of the clot. The haemoglobin has here been dissolved out from the red corpuscles by water, and the preparation has been stained by carbol fuchsin, followed by methylene-blue. The more copious tubercle growth at the inferior margin is related to the blood-clot having along this edge been in contact with air. Here, as also in the specimens depicted in Figs. III, IV, and VII, practically all the polynuclear and many of the large mononuclear leucocytes have emigrated to the walls, and only the small lymphocytes remain behind in the clot.

FIG. II.—Capillary tubercle-implanted plasma-clot. The preparation was obtained by implanting normal plasma with a centrifuged suspension of tubercle bacilli, by drawing this up into the stem of a capillary pipette, and incubating for four days. The clot was then simply blown out, dried on a slide, and stained with carbol fuchsin. The illustration shows a portion near the end of the clot where the microbes would have access to oxygen.

FIG. III.—Portion of a slide-cell clot from normal blood lightly implanted with tubercle and then incubated for 24 hours. Shows the aggregation of mononuclear leucocytes round a clump of tubercle bacilli.

FIG. IV.—Portion of a slide-cell clot similarly implanted and incubated for 48 hours. The specimen shows commencing 'cavernation' round an agglomeration of leucocytes which are centred upon a group of tubercle bacilli.

FIG. V.—Portion of a capillary plasma-clot which was lightly implanted with tubercle bacilli. This was lodged in an 'unlined' portion of a capillary stem and was then incubated for three days. The tubercle bacilli are here growing out unrestrained.

FIG. VI.—Another portion of the same clot which was lodged in a leucocyte-lined portion of the capillary stem. The leucocytes have here migrated from the walls on to the plasma-clot. Associated with this 'admigration' there is a notable restriction of the tubercle culture.

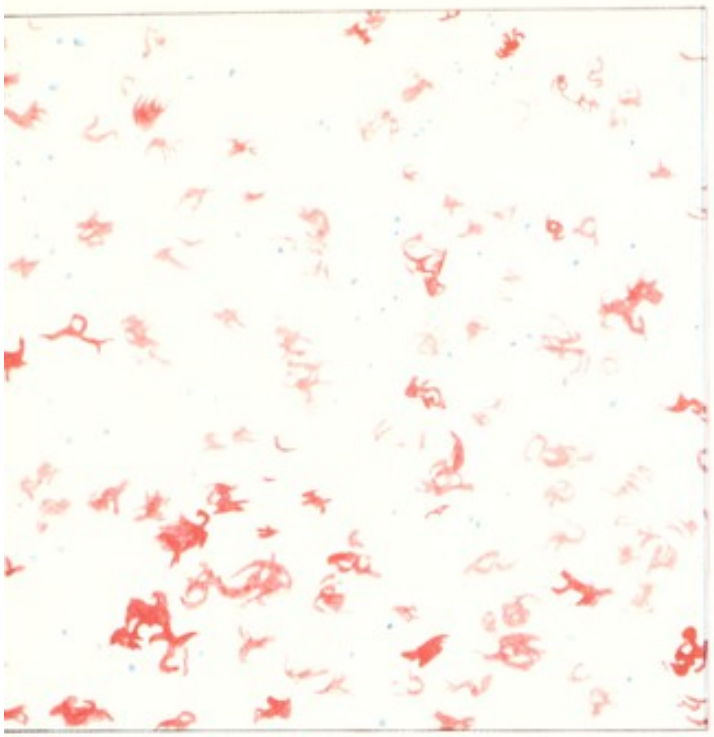


FIG. I

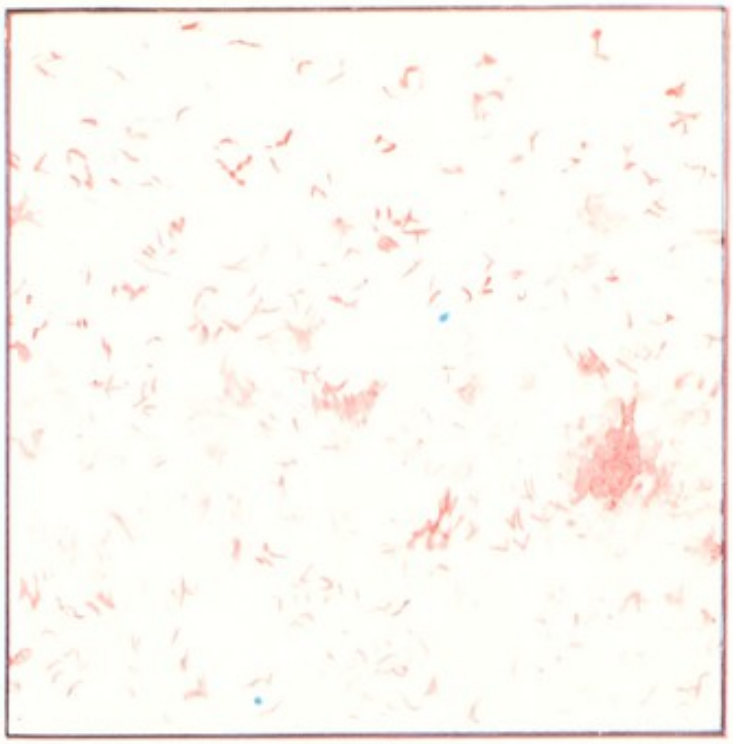


FIG. II

FIG. III

FIG. IV

FIG. V

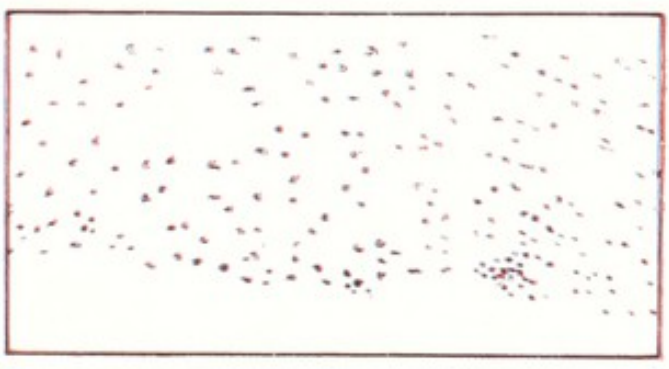


FIG. VI

and small mononuclear white corpuscles collect and form in concert with the disintegrating polynuclear phagocytes large agglomerations round the tuberculous foci exactly as described by Borrel in connexion with the intravenous injection of tubercle bacilli. These agglomerates of leucocytes—described by Borrel as miliary tubercles in the making—are shown in Figs. 3 and 4. A little later—in those regions where the leucocytes do not extinguish the infection—the fibrin meshwork begins to thin out and dissolve round the agglomerations of leucocytes which encompass the clumps of tubercle bacilli (Fig. 4). The beginning of this process of *cavernation* can be seen after 24 hours and after 48 hours definite cavities have formed, and the process goes on until the slide-cell clots are everywhere perforated with holes. The decolorised clots now look like miniature sections of a lung riddled with cavities (Fig. 9). In like manner tubercle-implanted capillary blood-clots are gradually eroded, and fall to pieces.

Ordinarily two factors—the tubercle bacillus on the one hand and leucocytes on the other—appear to co-operate in the process of cavernation. The tubercle bacillus by itself does not—this will be apparent on considering Figs. 1 and 2—furnish the necessary trypsin. Nor, judging by the case where leucocytes gather round insoluble foreign particles in a blood-clot, do aggregations of unpoisoned leucocytes do so. Nor, again, do the aggregations of leucocytes which may form round staphylococcus colonies dissolve the fibrinous meshwork. The cavernation would thus appear to be due to a quite special tryspin-liberating action exerted upon leucocytes by the tubercle bacillus. Exceptionally, where the blood has been very heavily implanted with tubercle, cavitation appears to occur independently of any intervention of leucocytes. We have in this case cavities filled with badly staining material in which tubercle bacilli are lying loose. Such cavitation is probably produced by a caseating necrosis.

Plasma Culture.

The tubercle bacillus having been found to grow freely in human blood, implantations were made into serum. The event, however, did not conform with expectation. The tubercle bacillus does not proliferate appreciably in serum. Implantations were, as a next step, made into plasma. The technique employed was to obtain blood from the finger to aspirate this into a paraffined pipette, and to centrifuge this without delay in a centrifuge bucket filled with cold water. This done the supernatant plasma was pipetted off, implanted with graduated dilutions of the centrifuged tubercle bacillus suspension, drawn up into capillary tubes, and incubated—control tubes being kept at the temperature of the air. The plasma-clots were then, after one or more days, blown out, were carefully washed to remove the adhering serum, and were then stained with carbol fuchsin and counter-stained. A mere glance at such preparations suffices to show that the tubercle bacillus grows very freely in the plasma-clots (Figs. 2 and 5). And when these are compared with blood-clots similarly implanted it is immediately seen that the tubercle bacillus proliferates much more freely and more uniformly in the former than in the latter. Whereas in the case of the blood-clot the colonies are irregularly distributed and come up larger and smaller according as the conditions are in one region favourable

and in another unfavourable, in the plasma-clot the colonies are regularly distributed, and are much more of one size. This is obviously what one would expect of microbes implanted into a nutrient medium which was everywhere favourable to their growth.

It would be improper to infer from the fact that the tubercle bacillus proliferates vigorously in a plasma-clot that it is cultivating itself in plasma. In the plasma-clot (and, of course, the same applies to the blood-clot) the microbe is in reality cultivating itself in a medium of serum. The proper conclusion from the facts would apparently be that the tubercle bacillus obtains from the fibrin of the clot some essential nutrient element which is not found in the serum.

Influence Exerted by Leucocytes Upon the Growth of the Tubercle Bacillus.

We have already seen that the tubercle bacillus grows more copiously and in more uniform distribution in the plasma-clot than in the blood-clot, and we have further seen that in the blood-clot the leucocytes aggregate round the implanted tubercle bacilli, and that the polynuclear leucocytes in particular ingest them. It was, however, desirable to determine by direct experiment whether we can by bringing in the agency of leucocytes check the growth of the tubercle bacillus. This can be put to the test in two different ways. It can be tested by drawing up tubercle-implanted plasma into leucocyte-lined capillary tubes, and comparing the growth in such tubes with that obtained in ordinary unlined capillary tubes. Leucocyte-lined tubes are made by filling blood into ordinary capillary pipettes, incubating, so as to allow the leucocytes to emigrate and fix themselves to the walls, and then drawing a stream of normal salt solution into the distal end of the capillary tube, thereby carrying the clot up into the barrel of the pipette and washing away the red corpuscles and other blood elements. Better for our purpose than tubes furnished with a complete lining of leucocytes are tubes in which only one-half of the stem is lined. And better still are tubes in which leucocyte-lined sections alternate with unlined sections. Such a disposition of leucocytes is obtained by drawing up into the pipettes an appropriately interrupted column of blood.

When tubercle-implanted plasma coagulates in a lined tube the leucocytes transfer themselves by *admigration* to the clot and form round it a cellular envelope which comes out uninjured when the clot is evacuated through the expanding upper portion of the capillary stem (Fig. 6). We can accordingly, when we have before us a clot derived from a lined tube, very easily distinguish those regions where the microbes have been subjected to the influence of leucocytes from those where these have not come into operation. Fig. 5 shows the growth of tubercle in a section of plasma-clot which has lain in an unlined region of the tube. Fig. 6 shows the growth in an adjoining lined portion. In those regions of a plasma-clot where a sufficient force of leucocytes—and here it is a question of polynuclear leucocytes only—comes into operation the culture of tubercle is, as shown in the figures, always very much less copious than in those regions of the clot where the leucocytes do not come into play.

Another method of investigating the effect exerted by leucocytes upon the

culture of the tubercle bacillus is to fill in tubercle-implanted blood into capillary tubes or flat emigration tubes and centrifuge immediately. We then obtain in the upper half of the tube plasma, and in the lower half of the tube red corpuscles intermixed with polynuclear leucocytes and small lymphocytes; and on the top of this a layer of large mononuclear leucocytes intermixed with the larger lymphocytes. When we now incubate these tubes the tubercle bacilli grow out freely in the plasma-clot; they are, when the implantation is not excessive, killed off in the substance of the red clot, and also, but not so completely, in those regions of the plasma-clot which are invaded by leucocytes.

While it holds true generally that leucocytes exert an inhibitory influence upon the growth of the tubercle bacillus, the rule is subject to important exception. In those regions of the clot where leucocytes succumb to tuberculous attack the growth of the tubercle bacillus is distinctly favoured. Examination of implanted blood-clots brings out the fact that polynuclear phagocytosis of tubercle bacilli is generally unsuccessful, and that it then furnishes new foci of infection, and that the colonies of tubercle that develop from these disintegrated leucocytes are quite markedly larger than those in other regions of the preparation.

Effect of Implanting Tubercle Bacilli into the Bloods of Phthisical Patients.

When similar implantations of tubercle bacilli are made into normal blood and into the blood of patients with pyrexial phthisis—or, to speak more generally, patients who have reacted to tubercle auto-inoculations—very remarkable differ-

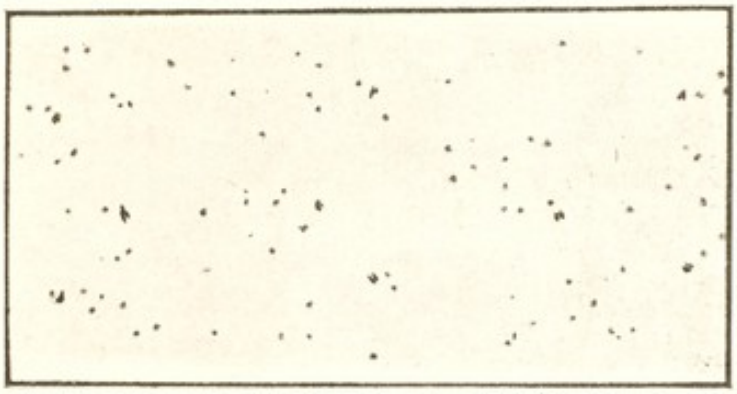


FIG. 7

Capillary clot from tubercle-implanted normal blood, blown out after three days' incubation, and then haemolysed by saponin and stained. Practically all the leucocytes have here emigrated from the clot, only a few lymphocytes being left behind. In association with this the tubercle bacilli have grown out unrestrained.

ences come to light. There is, in the first place, as will be seen on reference to Figs. 7 and 8, a difference with respect to the leucocytic reaction. Whereas the leucocytes of normal blood when the blood is heavily implanted collect only sparingly round the tubercle bacillus, most of them emigrating from the clot, the leucocytes of the tuberculous patient congregate in great masses round the bacilli. In correlation with this greater leucocytic reaction a more powerful destructive effect is exerted

upon the implanted bacilli, and when we compare tuberculous and normal blood, counting in each case the number of tuberculous colonies in the incubated and un-incubated blood we find there is in the blood of the tuberculous patient a much larger dissolution of microbes.

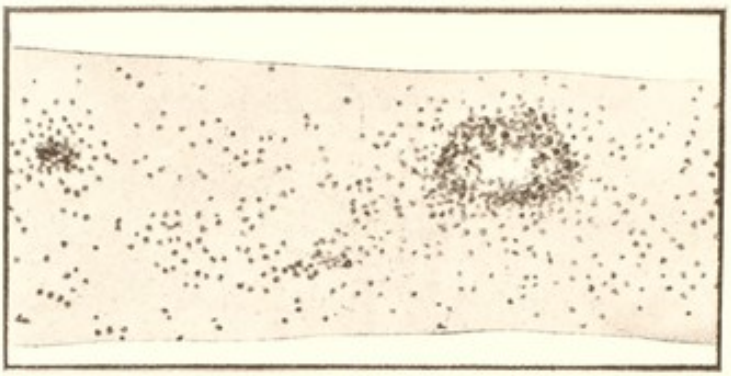


FIG. 8

A similarly implanted and treated blood-clot derived from a patient with phthisis showing leucocytic agglomerations round the tubercle bacilli. In association with this there is a greatly restricted growth of tubercle bacilli.

There is here much food for thought. It is in the first place obvious that though there are clinical similarities between pyrexial phthisis and chronic streptococcal septicaemia the immunological conditions are in the two profoundly different. In the septicaemic patient the haemo-bactericidal power is as compared with the normal, very conspicuously reduced. The treatment of streptococcal septicaemia should by consequence be *epiphylactic*. In other words, it must aim at increasing the bactericidal power of the blood. The pyrexial tuberculous patient here in view has, as compared with the normal, a greatly increased haemo-bactericidal power. *Epiphylaxis* is therefore here not the essential requirement. And we have to ask ourselves what can here be at fault and what treatment is required ? The answer to these questions would seem to be that the infecting tubercle bacilli are cultivating themselves in *ecphylactic* foci, and that *kataphylactic* treatment is called for. In other words, the bactericidal agents, that is the leucocytes, should here be transported from the blood into the regions of infection. That is precisely what we do when we introduce a tubercle-implanted plasma-clot into a lined capillary tube (Figs. 5 and 6).

The essential preliminary to treatment on these lines will obviously be the prosecution of research into the phenomena of emigration. We require to study the laws that govern the emigration of the leucocytes from the blood, or, as the case may be, blood-clot. We require to find out how to make leucocytes invade the tubercle foci as they *admigrate* to the bacilli in the bloods of tuberculous patients. And we require to study generally the chemotactic properties and the migrational movements of the different species of leucocytes.

Comparison of Normal Blood Inoculated *in Vitro* with Koch's Bacillary Emulsion with Uninoculated Normal Blood.

As an inducement to other workers to take up the study of this question, it may be placed upon record here that the increased leucocytic reaction and the increased tuberculo-bactericidal power which distinguish the blood of tuberculous patients who have reacted to auto-inoculations can be obtained by the addition of bacillary emulsion to the normal blood *in vitro*. But in connexion with this it must be borne in mind that here, as in all immunisation, the immunising result will

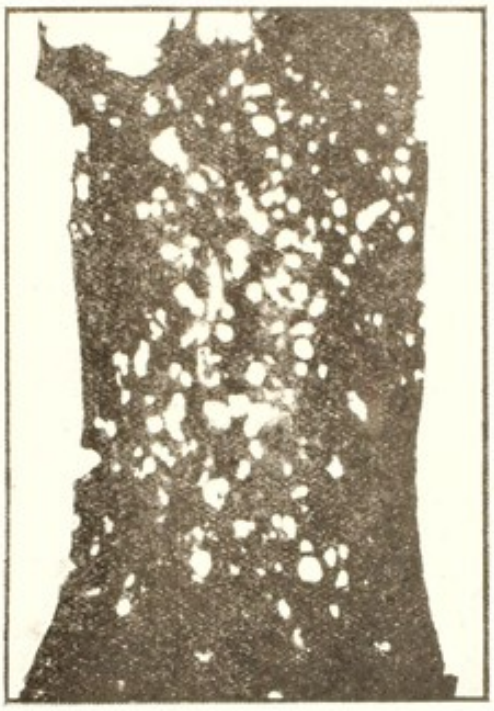


FIG. 9

Photograph of a slide cell tubercle-implanted blood-clot, which was incubated for four or five days and was then hæmolysed by saponin. By 'cavernation' the clot has been converted into a sort of honeycomb structure.

depend upon two factors operating in conjunction. The first is the dose of living microbes implanted. The second is the quantum of vaccine which is superadded. The following results apply to blood implanted fairly sparingly with living tubercle bacilli. With small additions of bacillary emulsion—i.e., doses ranging above and below 1 in 50,000,000 of bacillary emulsion in blood—very large reduction of the tuberculous growth is obtained. With larger doses—doses up to 1 in 5,000,000 of bacillary emulsion in blood—increased agglomeration of leucocytes round the tubercle bacilli is obtained. But with these larger doses the tubercle bacilli dispersed in the other regions of the preparation appear to grow more plentifully.

In conclusion, I desire to thank my friend and colleague Prof. Alexander Fleming for the drawings which illustrate this paper.

ON VACCINE THERAPY AND IMMUNISATION *IN VITRO*

BASED ON A LECTURE BY THE AUTHOR TO THE MEDICAL SOCIETY OF LONDON ON OCTOBER 27TH, 1930

(*Reprinted from 'The Lancet', Vol. II, 1931*)

The methods of combating infections in the organism can be divided up into (*a*) those which aim at removing or extirpating the microbes by mechanical means; (*b*) those which aim at killing the microbes by the agency of chemical antiseptics; (*c*) those which make use of the antibacterial powers which already exist in the organism, but do not concern themselves with increasing these; and (*d*) those which aim at developing increased bactericidal powers in the blood or passively supplementing these powers.

It will be useful to preface what I have to say about vaccine therapy by a brief survey of the alternative methods of treatment.

Methods of *surgical extirpation* may be distinguished into incomplete operations which confine themselves to the removal of a conspicuous focus of infection, and those which aim at the complete eradication of an infection combined with an excision of a *locus minoris resistentiae*.

Operations of the former kind were only a few years ago extensively employed in surgical tubercle. They were resorted to upon the idea that the organism would, when the full weight of the infection had been taken off, be competent to cope with the germs that were bound to be left behind. This optimism was, of course, more often than not disappointed, the operation being frequently followed by a recrudescence of the infection. This, in point of fact, is just what might have been expected. For operations upon infected foci must always carry with them a risk of producing a negative phase of diminished anti-bacterial power and favouring the spread of the infection.

Similar dangers attach to enucleation operations of the kind which leave open raw surfaces. Here, if the resistance of the organism is reduced, reinfection from outside is likely to follow.

Treatment by Antiseptics.

The history of antiseptic treatment is the history of what has followed from the erroneous assumption that chemical agents which kill microbes when brought into application in water will do the same when brought into application in serum-impregnated tissues, suppurative discharges, and blood. The ordinary medical man deems that assumption to be somehow right, and he fails to see any reason why it should not be. And he feels sure that if the assumptions he made were wrong, clinical observation would very quickly bring this out.

That confidence is quite misplaced. Probative evidence of the harmfulness of a treatment is furnished only when the clinical condition of a patient becomes conspicuously worse after the application of a treatment, and this happens again and again. Similarly probative evidence of the efficacy of a treatment is furnished only when the clinical condition of a patient is indubitably better after treatment, and this happens repeatedly. In every other case clinical observation leaves us in incertitude as to whether a method of treatment is doing a modicum of good, or a modicum of harm, or is doing neither good nor harm.

Once these inherent limitations of clinical methods are realised it becomes obvious that we must, if medicine is to make steady advance, find some method of distinguishing treatment which is in an inconspicuous manner useful; from treatment which is in an inconspicuous manner harmful; and from treatment which does neither harm nor good.

The first thing to lay to heart here is that it is hopeless to trust in such matters to our unaided senses, and that it is in the case of antiseptics fallacious to draw inferences from the clinical condition of the patient or the condition of a wound. If we seriously want to arrive at the therapeutics of a bacterial infection we must do two things.

First, we must experiment upon simpler biological complexes—complexes such as the blood or the pus or inflammatory fluids derived from a focus of infection, or, better still, upon separated blood fluids, transudation fluids, and leucocytes.

Secondly, we must, in order to discover the event of a therapeutic intervention, have recourse to laboratory technique and scientific apparatus.

Let me give a concrete illustration of the kind of therapeutic problem which I have here in view.

As an incident in an enterprise of research undertaken in 1911, with the aim of alleviating the formidable mortality from pneumonia in the mines of the Transvaal, I and my fellow-workers had, *inter alia*, to inquire into the utility of the internal administration of antiseptics, a method of treatment which was in vogue in some of the mine hospitals.

We had here to elect between two procedures—statistical inquiry into the results of treatment being one method; and inquiry into the effect exerted by antiseptics upon the pneumococcus suspended in serum being the other.

Let us consider what the former method of inquiry would have involved. It would have involved, first, obtaining control over the treatment in the mine hospitals. It would have involved choosing one particular antiseptic and one hard and fast scheme of dosage for all the cases, and imposing this upon all the medical officers. It would have involved further the carrying out of the experiments upon a very large number of cases, and arranging for a corresponding number of similar cases to be left untreated. It would have involved also the keeping of medical histories of all the cases and the verification of the diagnoses by post-mortem examinations of all who died.

Again, and this is a very important consideration in connexion with statistical inquiries into any treatment which is believed in by some observers and dissented from by others (and that was the case here), there is in all such cases a very strong

presumption that the treatment in question is in reality not very effective, and that the figures when they shall have been arrived at will exhibit a difference too small to be convincing.

Finally, if the aforementioned considerations do not clearly bar the method of statistical inquiry, one has to consider the length of time and the formidable number of patients which would be required for settling by statistics even a single item in a programme of treatment—for example, the superiority of one antiseptic over another, or of one scheme of dosage over another.

From all this it is apparent that the method of statistical inquiry is utterly impracticable, and that it would, if this had been the only method available to us, have been necessary to abandon all hope of determining whether the administration of this or that antiseptic is in some degree helpful ; or in some measure harmful ; or whether it leaves things just as they were. There remained, however, the alternative of recourse to laboratory experimentation.

On the Appraisement of the Value of Antiseptics by Laboratory Methods.

The earliest, or among the earliest, experiments on the question as to whether antiseptics can be turned to account in the treatment of bacterial infections, would appear to have been those of Koch. It was shown by him that though sublimate in a dilution of 1 in 1,000,000 inhibited the growth of anthrax in nutrient gelatin, sublimate in double that concentration does not in any way modify the course of anthrax infection in rabbits. Behring showed that this found its explanation in the fact that a dilution of 1 in 10,000 of sublimate was required to restrain the growth of anthrax in serum. And calculation shows that to achieve such a 1 in 10,000 concentration of sublimate in the blood fluids of a 70-kilo man it would (assuming these fluids to measure 2500 c.cm., and assuming also the full quantum of sublimate administered to come into application) be necessary to administer intravenously 0·25 g. of sublimate—i.e., about grs. 4.

Our experiments were carried out in essentially the same way as those of Behring. Graduated dilutions of antiseptics were made in water, on the one hand, and serum on the other, and then in each case the same quantum of pneumococcus culture was implanted. The details of these experiments are set out in my book entitled *The Treatment of Pneumonia by Drugs and Vaccines* (Constable, London, 1914). The upshot was that while lysol, creosote, and guaiacol—the three antiseptics experimented with—kill the pneumococcus in very high dilutions in water they would, for killing the pneumococcus in the patient's blood, have to be administered intravenously in frankly poisonous doses—in the case of lysol in a dose of over 75 minims and in the case of both creosote and guaiacol in a dose of 15 minims.

We now come to some further points in connexion with the testing the efficacy of antiseptics by *in vitro* experimentation. In particular, it must be realised that while the method of measuring of the bactericidal power of an antiseptic upon microbes implanted into serum is infinitely superior to the method of measuring the bactericidal power upon microbes suspended in water, it is, nevertheless, a method which fails to take into regard many very important physiological considerations.

My friend and fellow-worker, Prof. A. Fleming,[1] has very properly insisted that antiseptics which are destined for use in the blood should be tested upon microbes implanted into blood; and similarly, that antiseptics designed for use in a wound should be tested upon microbes contained in or implanted into pus.

Fleming further pointed out that antiseptics may be usefully tested by implanting staphylococcus into serum without and with antiseptics, and incubating measured quanta of such sera in capillary tubes lined with emigrated leucocytes and, for purposes of control, also in ordinary unlined capillary tubes.

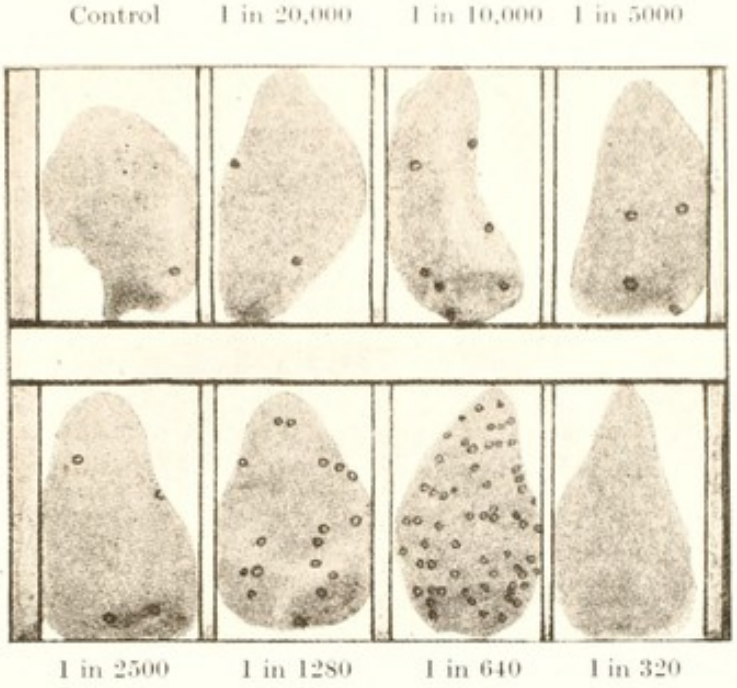


FIG. 1.

Carbolic Acid. Effect on staphylococci in blood. Equal volumes (25 c.mm.) of infected blood and dilution of antiseptic. 15 c.mm. of blood plated on agar gave 56 colonies.

The apparatus and technique employed in these testings having been elsewhere described,[2] I may deal directly with their results. And those experimental results can be most conveniently shown in the form of diagrammatic pictures.

Fig. 1 shows the effect of adding in slide cells an ordinary antiseptic to blood implanted with staphylococci. Infinitely more can be learned from an experiment of this kind than was learned from experiments in which microbes are implanted into serum. The experiments which I made by that method in South Africa taught us only that creosote, guaiacol, and lysol and such antiseptics do not kill microbes immersed in serum. From these of Fleming we learn that antiseptics added to blood in small doses render the blood more congenial to microbic growth, and that they, when added in the largest doses that could be used therapeutically,

[1] *Proc. Roy. Soc. B.*, 1924, xcvi, 171.

[2] Most of it in my *Technique of the Teat and Capillary Tube*, 2nd Edition, Constable, London, 1921.

convert the blood into a medium in which serophytic microbes grow unrestrained. (These results are due to the antiseptic entering into destructive chemical combination with the leucocytes.) Finally, we learn here, as in experiments such as those we made in Africa, that bactericidal effects are achieved only where frankly poisonous doses are resorted to.

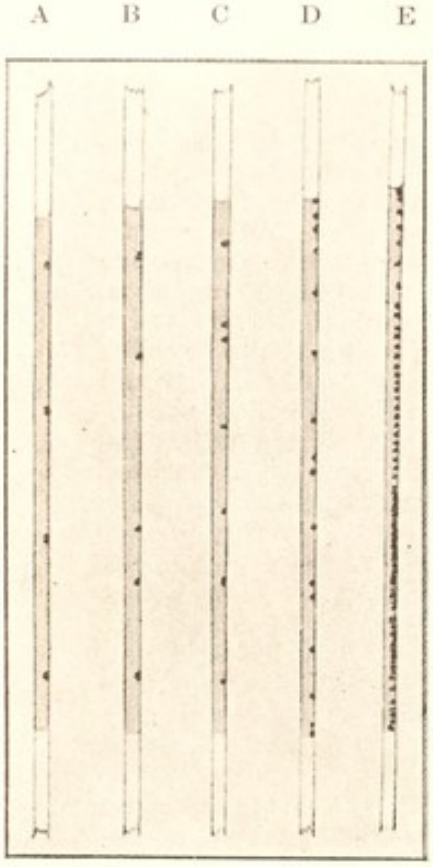


FIG. 2A

Leucocyte-lined capillary tubes filled in in each case with serum containing the same charge of staphylococcus and in the case of (B) (C) (D) and (E) an addition of carbolic acid.

(A) Serum with no carbolic.
(B) ,, ,, 1 in 3200 carbolic.
(C) ,, ,, 1 in 1600 ,,
(D) ,, ,, 1 in 800 ,,
(E) ,, ,, 1 in 400 ,,

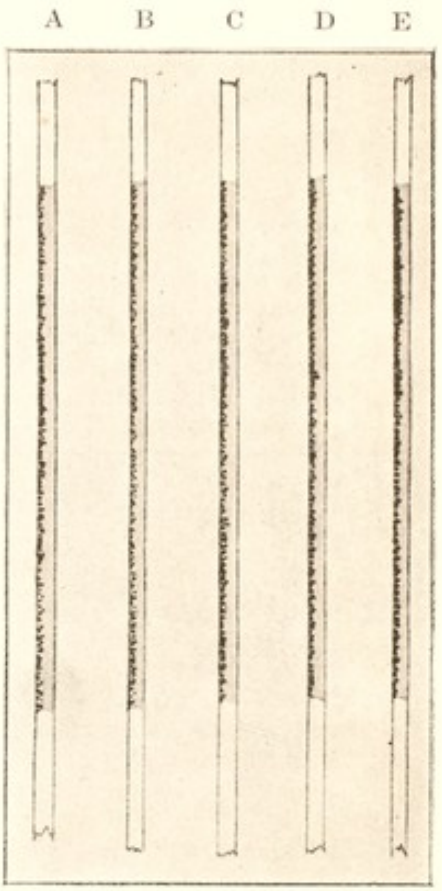


FIG. 2B

Unlined capillary tubes filled in in each case with the same charge of staphylococcus and in the case of tubes (B) (C) (D) and (E) an addition of carbolic acid.

(A) Serum with no carbolic
(B) ,, ,, 1 in 3200 carbolic.
(C) ,, ,, 1 in 1600 ,,
(D) ,, ,, 1 in 800 ,,
(E) ,, ,, 1 in 400 ,,

Exactly the same lessons are taught by Fleming's marvellously simple and ingenious experiments with pus.

Fig. 2 again enforces the same lesson. Here carbolised and non-carbolised sera are implanted in each case with staphylococcus. In Fig. 2A where the staphylo-implanted serum has been cultivated in tubes lined with emigrated leucocytes we see that the number of colonies which develop increases as more and more carbolic

acid is added—this result being as in the other experiments attributable to a poisoning of the leucocytes by the antiseptic.

In Fig. 2B where precisely the same sera are cultivated in unlined capillary tubes we see that the number of microbes which grow out into colonies is the same in all the tubes. In other words, carbolic acid in strengths up to 1 in 400 fails to inhibit the growth of staphylococcus in serum.

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Kataphylactic Methods.

Before I pass on to consider what can be done by vaccine therapy it will be well perhaps to gauge what can be done by other procedures which aim at killing microbes by what I have ventured to call *kataphylactic* measures, that is measures which determine to the focus of infection the bactericidal powers resident in the blood fluids and leucocytes. Examples of what I have called kataphylactic measures are the opening up of abscesses, the evacuation of infected discharges, the application of Bier's bandages and of hot compresses, all kinds of radio- and heliotherapy, and the drawing out of corrupted serum from an infected surface by the agency of hypertonic salt solution. These methods are in every case of service. But when all is said and done the degree to which they are useful in the particular case depends upon the antibacterial potency of the blood—this being the reservoir of antibacterial power which every kataphylactic measure draws upon.

This may be illustrated by the fact that the opening up of a localised tuberculous focus when the bactericidal power of the blood is below par does very little towards extirpating the infection while such a measure is more effective when the antituberculous power of the blood is well above par.

Principles of Vaccine Therapy.

I pass now to the subject matter proper of this discussion—the employment of vaccines for the purpose of enhancing the anti-bacterial power of the blood in infected patients.

Let me—for it is axiomatic that every therapeutic measure should repose upon a sound theoretical basis—begin what I have to say about vaccine therapy by harking back to its intellectual foundations, and justifying principles. The principle of vaccine therapy is the same as that of Pasteur's antirabic inoculations. Pasteur gave intellectual countenance to his antirabic inoculations by pointing out that a man who has been bitten by a rabid animal may, in the interval between the implantation of the virus and the development of overt symptoms, be quite properly classed as uninfected, and that he may consequently, with propriety, be preventively inoculated.

That was the principle to which I gave extended application by pointing out that all localised infections may be conceived of as precursors (generally, of course, abortive precursors) of septicaemic infections. And the way I pictured the situation

to myself was that, so long as an infection remains localised, the infected tissues combat the infection unaided, the uninfected tissues remaining in the meanwhile impassive and idle. Picturing things to myself in that way led inevitably to the thought that it would be possible by the aid of vaccines ' to turn to account in the interest of the infected tissues the unexploited immunising faculties of the uninfected tissues '.

In formulating that principle I had in view under the denomination of ' tissues ', the ' fixed tissues '. I meant, so far as I meant anything precisely, the subcutaneous tissues into which the vaccine was inoculated and the various cellular organs with which the vaccine would, after absorption, come into contact.

I would now propose to replace the term tissues in the above formula by the term leucocytes. That proposed change of wording is dictated by the consideration that conclusive evidence (some of this I propose to adduce) has been obtained of the elaboration of anti-bacterial substances by leucocytes, while we have, despite every sort of search, failed to find any indication of the generation of antibodies by the subcutaneous tissues.

With that I pass to another point. When it is claimed for therapeutic procedure that it will enhance the anti-bacterial power of the blood, that contention should in every case—I need hardly say how very rarely this is even attempted—be confirmed by evidence obtained by laboratory methods. To comply in the case of vaccine therapy with this requirement it was necessary to devise new methods of blood testing. This was necessary because the method of testing the bactericidal power of the serum which I had devised for use in connexion with anti-typhoid inoculation is inapplicable where we are dealing with serophytic microbes, such as the staphylococcus and streptococcus.

The ' Opsonic Index '.

It was to meet this new requirement my late friend and fellow-worker, W. B. Leishman, devised his method for measuring the phagocytic power of the blood. And the method for measuring the opsonic power of the serum, which was an outcome of Leishman's method, was also devised in the service of vaccine therapy.

Without these methods it would, except only in cases in which inoculation produces dramatic improvement or dramatic exacerbation of symptoms, have been impossible to decide whether a dose of vaccine was doing good or harm, whether it should be increased or diminished, and after what interval inoculation should be repeated.

But the guidance furnished in treatment of individual cases was not, as a matter of fact, the chief intellectual profit gained from our measurements of the opsonic index. (We made for many years 30,000 to 50,000 of such measurements annually at St. Mary's.) We learned from those measurements principles that seem to me to lie at the very foundations of medicine. Let me enumerate them.

(1) To begin with, we learned from these measurements that a succession of negative and positive phases such as I had described [1] in connexion with typhoid

[1] *Treatise on Antityphoid Inoculation*, Constable, London, 1904.

vaccination occurred when any vaccine was inoculated in quantity sufficient to produce appreciable constitutional disturbance. With regard to the negative phases, I had already in connexion with prophylactic inoculation pointed out that a negative phase means a temporarily lowered resistance to bacterial attack, and that, therefore, a negative phase should never be induced in a patient who is living in infected surroundings. The same principle holds *a fortiori* of a patient who is already the subject of an infection. Here the induction of a negative phase may lead to an exacerbation and a dissemination of the infection.

(2) The second general principle which was learned from measurements of the opsonic index (and Dr. John Freeman took a conspicuous part in the initiation and development of this research) was that *auto-inoculation* (the term had to be invented) may occur spontaneously, and may also be artificially evoked by a large variety of procedures. Such auto-inoculations produce negative and positive phases in exactly the same way as inoculations of vaccine.

We further learned in connexion with auto-inoculations that they can be produced in patients by active and passive movements, and by sending an ampler lymph stream through an infected focus (the misnamed *Stagnation Method* of Bier). Further, that they can be produced by helio-therapy, radio-therapy, and the application of heat. And we learned also, quite early in our investigations, that *Spontaneous Auto-inoculations* occur whenever a patient who is the subject of an extensive infection is allowed out of bed. Finally, it was in this connexion brought home to us that in a progressive infection such as phthisis—the same applies, of course, to all acute infections—there sooner or later supervenes a stage when the biography of the patient—even when he is kept completely at rest—resolves itself into a succession of spontaneous auto-inoculations. It is the signal merit of Dr. Marcus Patterson to have applied these principles to practice in the treatment of phthisis, and to have realised that success depends largely upon the regulation of the auto-inoculations by the regulation of exercise, and to have appreciated further that the most rapid and effective remedial treatment for pyrexia produced excessive auto-inoculations is absolute immobility in bed.

(3) A third advantage—and this was, of course, a question of purely practical as distinguished from theoretical importance—gained from our innumerable measurements of the opsonic index undertaken before and after inoculations undertaken upon patients, was that we learned what quanta of the various vaccines can be administered to slightly infected and non-pyrexic patients without risk of inducing a negative phase.

(4) The fourth advantage gained from our measurement of the opsonic indices of patients was that through these there was formulated a general schema or principle of dosage. The all-important principle which was arrived at is that the dose of vaccine administered to a patient should be inversely as the number of microbes affecting (i.e. delivering antigen) into his circulating blood. In other words, we should in constitutional infections give only minimal doses of vaccine ; should give intermediate doses to patients suffering from a strictly limited local infection ; and should exploit large doses which produce negative phases only in healthy patients and for prophylaxis.

'Opsonins' and Phagocytosis.

Having shown what important principles were learned from our measurements of the opsonic power of the blood-serum, I turn to the question whether we have in the opsonic index a true measurement of the phagocytic power of the blood.

My original idea—based upon investigations carried out with S. R. Douglas [1] —was that the opsonic index furnished a true measure of the phagocytic power of the blood. That conclusion was arrived at by taking from our out-patients (these were non-pyrexic and, therefore, probably non-auto-inoculating patients) bloods with high and bloods with low opsonic indices, and comparing the leucocytes of these bloods with those of our own bloods. We employed for that purpose what I have since called the *chiastic procedure*. That procedure consists of digesting with a microbic suspension: (1) the patient's washed corpuscles used in conjunction with his own serum; (2) his corpuscles used in conjunction with a normal serum; (3) washed normal corpuscles used in conjunction with normal serum; and (4) these corpuscles used with the patient's serum. The event of these operations carried out on our out-patients showed that in their case the amount of phagocytosis was determined almost entirely by the serum, and hardly at all by the corpuscles employed in the phagocytic mixture.

Afterwards S. G. Shattock and L. Dudgeon corrected these observations. They employed melanin granules instead of microbes, and carried out the tests upon pyrexic and auto-inoculating, instead as we had done upon patients who were non-pyrexic and, presumably, non-auto-inoculating patients. Shattock and Dudgeon's [2] investigations clearly demonstrated that the phagocytic avidity of the leucocyte is a variable factor, and that the phagocytic avidity of a patient's leucocytes may sometimes be much greater and sometimes much less than that of the normal leucocytes. Their investigations—their results were, as already mentioned, obtained with melanin granules—further established that phagocytic efficiency is a non-specific function of the leucocyte. These fundamentally important observations show that opsonic power is, at any rate, in pyrexic cases, a fallacious measure of the phagocytic power of the blood.

We have in the fact that the phagocytic avidity of leucocytes may suffer change a possible explanation of our having often found very high opsonic power in patients dying of phthisis. Examination might have shown these high indices to have been counterpoised by diminished phagocytic avidity. For in following up the observations of Shattock and Dudgeon we have found that the general rule in severe bacterial toxaemia is for the efficiency of the patient's leucocytes to be low while his opsonic index is high. It is easy to see a possible causal connexion between these two findings. The leucocytes may deplete themselves of anti-bacterial elements by parting with these to the blood fluids.

I now pass to an issue even more fundamental than that which was raised by Shattock and Dudgeon. We may ask ourselves whether a measurement of the phagocytic power furnishes in any case an adequate measure of the anti-bacterial

[1] *Collected Researches*, Vol. III, pp. 66 *et seq.*

[2] *Proc. Roy. Soc. B.*, 1908, lxxx, 165.

power of the blood. That question must be answered in the negative. And our answer must be in the negative—*first*, because the leucocytes can kill bacteria not only intracellularly, but also extracellularly, by excreting bactericidal elements into the surrounding fluids ; and *secondly* (and Douglas[1] has examined this question in detail), because phagocytosed microbes may fail to be digested in the leucocytes of one blood while they are in the leucocytes of another blood rapidly digested.

It follows that we cannot, if we wish to arrive at a true measure of the anti-bacterial power of the blood, content ourselves with measuring phagocytic power. We must measure the actual bactericidal effect exerted.

Anti-bacterial Power of Blood.

I accordingly set myself to devise methods for measuring, first, the haemo-bactericidal power exerted by the blood and its population of leucocytes upon serophytic organisms such as the staphylococcus, streptococcus, pneumococcus, and the tubercle bacillus. The problem I set myself here was to find, not a method of explantation to tell us what number of serophytic microbes are killed off without residue, but a method of inculturing to tell us what percentage of enumerated microbes implanted would grow out into colonies in the blood. The reason why the one and not the other kind of method is required to measure the effect exerted by the blood by serophytes will presently be apparent.

The slide cell method which makes use of defibrinated blood was one of the methods of inculturing devised for this purpose. It is illustrated in Figs. 1 and 3, and is one of the methods put to use by Fleming in his experiments on the action of antiseptics.

A second method devised for the same purpose is the following :

Graduated implantations of microbes are made into uncoagulated instead of into defibrinated blood, and the implanted blood volumes are then drawn up into capillary tubes. After overnight incubation the capillary clots are blown out, are treated with 1 per cent. saponin, and are then hung up in water until their colour is completely discharged. They are then dried and stained, and the colonies are enumerated under the microscope.

This, of course, gives—in particular in the case when the colonies are close-packed—a more accurate enumeration than the naked-eye observation which we trust to in the slide-cell method. It also gives us indications as to the rôle which phagocytosis plays in the killing of microbes. For the colonies found in the clots can be ranged into three classes : *naked colonies*—i.e., those which are not surrounded by leucocytes ; *ringed colonies*, colonies surrounded with leucocytes ; and what we may call blind, blank, or *obliterated colonies*—i.e., aggregations of leucocytes surrounding a presumably extirpated bacterial colony.

A third method—and this differs from the second only in the mode of implantation—is the ' wash and after-wash ' method. Here we draw up into a long capillary tube, first as a forerunner, a diluted bacterial culture, and then follow on with 10 equal volumes of blood separated off in each case by a bubble of air.

[1] *Proc. Roy. Soc. B.*, 1916, lxxxix.

I will now relate what these methods have taught us about immunisation and the killing of serophytic microbes by the blood. Let me first explain the following points :

To begin with, the normal serum constitutes a very excellent culture medium for the class of microbes we are here considering. The same holds of the plasma. This, since the plasma is always full of blood-platelets, means incidentally that these formed elements, in appraising the bactericidal powers of the blood, can here be dismissed from consideration. Further, the same holds of the red corpuscles, for the deleucocyted blood—i.e., the mixture of red corpuscles and serum which is obtained by passing blood through a close filter [1]—is as good a culture medium for serophytes as clear serum.

It follows from all that has just been said that the bactericidal action which is exerted by a blood upon serophytic microbes is neither more nor less than the bactericidal action exerted by its population of leucocytes.

The next issue which comes up in connexion with the killing of serophytes by leucocytes is that as to whether these microbes are killed exclusively by phagocytosis followed by intracellular digestion ; or whether they are killed also, and if so to what extent, extracellularly by bactericidal elements elaborated by the leucocytes and excreted by them into the serum.

Important data which have, as we shall see later, a bearing upon this question, are furnished by a study of the event of adding graduated quanta of serophytic microbes to equal volumes of blood.

Fundamental Experiments.

I have already published experiments bearing on this question. But it will be convenient to give a concrete illustration of what happens when my blood (it

TABLE I

Approximate number of staphylococci implanted [2]		No. of colonies which developed	Percentage of microbes that survived
Per c.cm.	Per 40 c.mm.		
6400	256	95, 96 | 95½	39
3200	128	57, 53 | 55	43
1600	64	34, 26 | 30	47
800	32	19, 11 | 15	46
400	16	11, 12 | 11½	72

[1] Wright, A. E. : *The Lancet*, 1926, i, 4 ; Fleming, A. ; *Brit. Jour. Exper. Path.*, 1926, vii, 281.

[2] The number of microbes implanted was here and in all the experiments which follow deduced from the number of microbes which grew out into colonies in serum.

is a blood of somewhat low staphylo-bactericidal power) is implanted with different quanta of staphylococcus. I subjoin protocols of two typical experiments confining myself for the moment to experiments carried out in slide cells.

Experiment 27.7.22.—Forty c.mm. volumes of A. E. W.'s defibrinated blood implanted with different quanta of staphylococcus culture and cultured in slide cells. (Table I.)

Experiment 1.8.22.—Graduated dilutions of staphylococcus culture were implanted into measured volumes of A. E. W.'s blood. The blood was then tested by two observers. (Table II.)

TABLE II

Observer A. E. W.

Implantation per c.cm. of blood	No. of microbes implanted into 40 c.mm. of blood	No. of microbes which developed into colonies	Percentage of survivors
8400	336	64	19
4200	168	28	17
2100	84	24	28
1050	42	7·5	18 } 21·2
525	21	11·5	55
262	10½	5	50
131	5	5	All the implanted
66	3	7	microbes grew
33	1·5	1·5	out into colonies
	671	153	22·8

Observer L. C.

9600	384	74	19
4800	192	44	23
2400	96	27	28
1200	48	13	27 } 23·7
600	24	15	63
300	12	6	50
150	6	5	All the implanted
75	3	5	microbes grew
38	1·5	2	out into colonies
	766	191	24·9

The explanation of the fact that the implanted microbes are here in no instance killed off without residue is furnished by the consideration that extra-cellular killing involves close proximity, and phagocytosis actual contact, between the microbes and leucocytes. In connexion with these requisites attention must be given to the following series of facts: (1) there are always in the blood large areas

unoccupied by leucocytes (the volume of the contained leucocytes stands to the volume of the containing blood in the ratio of about 1 to 300); (2) these regions are for the major part leucocytically unpatrolled or very ineffectively patrolled; and (3) since it can, by appropriate experiments, be shown that leucocytes are insensitive to the feeble chemotaxic stimuli which emanate from single microbes, the finding of isolated leucocytes by microbes must—even in patrolled areas in the blood—be a matter of chance. In other words, it depends upon the microbe happening to lie directly in the path taken by the leucocyte.

The fact that with larger and larger implantations larger and larger numbers of microbes are killed, shows that the blood develops under the impulsion of microbic stimulation increased bactericidal power.

Interpretation of the Data.

From this then follows the important principle that our methods of haemobactericidal measurement are not in the same category as quantitative chemical tests. They are, in reality, biological tests; tests which tell us not what amount of bactericidal energy is actually resident in a sample of blood, but what bactericidal energy can, under the impulsion of microbic stimulation, be developed in that blood. In other words, they are comparable to tests made to find out what amount of muscular power can, under electrical stimulation, be put forth by a muscle *in vitro*.

It is pertinent to note in passing that the general principle of which we have illustration here may hold true, not only of bactericidal, but also of opsonic power. It is conceivable that the values for opsonic power which are registered when microbes are incubated with a mixture of serum and leucocytes may represent, not as hitherto assumed, the native opsonic power of the serum, but native opsonic power supplemented by opsonins excreted into the serum by leucocytes under the impulsion of a microbic stimulus. Experimental data which bear upon this question will come up for consideration later. For the moment it will suffice to point out that by centrifugalising instead of incubating a phagocytic mixture, and by conducting our phagocytic operations at the temperature of the air instead of at blood heat, we can reduce the exposure of the leucocytes to microbic stimulation to a minimum.[1]

The Immunising Response.

Now that our measurements of the bactericidal power of the blood have brought us face to face with the conception of immunising response to the inoculation of microbes into the blood *in vitro* it will be appropriate to explain the genesis of that idea.

My first experiments on the question of immunising response made by the blood were prompted by the fact already mentioned that we had, despite strenuous experimental search, failed to find any indication of the production of anti-bacterial substances by the fixed tissues. In view of that I performed the following experiment :—

[1] Fleming: On Wright's 'Centrifuge Method of Estimating Phagocytosis', *Brit. Exp. Path.*, 1927, viii, 50.

Having placed double ligatures loosely round the jugular vein of a rabbit and having tied off all tributaries, vaccine was introduced into the vein of the opposite ear, and thereupon the double ligatures were drawn tight so as to isolate a portion of the vaccinated blood within the jugular vein. At the same time a sample of the vaccinated blood was withdrawn from the carotid and was placed in the incubator.

Experiments of this kind showed that the anti-bacterial response was obtained, not only in the circulating blood, but also in the blood isolated in the vein ; and in addition in the blood which was incubated *in vitro*.

These experiments were followed by others in which graduated doses of a staphylococcus and also of a streptococcus vaccine were added to my own blood *in vitro*. The inoculated samples of blood were then incubated for six hours and afterwards the sera were pipetted off, and then using the ' wash and after-wash ' procedure 10 volumes of each serum were implanted with a mixture of staphylococci and streptococci. The separate volumes of serum in question were then, after overnight incubation, explanted upon agar. These experiments showed (*a*) that the serum derived from blood implanted with an appropriate dose of a staphylococcus or streptococcus vaccine becomes bactericidal to *both species* of microbe ; and, further (*b*) that the sera of the bloods implanted with an overdose of vaccine furnish, as compared with the serum of the uninoculated blood, a more favourable culture medium for both kinds of microbe.

The next important step undertaken—but this had to wait until the slide cell and other haemobactericidal methods had been devised—was to investigate, as we have just done, the effect of adding graduated quanta of serophytic microbes to the blood.

Immunising Response in Vitro.

I have now, for 12 and more years, experimented continuously upon the immunising response of blood *in vitro*.

In addition to examining for augmented and reduced bactericidal power in the blood taken as a whole, I have analysed the results which relate to this by chiastic bactericidal experiments. Further, I have searched for augmented and reduced bactericidal power in the serum—I use here the term *reduced* as well as the term *augmented* for the reason that the normal serum, though it is as good a culture medium for staphylococci as nutrient broth, furnishes, there is reason to think, less than 100 colonies for 100 implanted staphylococci. I have further looked for increased and decreased phagocytic response ; for increased and diminished opsonic power ; and for negative and positive changes in the phagocytic avidity of the leucocytes—keeping in view all the time the problem of the rôle played by each of these items in the general bactericidal power of the blood.

I have employed also as antigens, both living and dead, and in some cases, autolysed microbes, testing the resulting immunising response upon both homologous and heterologous cultures.

Moreover, I have experimented upon the blood before clotting, upon coagulated blood, upon defibrinated blood, and upon isolated leucocytes—either

leucocytes which had emigrated from the blood, or leucocytes separated by centrifugalisation.

Again, I have brought living or dead vaccines into operation sometimes by incorporating them into the blood ; and at other times by placing a blood containing vaccine upon or side by side with a blood containing a test-quantum of microbes (*Method of Welded Clots*) ; and, finally, I have—and this is also an effective method of vaccination—inoculated the leucocytes intracellularly. The procedure here in question (I call it the '*baiting method*') consists in taking two samples of blood, giving the leucocytes in one sample a somewhat sparse phagocytic meal of microbes; and then supplying, after an interval of incubation, both the fed and the unfed populations of leucocytes with a full phagocytic test-meal.

In view of the wide domain covered by these experiments, I must confine myself to setting out here only the most important experimental findings.

Let me begin with the results of the chiastic experiments.

Experiment 1, 29.8.23.—Simple bactericidal experiment in slide cells and also chiastic experiment. (Observer—L. C.) (Tables III and IV.)

Female student inoculated with 1000 million staphylococci subcutaneously. *Blood A* taken before the inoculation ; *Blood B* four hours after. Both bloods were tested with three different suspensions of staphylococcus—2·5 c.mm. of each being added in each case to 50 c.mm. of blood.

TABLE III.—*Haemo-bactericidal Experiment*

Tests of samples of blood before and after inoculation in vivo

No. of staphylococci implanted	747	249	83
	No. of colonies which developed		
Blood A	13	5	1
Blood B	4	4	2

TABLE IV.—*Chiastic Slide Cell Bactericidal Tests*

No. of staphylococci implanted	420	140
	No. of colonies which developed	
Corpuscles from Blood (A) with serum of Blood (A)	27	11
,, ,, ,, (B) ,, ,, (B)	9	2·5
,, ,, ,, (A) ,, ,, (B)	16·5	9·5
,, ,, ,, (B) ,, ,, (A)	8·5	5·0

Blood B.—Index of haemo-bactericidal efficiency 3·3; Index of the bactericidal efficiency of the serum, 1·45 ; Index of bactericidal efficiency of the leucocytes, 2·8.

Experiment II.—Inoculation *in vitro*.—A 100 c.cm. volume of R. T. M.'s unvaccinated defibrinated blood, and also a 100 c.mm. volume of his blood vaccinated with 1,000,000 of dead staphylococci per c.cm. These bloods were incubated for one hour. After that the sera were pipetted off and the corpuscles washed in saline. Then in each case one volume of washed corpuscles was mixed with one volume of serum, and then 50 c.mm. volumes of each mixture were implanted with one-twentieth volume of graduated dilutions of staphylococcus culture. The implanted bloods were then cultivated in slide cells. (Table V.)

TABLE V.—*Chiastic Slide Cell Bactericidal Tests*

No. of staphylococci implanted		736	368	184	92	66
		No. of colonies which developed				Total
Serum and corpuscles of	Normal blood	25	12	5	4	46
	Vaccinated ,,	36	19	9	7	71
Serum of normal and corpuscles of vaccinated blood		54	20	15	6	95
Serum of vaccinated and corpuscles of normal blood		12	6	3	2	23

Vaccinated Blood.—Index of haemo-bactericidal efficiency, 0·64; Index of the bactericidal efficiency of the serum, 2; Index of the bactericidal efficiency of the leucocytes, 0·5.

The results, you will see, of these experiments are inconclusive as to the relative importance of the part played by the leucocytes and the serum in the general bactericidal effect.

I must therefore pass on to other experiments. And let me, in order that these may be followed properly, point out that the number of serophytic microbes killed by the blood is determined largely by the disposition of the population of leucocytes relative to the population of microbes, and this disposition is, of course, modified by every physical force that comes into operation upon the blood.

The forces which here require to be considered are: (*a*) the force of gravity; (*b*) the compressional force exerted by the contraction of the clot; (*c*) the effect of centrifugalisation respectively upon uncoagulated, defibrinated, and coagulated blood; and (*d*) the spontaneous movements of the leucocytes.

With regard to the effect of gravity this comes into consideration in particular when we are operating with defibrinated blood. For here the red corpuscles and leucocytes settle to the bottom; and the microbes are left floating in the supernatant serum. They are there safe from phagocytic attack, and can, if the serum continues to be non-bactericidal, there develop into colonies.

The spontaneous contraction of the clot influences the results by bringing closer together the leucocytes and microbes which were in the unclotted and freshly clotted blood comparatively far apart. In connexion with this it will be recalled that half the volume of blood consists of fluid—and that this in the unretracted clot forms pools in which the microbes are, as in the supernatant serum of

defibrinated blood, safe from phagocytic attack. The degree to which an individual clot is compacted by the spontaneous retraction of the fibrin meshwork depends, of course, upon all sorts of variable and incalculable factors, the content in salts being one.

Centrifugalisation—we may consider first the centrifuging of uncoagulated or defibrinated blood—altogether alters the distribution of the leucocytes in those bloods. When uncoagulated blood is centrifuged the large mononuclear leucocytes and the lymphocytes are brought to the surface, and are with a proportion of the blood-platelets agglomerated into a leucocytic cap. Most of the polynuclear leucocytes are assembled immediately underneath. When defibrinated blood is centrifuged for a long period (let us say, a quarter of an hour) in a high speed centrifuge, the polynuclear leucocytes are brought to the top. When microbes are implanted into serum or plasma or are implanted into blood which does not make immunising response, the microbes are not carried down in full proportion by ordinary centrifuging. Many are left in suspension in the supernatant fluid.

When a coagulated blood is centrifuged the clot is, of course, compacted, the lacunae are abolished, and in the case of an implanted blood the microbes and leucocytes are brought into much closer proximity than in the uncompacted clot.

The results obtained by the centrifugalisation of implanted capillary clots furnish a striking manifestation of the influencing of the haemo-bactericidal effect by mechanical factors.

TABLE VI

No. of staphylococci implanted	520	1560
	No. of colonies which developed	
Defibrinated blood in slide cells ..	30, 52, 43, 46 — 43	93, 121, 114, 111 — 110
Unpacked capillary blood-clots ..	48, 40 — 44	124, 129 — 126½
Packed capillary blood-clots ..	4, 3 — 3½	11, 48 — 29½

Experiment 7.11.28.—Comparison of Packed and Unpacked Blood and Slide Cells.—Four 50 c.mm. volumes of R. M. F.'s blood implanted with an 1800-fold and four others with a 600-fold dilution of staphylococcus, 2.5 c.mm. of staphylococcus dilution being added to each 50 c.mm. of blood. The implanted bloods were then filled into capillary tubes and were kept in the incubator for six minutes to allow time for clotting. Then two tubes from each set of four were centrifuged for two minutes at full speed in the electric centrifuge. All the capillary tubes were then incubated for 18 hours. The clots were then decolourised and the colonies in them were counted.

Four 50 c.mm. volumes of defibrinated blood to which the same addition of staphylococcus had been made were then cultured in slide cells. Higher dilutions of staphylococcus were enumerated in slide cells in watered blood. (Table VI.)

Experiment 16.11.27.—Similar experiment and technique to above. (Table VII.)

TABLE VII

—	Volume of staphylococcus implanted		
	$\frac{1}{18}$ Wash	$\frac{1}{6}$ Wash	$\frac{1}{4}$ Wash
	No. of colonies which developed		
Defibrinated blood in slide cells ..	48, 38 } 43	113, 108 } 111	∞, ∞ } ∞
Uncentrifuged capillary blood-clots ..	15, 13 } 14	39, 65 } 52	149, 225 } 187
Centrifuged capillary blood-clots ..	0, 2 } 1	0, 0 } 0	32, 16 } 24

Having got these preliminary matters out of our way we may consider the outcome of repeating the fundamental experiments, varying, however, the technique, in using here for our implantation instead of defibrinated blood or blood fresh from the circulation, leucocytes derived from centrifuged blood suspended in serum. The following protocols of experiments show that we obtain when we operate with leucocytic suspensions exactly the same results as when we operate with the whole blood.

TABLE VIII

Dilution of staphylococci implanted	No. of colonies which developed in a leucocyte-free serum	No. of colonies which developed in the leucocytic suspension	Proportion of colonies in column 3 to those in column 4
Staph. / 100,000	8, 8 } 8	10, 8 } 9	100 per cent.
Staph. / 50,000	16, 17 } 16·5	2, 2 } 2	12 ,, ,,
Staph. / 25,000	(33)	6, 4 } 5	15·5 ,, ,,

Experiment 31.10.30.—A. E. W.'s blood. Citrated blood was prepared by adding $\frac{1}{2}$ per cent. of citrate of soda to 50 c.mm. volumes of blood. The blood samples were then centrifuged in capillary tubes and the plasma was pipetted off. The capillary tubes were now cut across immediately below the leucocytic cap and the agglomerated leucocytes dispersed in 30 c.mm. of serum. These serum suspen-

sions of leucocytes were then implanted with, in each case, 5 c.mm. of a diluted staphylococcus culture, and were then incubated in capillary tubes disposed nearly horizontally. Similar quanta of staphylococcus were, for purposes of enumeration, implanted into leucocyte-free serum. (Table VIII.)

Experiment 7.11.30.—50 c.mm. volumes of R. H.'s blood were centrifuged after the addition of $\frac{1}{2}$ per cent. citrate of soda. The plasma having been pipetted off the leucocytes composing the leucocytic cap were dispersed in 30 c.mm. of serum, and these suspensions were implanted with one-tenth of their volume of graduated dilutions of staphylococcus, and were then incubated in capillary tubes disposed nearly horizontally. (Table IX.)

TABLE IX

Dilution of staphylococcus implanted	No. of microbes implanted into each volume of leucocytic suspension	No. of colonies which developed in each tube	Percentage of survivors
$\frac{\text{Staph.}}{200{,}000}$	25	1, 1 | 1	4
$\frac{\text{Staph.}}{100{,}000}$	50	0, 1 | 0·5	1
$\frac{\text{Staph.}}{50{,}000}$	100	1, 2 | 1·5	1·5
$\frac{\text{Staph.}}{2{,}500}$	2000	9, 7 | 8	0·4

Experiment 5.11.30.—R. T. M.'s blood. Leucocytic suspension made by suspending the leucocytes from 50 c.mm. of centrifuged blood in 15 c.mm. of serum.

TABLE X

No. of microbes implanted	No. of colonies which developed	Percentage of survivors
36	9, 12 | $10\frac{1}{2}$	28
72	20, 18 | 19	26
144	18, 18 | 18	12
288	58, 30 | 44	15
576	60, 58 | 59	10

Experiment 3.12.30.—R. M. F.'s blood. Graduated implantations of staphylococcus into a weak leucocytic suspension.

Leucocytic suspension prepared in bulk from R. M. F.'s citrated blood and diluted with serum to one-eighth: $2\frac{1}{2}$ c.mm. volumes of staphylococcus dilutions were then implanted into 20 c.mm. of this leucocytic suspension—in triplicate and

TABLE XI

Dilution of staphylococcus	Count	Calculated implant	Colonies developed	Percentage of survivors
Staph. 1,000	—	720	246, 272 | 259	36
Staph. 3,000	—	240	72, 90, 98 | 87	36
Staph. 10,000	—	72	32, 33, 22 | 29	40
Staph. 30,000	18, 29, 18, 30, 26	24	8, 6, 22 | 8	33
Staph. 100,000	—	7	5, 6, 7 | 6	86

Similar experiment and technique as above, 4.12.30

Staph. 10,000	91, 81, 103, 93, 78	89	6, 3, 12, 4 | $6\frac{1}{4}$	7
Staph. 20,000	—	44	8, 6, 8, 4 | $6\frac{1}{2}$	14·8
Staph. 40,000	20, 14, 24, 32	$22\frac{1}{2}$	4, 5, 2, 15 | $6\frac{1}{2}$	29
Staph. 80,000	—	11	1, 6, 3, 5 | $3\frac{3}{4}$	34
Staph. 160,000	—	6	3, 3, 2, 1 | $2\frac{1}{4}$	37·5

the suspensions were then incubated as above. Five counts made of 1/30,000 dilution in normal serum. (Table XI.)

We pass now to other experiments in which we employ, as before, graduated quanta of microbes, and operate again upon blood or separated leucocytes; but we now arrange our experiments so that the acquirement of bactericidal power of the serum and the effect exerted by it shall be separately manifest.

The simplest and most obvious way of achieving this is to bring the vaccine of dead or living microbes into operation upon the blood, to incubate the clots, and then pipette off the serum and test it by implanting it with *very small* numbers of living microbes.

The two following experiments are of this kind :—

Experiment 17.12.30. A. E. W.'s blood.—5 c.mm. of salt solution or diluted staphylococcus culture were incorporated into 50 c.mm. volumes of blood, and the bloods were then incubated for 20 minutes. After this they were centrifuged and the sera pipetted off and tested by the wash and after-wash method—the forerunner volume being here a 10 c.mm. volume of $\frac{\text{Staph.}}{1000}$ and the after-runner volumes three 5 c.mm. volumes of serum. The results were as shown in Table XII.

TABLE XII

—	No. of colonies which developed in the three after-runner volumes			Total
	1	2	3	
Serum from control blood ..	27 26	20 27	13 15	60 68 } 64
Serum from blood implanted with—				
320 staph. per c.cm. ..	30	25	13	68
640 staph. per c.cm. ..	30	24	1	55
1280 staph. per c.cm. ..	23	16	10	49
2560 staph. per c.cm. ..	18	12	0	30

Calculation shows that here, in the most favourable case, the 15 c.mm. of serum here used acquired sufficient bactericidal power to kill at the rate of 2500 staphylococci per c.c.

Experiment, 2.12.30. A. E. W.'s blood.—50 cmm. volumes of blood centrifuged after clotting. The sera were drawn off and were, after addition of Tuberculin B.E., reimposed upon the clots. After 24 hours' incubation the sera were a second time drawn off, and were tested by the wash and after-wash method, using a 5 c.mm. volume of $\frac{\text{Staph.}}{1000}$ as a forerunner and three 5 c.mm. volumes of serum as after-runners. (Table XIII.)

TABLE XIII

—	No. of colonies which developed in the three after-runner volumes			Total	
	1	2	3		
Control normal serum ..	24	15	4	43	48
	34	13	6	53	
Serum from blood vaccinated with—					
B.E.	13	7	4	24	27½
2000 million	21	6	4	31	
B.E.	17	9	0	26	24
1000 million	14	8	0	22	
B.E.	18	3	2	23	30
500 million	22	12	3	37	
B.E.	13	2	0	15	16
100 million	9	4	4	17	
B.E.	15	8	5	28	24½
50 million ..	16	5	0	21	
B.E.	8	10	7	25	19½
25 million	9	3	2	14	

Calculation shows that here again in the most favourable case the serum acquires sufficient bactericidal power to kill just over 2000 staphylococci per c.cm.

Experiment 21.3.30.—100 c.mm. volumes of A. E. W.'s uncoagulated blood implanted with 10 c.mm. of normal salt solution or staphylococcus culture were incubated for 25 minutes, and then centrifuged. The sera were now pipetted off. 5 c.mm. volumes of a 50,000-fold diluted staphylococcus culture were added in each case to 40 c.mm. of serum. (Table XIV.)

TABLE XIV

—		No. of colonies which developed
Serum of control blood	(1)	51
	(2)	50
Serum from blood vaccinated with—		
5 staph. per c.c.		39
50 staph. per c.c.		15
500 staph. per c.c.		22
1,000 staph. per c.c.		17
2,000 staph. per c.c.		17
5,000 staph. per c.c.		12
20,000 staph. per c.c.		26

The serum here killed off, at the best, 950 staphylococci per c.cm.

Another way of studying the effect of the serum separately is illustrated by the next two experiments.

Experiment 9.12.30. A. E. W.'s blood.—50 c.mm. volumes of blood centrifuged after clotting in capillary tubes. The sera were then drawn off, 2·5 c.mm. of diluted staphylococcus culture were added to each volume of serum, and the sera were then replaced on the clots and lightly centrifuged. The containing tubes were then disposed almost horizontally and incubated overnight. (Table XV.)

TABLE XV

Dilution of staphylococcus culture implanted	No. of microbes implanted into each volume of serum	No. of colonies which developed	Percentage of survivors
Staph. / 160,000	6	6, 4 } 5	84
Staph. / 80,000	12	2, 6 } 4	32
Staph. / 40,000	25	9, 17 } 13	48
Staph. / 20,000	50	21, 19 } 20	40
Staph. / 10,000	100	55, 21 } 38	38

Calculation shows that here the serum in the most favourable case killed 2500 staphylococci per c.cm.

Experiment 23.9.30. A. E. W.'s blood.—50 c.mm. volumes were filled into capillary tubes and allowed to clot. They were then centrifuged and the sera pipetted off. The volumes of sera were then inoculated with Tuberculin B.E. and then implanted in each case with 58 staphylococci and replaced on the clots. The containing tubes were then sloped so as to form a small angle with the horizontal and were incubated in that position. The number of colonies which developed in the sera were then enumerated. (Table XVI.)

Yet another way of arranging the experiment (there is also a fourth, which will be considered elsewhere) is as follows :—

We take a series of capillary tubes upon the stems of which we have marked off two segments of, let us say, 25 c.mm. capacity. We then fill into the distal segments blood from the finger, incubate for 20 to 30 minutes and then expel the clots. Having thus provided ourselves with capillary tubes divided into two segments, of which the distal one is carpeted with leucocytes and the proximal one uncarpeted, we introduce into these combined segments through the proximal ends of the tubes in each case a 50 c.mm. volume of serum—the sera employed for the purpose having been implanted with graduated quanta of staphylococcus. The tubes are now sealed up without displacing the columns of serum, and, the unlined

TABLE XVI

		No. of colonies which developed
Control serum	(*a*)	52
	(*b*)	52 } 52
Serum containing—		
B.E. / 2400 million	.. ..	51 / 47 } 49
B.E. / 1200 million	.. ..	42 / 41 } 41½
B.E. / 600 million	.. ..	47 / 44 } 45½
B.E. / 300 million	.. ..	35 / 37 } 36

Calculation shows that the serum killed 640 staphylococci per c.cm.

segments being kept uppermost, are sloped so as to form a small angle with the horizontal; they are then placed in the incubator. The protocols of two experiments of this kind are subjoined.

Experiment 2.1.31.—R. M. F.'s blood. (Table XVII.)

TABLE XVII

Dilution of staphylococcus culture implanted into the serum	No. of microbes suspended in the serum in the		No. of colonies which developed in the serum in the		Percentage of survivors in unlined segment
	Lined segment	Unlined segment	Lined segment	Unlined segment	
Staph. / 180,000	12	12	0 / 0	15 / 7 } 11	90
Staph. / 90,000	24	24	0 / 0	7 / 5 } 6	25
Staph. / 30,000	72	72	0 / 0	33 / 34 } 33½	48
Staph. / 10,000	216	216	0 / 0	73 / 82 } 77	30
Staph. / 3,000	720	720	0 / 0	120 / 120 } 120	17

Here the serum in the unlined segment of the tubes killed in the most favourable case, 24,000 staphylococci per c.cm.

Experiment. A. E. W.'s blood.—(Same technique as in the above.) (Table XVIII.)

TABLE XVIII

Dilution of staphylococcus culture implanted into the serum	No. of microbes implanted into the serum in the		No. of colonies which developed in the serum in the		Percentage of survivors in unlined segment
	Lined segment	Unlined segment	Lined segment	Unlined segment	
$\frac{\text{Staph.}}{160{,}000}$	6	6	0 0	0 3 } 1·5	—
$\frac{\text{Staph.}}{80{,}000}$	12	12	0 0	12 9 } 10·5	87
$\frac{\text{Staph.}}{40{,}000}$	24	24	0 0	19 19 } 19	80
$\frac{\text{Staph.}}{20{,}000}$	48	48	0 0	38 34 } 36	75
$\frac{\text{Staph.}}{10{,}000}$	96	96	0 0	50 67 } 58	60
$\frac{\text{Staph.}}{5{,}000}$	192	192	0 0	73 70 } $71\frac{1}{2}$	37

Here the serum in the unlined segment of the tubes killed, in the most favourable case, 4800 staphylococci per c.cm.

When we review these experiments we recognise that *the law*—for we may now call it so—*of zero, or all but zero, bactericidal response to minimal microbic implantations, and of progressively greater response to increasing microbic stimulation*, holds not only of implantations into blood, but also of those made into leucocytic suspensions. And we have further seen that precisely similar effects are obtained with sera pipetted off from respectively lightly and heavily vaccinated bloods, and also with sera imposed upon respectively lightly and heavily vaccinated leucocytes.

The problem as to whether the increasing killing of serophytic microbes witnessed in effectively vaccinated blood is attributable to increased phagocytic response, or to a development of bactericidal powers in the serum, would appear herein to find its answer.

We shall have to consider later whether the increased phagocytic response which is concurrently obtained does or does not in cases where the action of phagocytes is not mechanically barred, contribute its quota to the observed result.

We may now, taking our cue from the experiments set out in Tables XIII and XVI, dispose of one further general point before entering deeper into the question of phagocytic response in *vitro*. On studying the protocols in question you will note two outstanding points of difference between these and those previously considered. It will be noted, first, that instead of the vaccine being directly incorporated into the blood it was here merely placed in abutment against the blood-clots. And it will be noted further, that the smaller and larger doses of vaccine here brought into application do not here, as they do when incorporated into the blood,

produce opposite effects in the form of positive and negative responses. Instead of that we have here with an extensive range of doses (in the one case with doses ranging from 1 to 2000 million to 1 in 25 million, and in the other case with doses ranging from 1 in 2400 million to 1 in 300 million of Tuberculin B.E.) with every dose a positive bactericidal response.

There is not in reality any matter for surprise in this. For consideration will show that when a vaccine is merely superposed upon a clot there will, in the initial stages of the experiment, come into operation upon the leucocytes only such fraction of the total antigen as may diffuse into the clots. And in conformity with this there will, in the stage of the experiment we are considering, come into lethal operation upon the microbes disposed outside the clot, only the bactericidal substances elaborated in response to this first instalment of antigen.

A number of theoretically interesting points connected with this mode of bringing vaccines into application must be reserved for treatment in a separate paper, but it is of practical interest to reflect that the two contrasted methods of applying a vaccine which we have here been considering have their analogues, the one in intravenous administration and the other in subcutaneous application of vaccines. In the case of the first there is in respect to dosage very little margin of latitude. If we inject a little too much we produce an immediate negative phase. Contrariwise, where a vaccine is applied hypodermically the patient may, even in despite of the administration of an excessive dose, reap benefit from it. For the first instalment of antigen absorbed may achieve the therapeutic aim we have in view, and our aim once achieved any negative phase which may thereafter supervene would be quite innocuous.

Increased Phagocytic Response in Vitro.

We may now pass to the consideration of the phagocytic response obtained *in vitro*.

Perhaps when all things are taken into consideration the most dramatic demonstrations of increased phagocytic response obtained *in vitro* by vaccines are those which are obtained by the ruse of employing in our phagocytic mixtures instead of unheated serum, heated serum—i.e., serum which is almost denuded of opsonin. It will suffice here to cite a single experiment.

Experiment 5.5.30. A. E. W.'s defibrinated blood.—The blood was centrifuged. The serum was then pipetted off and heated for 45° to 60° C. The corpuscles were washed several times in normal salt solution. Two volumes of heated serum were then mixed with one volume of washed corpuscles and one volume of a six-fold diluted staphylococcus broth culture, and there was then added to the control phagocytic mixture one wash of physiological salt solution, and to the other mixtures in each case one wash of a dilution of Tuberculin B.E. These mixtures were then, after incubation for 20 minutes, centrifuged and recentrifuged for 45° in each direction. Phagocytic films were then made from each sample of blood. (Table XIX.)

In order to appraise at their proper value the figures set down in the first two columns of the above protocol account has to be taken of the fact that in counting

the films any ingest of over 20 staphylococci was entered as an ingest of 20. If it had been possible to count the full number of microbes ingested (the percentage of leucocytes containing uncountable numbers is recorded in column 4 of the protocol) the phagocytic indices would have come out very much higher. The phagocytic counts with the optimum additions of vaccine would certainly have reached astonishing figures—let us say an average of 30 or 40 per cell, and the phagocytic indices would have reached 7·5 or 10.

TABLE XIX

A = Percentage of leucocytes which phagocytose.
B = Percentage of leucocytes containing an uncountable number of staph.

—	Phagocytic		A	B
	Count	Index		
Heated serum + corp's + $\frac{\text{staph.}}{6}$ + 1 Wash of normal salt	2 3·3 } 2·6	1	42 57	5 5
Heated serum + corp's + $\frac{\text{staph.}}{6}$ + 1 Wash (= 1/100th) of $\frac{\text{B.E.}}{256 \text{ million}}$	6	2·3	58	16
" " " $\frac{\text{B.E.}}{128 \text{ million}}$	2·6	1	10	8
" " " $\frac{\text{B.E.}}{64 \text{ million}}$	7·7	3	67	21
" " " $\frac{\text{B.E.}}{32 \text{ million}}$	10	3·9	66	19
" " " $\frac{\text{B.E.}}{16 \text{ million}}$	7·1	2·8	60	21
" " " $\frac{\text{B.E.}}{8 \text{ million}}$	6·1	2·3	49	21
" " " $\frac{\text{B.E.}}{4 \text{ million}}$	12·0	4·6	88	48
" " " $\frac{\text{B.E.}}{2 \text{ million}}$	5·6	2·2	56	18

An experiment like the foregoing furnishes conclusive evidence that leucocytes when treated with vaccines, respond, not only (as seen in previous experiments) by excreting bactericidal substances into the serum, but also by an increased phagocytosis. Similar, but owing to the larger phagocytic intake in the control, quantitatively less striking differences between the vaccinated bloods and the controls are obtained by adding appropriate quanta of vaccines to phagocytic mixtures containing, instead of heated, unheated serum. And analysis of these results by the chiastic method has shown that increased phagocytic response obtained is, in most cases, due to a combination of increased opsonic power developed in the serum and to

increased phagocytic avidity in the leucocytes. Not infrequently, however, the opsonic power of the serum is increased, while the phagocytic avidity of the leucocytes is reduced; and again, sometimes the phagocytic avidity of the leucocytes is increased while the opsonic power of the serum remains the same or is reduced.

There is, however, in these respects, very little agreement between one experiment and another, and very little also between the figures which represent the phagocytic index of a blood and its component factors—i.e., the figures which represent (*a*) the opsonic index of the serum, and (*b*) the phagocytic avidity of the leucocytes. This is no doubt due to the fact that the washing, centrifuging, and standing in salt solution to which the leucocytes are exposed in chiastic and opsonic experiments all affect their intake, and it is probably also due to the serum of vaccinated bloods containing, in addition to opsonins, antigens which can evoke immunising response in the leucocytes used for testing. There is at this stage no need, I think, to labour the point that the leucocytes of a phagocytic mixture are responsive elements, and are not, in any sense, the analogues of chemical indicators.

Importance of the Factor of Phagocytosis.

I now pass to consider certain general problems which require to be carefully pondered.

The first of these is the problem—already envisaged above—as to whether enhanced phagocytosis or the development of bactericidal power in the blood-fluids is the more important factor in the immunising response which eventuates in increased killing of serophytic microbes in the blood. In connexion with this arithmetical considerations make clear that inasmuch as the blood normally contains $7\frac{1}{2}$ millions of leucocytes per c.cm. there would, if we implanted 100 microbes (and 100 microbes per c.cm. represents a formidable septicaemic infection), be an army of 75,000 leucocytes available for the phagocyting of each microbe. The same sort of considerations apply to the opsonic capacity of the normal blood fluids. That opsonic capacity as measured (rightly or wrongly) by the ordinary phagocytic technique [1] easily permits of ingests of 10 to 20 microbes per leucocyte of normal phagocytic avidity—and to a total of 75 and 150 millions of microbes ingested per c.cm.

It will be obvious from these data that the normal blood already possesses a phagocytic capacity far in excess of all conceivable requirements. It follows that the explanation of inadequate phagocytosis of implanted or infecting microbes must be sought in the difficulty of leucocytic access to the implanted microbes. When we meditate upon these things and reflect that chemical access (that is access of dissolved bactericidal substances) to implanted microbes must, in the nature of things, be more easily realisable than leucocytic access, and consider further that such chemical access is, in the case of the circulating blood, secured, it comes home to us convincingly that the development of an even moderate amount of bactericidal power in the serum must count for more in the killing of serophytic microbes than any reinforcement of phagocytosis.

[1] It will be shown elsewhere that quite different results are obtained when we employ (and the centrifugalisation method makes this possible) plasma instead of serum in our opsonic measurements.

The further questions we have to consider are : (1) the question of immediate (I have before called it '*subitaneous*') immunising response ; (2) the question of specific or non-specific immunity (we shall see that specific and non-specific are ambiguous terms) ; and lastly (3) the question of dosage.

The Immediate Response.

One of the things I had to do in connexion with antityphoid inoculation was to refute with data obtained by measuring the bactericidal power of inoculated men before and after inoculation, the traditional belief that ten days must go over before the effect of a vaccination process becomes manifest. I showed there that the effect of inoculation upon the blood was already plainly manifest 24 hours after the administration of the vaccine. A further scaling down of the time deemed requisite for the manifestation of the effects of inoculation was achieved when it emerged from our measurements of the opsonic index that the effects of subcutaneous inoculation and after auto-inoculation were already well developed within an hour after the application of the ictus immunisatorius. And further research has shown that immunising response to vaccines is, in the case of intravenous inoculations, well developed within 10 minutes after the inoculation, and finally, both bactericidal and phagocytic experiments on the blood *in vitro*—which, of course, lend themselves better to accurate timing—have shown that there is instantaneous response to the introduction of vaccines into the blood. You will, when we come to consider the question of dosage, see that the time requisite for the development of maximum immunising response is not a simple function of the quantum of antigen incorporated, but is largely determined by the soluble or insoluble nature of the antigen and the lytic forces which the blood brings to bear upon it.

Specific and Non-specific Immunity.

It has already been indicated above that the terms 'specific' and 'non-specific' are terms of ambiguous signification. Let me justify this.

When we speak of 'specific' immunising response, we may mean that infection or its equivalent—i.e., the inoculation of a vaccine—renders the blood bactericidal to the homologous microbes, and only to these ; and when we attach this meaning to 'specific immunising response' we should, of course, mean by 'non-specific immunising response' the acquirement of bactericidal properties for heterologous, as well as homologous microbes.

We may, however, when we use the terms 'specific' and 'non-specific' be thinking not of the question as to whether immunising response gives protection against one or more varieties of microbes, but of the question as to whether the anti-bacterial substances brought into play operate 'specifically'—each affecting only one species of microbe, or whether they operate 'non-specifically'—each affecting more than one species.

Having realised that the terms 'specific' and 'non-specific' are terms which carry these two distinct meanings, it will avoid confusion if we call the thesis that infection and vaccination immunise only against homologous microbes : '*the doctrine of purely homologous immunising response*', and the antithetic thesis that

protection is acquired also against heterologous microbes: '*the doctrine of conjointly homologous and heterologous immunising response*'. Similarly, we should do well to call the thesis that there are special anti-bacterial substances for each kind of microbe: '*the doctrine of specific antibodies*'; and the antithetic thesis that there are anti-bacterial substances which operate upon more than one kind of microbe: '*the doctrine of non-specific antibodies*'.

Is the Immunising Response Specific?

Having clarified the issues we may now make inquiry from the facts, first, as to whether immunising response is specific or non-specific, and secondly, as to whether the antibodies which are native to the blood or are engendered there by infection and vaccination, are specific or non-specific. The answer to the former question is given in many of the protocols of immunisation *in vitro* already adduced or to be adduced later, as well as in numerous others published and unpublished. A study of the protocols referred to shows that Tuberculin B.E. gives immunising response upon staphylococcus; that staphylococcus vaccine gives immunising response upon streptococcus, and typhoid vaccine operates in the same way upon staphylococcus. Further, it would seem—at any rate in the case of my own blood—that better immunising response is obtained upon staphylococcus with Tuberculin B.E. than with staphylococcus vaccine.

I shall presently have something to say with respect to this last finding.

When we pass from immunising operations *in vitro* to immunising operations *in vivo* we again find clear evidence of 'non-specific'—i.e., heterologous response. Fig. 3 gives a very convincing example of response obtained upon staphylococcus

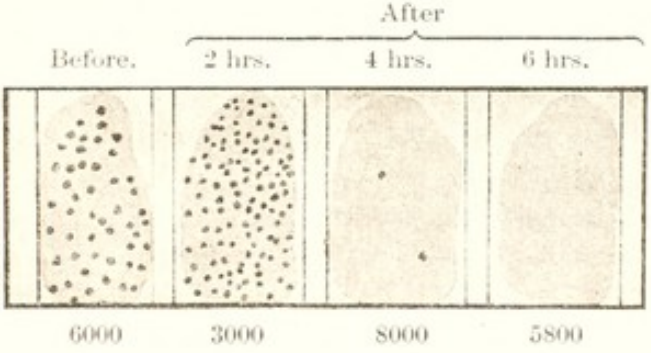


FIG. 3.

The effect of the inoculation was tested by implanting 225 staphylococci into 50 c.mm. vols. of blood; and then incubating in a slide cell. At the same time the leucocytes of the blood were enumerated, the numbers found being those entered in the figure.

by an intravenous inoculation of 200 millions of sterilised coliform microbes. We have here first a negative phase of diminished and then a positive phase of increased haemobactericidal action exerted upon the staphylococcus.

And let me, in this connexion, call attention to two points. The first is a question of terminology. It relates to the fact that severe and rapidly supervening constitutional symptoms, such as follow intravenous inoculation, and followed it

here, are in the current jargon of medicine, designated ' protein shock '. This terminology is to be deprecated because there attach to this term quite erroneous implications. (1) The term suggests that in the case where vaccines produce protein shock this is produced by active agents other than those which produce ordinary immunising response. (2) The term further suggests—for it is employed only in connexion with intravenous injections—that protein shock is produced only by intravenous inoculations. Those who have administered large doses of typhoid vaccine hypodermically, and have witnessed the resulting supervention of shock in patients, and in particular in patients who have immediately after inoculation engaged in active exercise, will not require to be told that shock can follow when a severely toxic dose of a vaccine given by the subcutaneous channel has been carried into the circulating blood. Another vice of the term ' protein shock ' lies in its assimilating intravenous inoculations of vaccine to intravenous inoculations of milk, foreign serum, and such like, in lieu of assimilating these injections of foreign albuminous substances to inoculations of vaccine. And an even more serious vice is that the term ' protein shock ' carries with it the suggestion that non-specific immunising response is a product peculiar to intravenous injections of foreign proteins when it is, in point of fact, a general property of vaccines.

A final point to which I want to draw attention relates to the leucocytic counts inserted at the foot of Fig. 3. These figures illustrate the point that inoculations of vaccine, and in particular, severe inoculations, such as that here in question, are followed by a phase of leucopenia, followed by a phase of leucocytosis ; and the figures are inserted here to bring out the point that the increased bactericidal effect shown in the *fourth* compartment of the slide cell is achieved quite independently of any leucocytosis.

Immunising response *in vivo* is, I need now hardly say, not confined to the case of coliform vaccines. We have evidence that streptococcus gives immunising response *in vivo* upon staphylococcus, and similarly, staphylococcus vaccine upon streptococcus. And these are results obtained by measurements of phagocytic and of sero-bactericidal response—in other words, measurements which cannot be falsified by variations in the number of circulating leucocytes. Let me defer for a moment what I have to say about the importance of these findings for vaccine therapy.

For there can be set off against the protocols of experiments showing non-specific immunising response others which favour the idea that immunising response is specific. I am thinking here of the experiments set out in Charts 32 and 44 [1] reproduced in Vol. III of these *Collected Researches* (pp. 362 and 368), and I have in view also some unpublished experiments, and among these a peculiarly striking one made by Dr. John Freeman. In the experiments in question (they were, in each case, massage experiments) the patients' bloods were tested to two microbes (in Dr. Freeman's case the blood was tested to five), and immunising response was obtained in each case only to that microbe which was from the clinical and bacteriological evidence adjudged to be casually associated with the patient's malady. But obvi-

[1] These charts should, however, be studied in conjunction with Charts 7 and 8, pp. 337 and 338, loc. cit.

ously, we have no reason to suppose that every species of microbe can immunise against every other species.

Are the Antibodies Specific?

The study of the question as to whether the antibodies which are native to the blood and also those engendered by inoculation operate upon one kind of microbe to the exclusion of all others has, for the immunisator, only a subordinate interest. A few points may, however, here be referred to. W. Bulloch and G. T. Western,[1] dealing with antibodies native to the blood, arrived at the conclusion that the opsonins which operate upon the tubercle bacillus can be removed from the serum without removing the opsonins for staphylococcus and vice versa. They further arrived at the conclusion that the opsonin for staphylococcus can be removed without removing the opsonin for the *Bacillus pyocyaneus*. On the other hand, it has been shown (the data will be found in Vol. III of these *Collected Researches*, pp. 66 and 67) that treatment of the serum with dead cultures of typhoid and cholera extracts the bactericidins for both species of microbe.

Let me now supplement the points already brought forward by one more. The work of Shattock and Dudgeon—it was, it will be remembered, carried out with melanin particles—suggested that 'phagocytic avidity' was non-specific. This conclusion is confirmed by the observations of my sometime fellow-worker, Dr. R. Hare,[2] dealing with the bloods of cases of puerperal septicaemia. These observations show that increased and diminished phagocytic avidity to streptococcus goes hand in hand with increased and diminished phagocytic avidity to staphylococcus.

General Conclusions.

I should now like to set out the conclusions which seem to me to follow from the data which we have been considering.

Doubtless it seemed paradoxical, and, therefore, doubtful and unacceptable, when I said that when I operate upon my blood with a tubercle vaccine (B.E.) I get better—that is, more emphatic, and for the same dose employed more consistent—immunising response than when I operate with staphylococcus vaccine. In connexion with it I am not going to be rash enough to suggest that the reader should abate anything of his faith in its being a general law of nature for the best immunising response to be obtained by the use of a homologous vaccine. I do not want to do anything more than suggest that a rule such as this might quite well be open to exception. It might be open to a general exception if the heterologous vaccine in question were, as compared with the homologous vaccine, more easily soluble in the blood fluids. And it might be open to an individual exception if a particular patient's blood exerted less lytic action upon a vaccine made with his own microbe than it did upon a vaccine made from a microbe other than the infecting microbe.

Anyone who goes as far as to accept the possibility of such exceptions to rule will be prepared to let the superiority of a vaccine A over a vaccine B—even if the vaccine A should be heterologous and the vaccine B homologous—be decided by

[1] *Proc. Roy. Soc. B.*, 1906, lxxvii. [2] *Vide infra*, p. 194.

laboratory experiment and, moreover, will be prepared to look upon such experiments without *parti pris*. But while I would plead that the question of superiority of one kind of vaccine over another is one that can be settled only by experiment, none the less I want to put two general points. They are the following :—

In cases where the nature of the infecting agent cannot be determined, advantage very often accrues from inoculation with vaccines made from microbes which are, in all probability, different from those with which the patient is infected. When I say this I have in view (well knowing that these and all other bacteriologically undiagnosed affections are of debatable aetiology) articular rheumatism and phlyctenules of the cornea. It would, I think, be sheer obstinacy to let doubt respecting the aetiology of these affections, and a passionate conviction that immunising response is purely specific, hold us back from inoculating in the one case vaccines made from intestinal streptococci, and in the other case minimal doses of Tuberculin B.E. Essentially the same reasoning would, I think, apply in dealing with infections such as glanders, in which homologous vaccines have been tried time and again without success. One might, I think, surmise that such a microbe as that of glanders furnished a very ineffective antigen, and one might, by consequence, reasonably try whether anything useful could be done with a non-homologous vaccine.

Principles of Dosage.

I turn now—and this will complete what I propose to say here on the subject of vaccine therapy—to the question as to whether we can, with the knowledge gained by the study of immunisation *in vitro*, add anything to the principles of dosage which were learned when we had only the clinical symptoms and measurements of the opsonic index to guide us. It will be remembered that the most important of the general principles of dosage arrived at by watching and measuring the opsonic index of patients was that the doses of vaccine employed should be inversely as the amount of infection. Further, that, with a view to avoiding negative phases, the doses given to heavily infected patients should be much smaller than those given to the lightly infected, and should represent a very small fraction of the doses administered in prophylactic operations undertaken upon the healthy. This principle finds interesting confirmation when we carry out experiments *in vitro*. We take a series of equal measured blood volumes and implant into these graduated quanta of living microbes, and then add to each implanted volume one and the same dose of vaccine. It does not require a great effort of imaginative vision to discern that we have here analogous conditions to those which prevail in lightly and heavily infected patients. Instructive illustration of the effects produced is furnished in the subjoined protocols.

Experiment.—Capillary tubes were prepared with a stem about a foot and a half in length and of almost uniform bore, and a 10 c.mm. division mark was then placed on the distal end of the stem. Blood was then obtained from my finger, and then there was aspirated into one of the capillary tubes as a forerunner 10 c.mm. of a 1000-fold dilution of a broth culture of staphylococcus ; and then, as after-runners, ten 10 c.mm. volumes of blood. The same procedure was carried out with two

other portions of blood, the one inoculated with a smaller and the other with a larger dose of Tuberculin B.E. After 24 hours' incubation the blood-clots were blown out in order, each series arranged in a row upon a separate strip of filter paper. The clots were then, as soon as they had by absorption of fluid become adherent to the filter paper strips, treated with 1 per cent. saponin, and the strips were then introduced into a beaker full of water, being applied to its interior wall after the fashion of a cornice. When haemolysis was complete the filter paper strips were placed in a Petri dish containing water, and the decolourised clots were then aspirated into a capillary tube and transferred in proper order to a microscopic slide. They were then dried and stained. The number of colonies which developed in each of the three series of clots is set out in Table XX.

TABLE XX

—	No. of colonies of staphylococcus in volumes										No. of colonies in—		
	1	2	3	4	5	6	7	8	9	10	The whole 10 vols.	Vols. 1–5	Vols. 6–10
Control blood	1	9	5	2	1	2	1	1	0	0	22	18	4
Blood inoculated with B.E. 1 in 1000 million	1	3	4	1	2	0	2	0	0	0	13	11	2
Blood inoculated with B.E. 1 in 500 million	4	10	9	15	3	1	0	0	0	0	42	41	1

Note.—The small number of colonies in Vols. No. 1 is no doubt due to this volume of blood being very highly diluted by the forerunner volume of tuberculin in salt solution.

When we glance, to begin with, at the rubric in which are set down the aggregate number of staphylococcic colonies which developed in the three capillary tubes, we see that the aggregate is, in the case of the blood containing 1 part of B.E. in 1000 million, less by nearly 50 per cent. than it is in the control blood, and that the aggregate number of colonies which developed in the blood which contained 1 part of B.E. in 500 million is nearly double that in the control. If demonstration were necessary of the impossibility of judging a dose of vaccine by the epitomal result produced upon a series of different bloods of patients, such demonstration is furnished here. For, judged by the epitomal effect produced in the whole series of volumes, a dose of 1 part in 500 millions of tuberculin is a large overdose for my blood. Very different are the conclusions which emerge when we analyse the results and consider separately that which applies to the first five and that which applies to the last five volumes of the series. We then see that in the case of the heavily implanted blood volumes the effect of the larger dose of vaccine is to give us in these volumes more than twice as many colonies as in the control blood, and nearly four times as many as in the less heavily vaccinated blood. But when we turn to the effect produced upon the more lightly implanted bloods we see that the larger dose of vaccine enhances the bactericidal power more than does the smaller dose.

An essential similar experiment is set out in Table XXI.

Experiment 8.4.31.—Same technique. Forerunner 15 c.mm. volumes of $\frac{\text{staph}}{1000}$; afterrunners, ten 15 c.mm. volumes of A. E. W.'s blood.

TABLE XXI

—	No. of staphylococcus colonies in volumes [1]								Whole 8 vols.	1st 4 of those vols.	2nd 4 of those vols.
	3	4	5	6	7	8	9	10			
Control—											
Unvaccinated blood	24	16	9	3	0	0	1	1	54	52	2
	29	12	2	3	0	0	0	0	46	46	0
	20	9	20	0	4	1	0	0	54	49	5
	29	7	3	3	0	3	0	0	45	42	3
									50	$47\frac{1}{2}$	$2\frac{1}{2}$
Blood vacc. with—											
B.E. / 1000 million ..	2	0	2	1	0	1	0	0	6	5	1
B.E. / 500 million ..	11	8	5	0	1	0	0	0	25	24	1
2000 killed staph. ..	15	4	6	2	0	0	1	0	28	27	1
4000 ,, ,, ..	23	14	14	14	4	0	0	0	69	65	4

[1] Nos. 1 and 2 nil. Staph. = staphylococci.

Increased Phagocytosis Obtained by Inoculation of Blood *in Vitro*.

Precisely similar effects—and effects which show that we are here dealing with a general law—are seen in phagocytic experiments in which an inoculated blood is tested with progressive dilutions of a staphylococcus culture.

Phagocytic Experiment.—A. E. W.'s blood centrifugalised with different strengths of Tuberculin B.E. and different strengths of staphylococcus vaccine. (Table XXII.)

TABLE XXII

—	Phagocytic indices tested with—	
	5-fold diluted sterilised culture	15-fold diluted sterilised culture
Control blood ..	1·0	1·0
Blood with—		
B.E. / 1000 million ..	0·76	1·63
B.E. / 200 million ..	0·66	1·0

No preliminary incubation

Phagocytic Experiment.—A. E. W.'s defibrinated blood mixed with different strengths of Tuberculin B.E. and different dilutions of staphylococcus and then centrifugalised immediately after mixture. (Table XXIII.)

TABLE XXIII

—	Phagocytic indices tested with—			
	Undiluted culture	3-fold dilution	9-fold dilution	27-fold dilution
Control blood	1·0	1·0	1·0	1·0
Blood with—				
$\frac{\text{B.E.}}{1000 \text{ million}}$	1·5	2·45	1·65	1·2
$\frac{\text{B.E.}}{5000 \text{ million}}$	1·4	1·0	1·0	0·96

Phagocytic Experiment.—A. E. W.'s defibrinated blood implanted with Tuberculin B.E. and centrifugalised immediately after mixture with progressive dilutions of staphylococcus. Where the higher dilutions of staphylococcus were employed the length of centrifugalisation was increased in order to obtain a conveniently large ingest. (Table XXIV.)

TABLE XXIV

—	Phagocytic indices tested with—			
	An undiluted culture	3-fold dilution of the culture	9-fold dilution of the culture	27-fold dilution of the culture
Control blood	1·0	1·0	1·0	1·0
Blood with $\frac{\text{B.E.}}{1000 \text{ mill.}}$	1·05	1·1	1·35	1·4
Duration of centrifugalisation	½ min.	1 min.	2 min.	2 min.

The rationale of these results is obvious. They show us that when the blood is implanted with a moderate quantum of living microbes and a moderate quantum of vaccine is added, the antigens derived from these twin sources may constitute, taken together, an optimum immunising stimulus. They show us further that when the implant of living microbes is cut down, and the antigen derived from this source is correspondingly diminished, the quantum of vaccine may be profitably increased. And vice versa, when the implant of living microbes is increased the quantum of vaccine must be correspondingly cut down.

Before leaving this question a word may be said concerning the situation in which we find ourselves when dealing with a patient who has an acute infection, and we are in doubt as to whether he can still make response to a vaccine, and if so as to what quantum can be safely administered. In this situation we can, by recourse to what I have called the Vaccine Response Test, obtain the required information.

The '*Vaccine Response Test*' consists in adding to the patient's blood *in vitro* graduated quanta of the vaccine which it is proposed to use, and seeing whether any of the doses employed enhance, after some arbitrarily fixed period of incubation, the phagocytic power of the patient's blood, or whether all doses of vaccine impair that power.

I have elsewhere[1] published data showing the help that can be obtained from the vaccine response test. But I should like to say here that when in a rapidly advancing infection we needs must decide in the dark, it is always advisable to try the effect of inoculating with a very small dose of vaccine. For the advantage that may accrue if the patient's blood is still capable of making immunising response far outweighs the harm that might result if his were incapable of making immunising response, and the case already hopeless.

Let me now come back from this digression and consider yet another question in connexion with the dosage of vaccines. I can best introduce the point by recalling that while in the case of ordinary vaccines some of the antigen is in solution, the bulk of it is present in the form of particulate matter which is (I am thinking here in particular of vaccines made from serophytic microbes) not very readily soluble in the blood fluids. It follows that if there is, when the vaccine comes into operation upon the blood, any immediate immunising response, this will be response to that fraction of the total antigen which is in solution in the vaccine, or is, as the case may be, immediately brought into solution by the blood fluids. Such subitaneous response takes in the typical curve of opsonic response (figured in Vol. III of these *Collected Researches*, Vol. III, p. 331, Chart I), the form which I there called a 'false rise', and this 'false rise' is, according as the quantum of antigen afterwards dissolved is appropriate or excessive, followed by either a positive phase unpreceded by a negative, or a negative phase followed by a positive. All this is very clearly shown in the subjoined series of protocols.

TABLE XXV

	Phagocytic indices		
	Immediate result	After 1 hr. 45 min. on bench	After a further 45 min. at 37° C.
Control blood	1·0	1·0	1·0
Blood inoculated with 10,000 dead staph. per c.cm.	1·23	0·55	0·18
Blood inoculated with 5 million dead staph. per c.cm.	1·33	0·77	0·31

[1] *Vide supra*, pp. 110–112.

Phagocytic Experiment.—A. E. W.'s defibrinated blood treated with staphylococcus vaccine and tested with staphylococcus by the method of centrifugalisation at intervals after vaccination. (Table XXV.)

Phagocytic Experiment.—A. E. W.'s defibrinated blood vaccinated with Tuberculin B.E., and tested at intervals after vaccination by centrifugalisation with staphylococcus. (Table XXVI.)

TABLE XXVI

—	Phagocytic indices	
	Tested immediately after vaccination	After 1 hr. 20 min. incubation
Control blood	1·0	1·0
Blood inoculated with—		
$\frac{\text{B.E.}}{\text{1000 million}}$	1·5	0·6
$\frac{\text{B.E.}}{\text{200 million}}$	0·57	0·7
$\frac{\text{B.E.}}{\text{10 million}}$	0·53	0·7

Phagocytic Experiment 22.10.25.—A. E. W.'s blood and the same blood treated with two strengths of Tuberculin B.E. Tested phagocytically by centrifugalisation with staphylococcus at different intervals after vaccination. (Table XXVII.)

TABLE XXVII

—	Phagocytic indices					
	5 min.	10 min.	20 min.	40 min.	90 min.	140 min.
Control blood	1·0	1·0	1·0	1·0	1·0	1·0
Blood inoculated with—						
$\frac{\text{B.E.}}{\text{1000 million}}$	0·82	0·94	1·0	1·15	1·63	1·6
$\frac{\text{B.E.}}{\text{50 million}}$	0·6	0·56	1·0	0·8	0·76	0·86

Experiment 23.10.27.—A. F.'s defibrinated blood implanted with Tuberculin B.E. and kept in the incubator and tested at progressive intervals after vaccination by centrifugalisation with staphylococcus. (Table XXVIII.)

TABLE XXVIII

—	Phagocytic indices			
	5 min.	10 min.	1 hr.	1 hr. 20 min.
Control blood	1·0	1·0	1·0	1·0
Blood inoculated with—				
B.E. / 500 million	0·89	0·86	1·53	1·64
B.E. / 5 million	1·25	1·1	1·25	1·0

Experiment (undated).—A. F.'s defibrinated blood incubated with Tuberculin B.E. and tested at progressive intervals after vaccination by centrifugalisation with staphylococcus. (Table XXIX.)

TABLE XXIX

—	Phagocytic indices				
	10 min.	14 min.	20 min.	25 min.	30 min.
Control blood	1·0	1·0	1·0	1·0	1·0
Blood with B.E. / 300 million	1·46	1·47	1·31	1·30	0·90

There remains one last point which I should like to call attention to. It is again a point of similarity between the effects produced by infection *in vivo* and those produced by inoculation *in vitro*. It will perhaps be remembered that I have from the very outset called attention to the fact that patients suffering from lupus and generally from strictly localised forms of tuberculosis,[1] unaccompanied by constitutional disturbance, have a very low opsonic index to tubercle.

The phenomenon is clearly not one of the same order as the negative phase. For negative phases supervene, not upon minimal auto-inoculations, such as can here come in question, but only upon large inoculations or auto-inoculations. Further, a negative phase is practically always followed by the response in the form of a positive phase, and the indices of these patients remain, as can be inferred from the fact that their tuberculo-opsonic indices are uniformly low, chronically below standard. Every one of these points finds its parallel in the results obtained by the inoculation of the blood with minimal quanta of vaccine.

It has already been seen in one of the protocols of haemobactericidal experi-

[1] The data relating to this question and also important confirmation of these data by Prof. W. Bulloch are to be found in Vol. III of these *Collected Researches*, pp. 144 *et seq.*

ments which I have shown—and I could have very easily produced others showing the same—that when minimal quanta of living microbes are implanted into the blood on the one hand and serum on the other, more microbes develop into colonies in the blood than in the serum. The same thing may be seen when the sera of bloods which have been treated with minimal quanta of vaccines are tested for bactericidal power by implantation with staphylococcus. Such sera are very often found to furnish a better culture medium for staphylococcus than the control sera. And again, the addition of minimal quanta of vaccine to a phagocytic mixture often lowers the phagocytic index.

In conclusion, I have to express my gratitude to my fellow-workers, and in particular Prof. A. Fleming, Dr. Leonard Colebrook, Dr. R. M. Fry, and Dr. R. Mummery, for having very generously placed not only their work, but also their ideas at my disposal.

IMMUNITY

BY THE AUTHOR

(*Reprinted from 'The Encyclopaedia Britannica', 13th Edition*)

The term 'immunity' is used in science in the technical sense. An animal is described as *naturally immune* against the microbe of disease if the microbe in question cannot establish itself in the organism and as *artificially immune* if it was naturally susceptible and has been rendered insusceptible. Similarly, an animal is described as *naturally immune* against a poison if its organism is naturally proof, and as *artificially immune* if its organism has been rendered proof against the poison.

Natural Immunity.

A few words may be said first with respect to natural immunity against infection. We may attribute the fact that the body normally remains free from microbic infection to a conjunction of causes:—*First*, to the fact that its external and internal coatings furnish mechanical protections against infection (this point need not be further considered here); *secondly*, to the fact that the body is equipped with special machinery for the destruction of microbes (the nature of this machinery will be discussed below in connexion with acquired immunity); and, *thirdly*, to the fact that certain of the conditions which normally prevail in the animal organism are inimical to the growth of microbes as a whole or at any rate to the growth of certain classes of microbes. Of the conditions here in question two which are of quite dominant importance were discovered in the course of researches on wounds carried out in the World War.

Antitrypsin.

The first and more generally important is the antitryptic power of the blood fluids. Such antitryptic power will in every case hamper, and in the ordinary case completely inhibit, bacterial growth. It will achieve this by neutralising, in part or completely, the digestive ferments of the microbes which would otherwise convert the unassimilable native albumens of the blood-fluids and serous discharges into assimilable nutrient materials. Antitrypsin in the blood fluids will therefore mean for the microbes therein implanted, either, a restriction of their food supply, or complete starvation. The facts, as far as known, all comport with this inference.

To begin with, only a few species of microbes (the staphylococcus, streptococcus, tubercle bacillus, pneumococcus and perhaps the microbes of all genuinely septicaemic diseases) can proliferate in antitryptic blood fluids. Further, these microbes (we may conveniently call them *serophytic* microbes) produce when grown in plasma (we are here generalising from observations made with staphylococcus and

streptococcus) trypsin which quenches the antitryptic power of the surrounding blood fluids and then digests vacuoles in the surrounding clot. Again, all serophytic microbes grow very much more vigorously (the streptococcus for example very many thousand times more vigorously) in the blood fluids when these microbes have, by an artificial addition of trypsin, been relieved from the task of themselves producing enough trypsin to quench the antitryptic power of the surrounding medium. And finally, all those kinds of microbes which are unable to grow in antitryptic blood fluids can pullulate there the moment trypsin is added—and let it be noted here that a spontaneous addition of trypsin occurs regularly in wounds as soon as the emigrated leucocytes are broken down under the influence of bacterial growth and unfavourable external influences.

Effect of Alkaline Reaction.

Again—and here the second of the restraints on microbic growth spoken of above comes into question—in the course of research work on wound infections conducted in the course of the War it was established, in connexion with the microbe of gas gangrene, that the proliferation of this bacillus in the blood fluids and serous effusions is inhibited not only by the antitryptic power but also by the normal alkaline reaction of these media. Proof of this is furnished by the fact that as soon as the alkaline reaction in question is blunted off by the ante- or post-mortem formation of lactic acid in muscles deprived of their blood supply, or more directly by an infusion of any acid into the blood, the bacillus of gangrene multiplies without restraint.

Natural Susceptibility.

The problem as to why a particular animal is naturally susceptible to particular poisons is likewise a chemical problem, but a chemical problem of a different order. Formulated in the technical terms which were introduced by Ehrlich the problem is that as to why the tissues of the insusceptible animal contain no *receptors* for the poison, that is to say, no organic substratum upon which that poison can anchor itself. This is a question of the aboriginal chemical constitution of the body—a question as to why a particular species of animal was constructed of one kind of chemical substances rather than of another.

Acquired Immunity.

The problem presented by acquired immunity to infections or poisons differs in important respects from that presented by natural immunity. With respect to such reinforcement of the natural resistance it will be well to note at the outset (*a*) that it can be achieved apart from any violent physiological commotion ; (*b*) that acquired immunity does not betray itself in any altered habit of body ; and (*c*) that the condition may in many cases be present to-day and gone to-morrow. All these facts indicate that the acquired immunity cannot involve anything in the nature of a revolutionary physiological upheaval such, for example, as a fundamental alteration in the blood elements, or a cutting out of receptor elements from cells.

Anticipating here, we may say that later it will be shown with regard to acquired immunity to infections that this depends upon the development of (*a*) increased antibacterial substances in the blood fluid, (*b*) increased efficiency in the leucocytes and (*c*) increased capacity for producing antibacterial substances in response to infection. Similarly it will be shown with regard to acquired immunity to poisons that this depends upon the appearance of neutralising elements (so-called antitoxins) in the blood coupled with the acquirement of increased capacity for elaborating these elements in response to an incorporation of the corresponding poisons.

Early Doctrines.

Confining ourselves to the problem as to how the bacterial infections are combated in the organism, we may begin by considering the two chief opposing doctrines which were promulgated, when, under the prompting of Pasteur's practical achievements in the field of prophylaxis, problems of immunity began to be first seriously considered. These theories included natural as well as acquired immunity.

Humoral Theory.

In the theory which goes by the name of the humoral theory, natural immunity is attributed to the bactericidal substances contained in the normal blood fluids, and acquired resistance to the acquisition or increase of such bactericidal power. The general thesis, at any rate, of this theory stands secure. Where microbes are killed otherwise than by physical agencies or by inanition, their destruction must inevitably result from some form of chemical action. So, too, the acquisition of greater power of destruction must of necessity be imputed to the achievement of more potent chemical powers. On the other hand, it cannot of course be *a priori* certain that the anti-bacterial elements concerned in the destruction of microbes will be found in the blood fluids and only in these.

That the blood fluids do, in point of fact, possess bactericidal power was demonstrated by Fodor and Nuttall, and it was at first thought, though a careful study of Nuttall's results should have prevented this, that such bactericidal action was exercised upon microbes without distinction of kind. Later research has, however, shown that the blood fluids are directly poisonous only for certain species of microbes such, for example, as the typhoid bacillus and the cholera vibrio. Certain other species of microbes, and in particular the microbes of specifically septicaemic diseases, are, as we have seen, serophytic—the number that grow out in serum being as great as the number which grow out in the best artificial nutrient medium. Thus the so-called *humoral theory*, though it would account for the organism possessing considerable resistance to typhoid and cholera, leaves unexplained the fact of the normal organism offering very considerable resistance to serophytic microbes.

What applies to natural, applies also to acquired immunity. There is nothing to show that increased resistance to serophytic microbes and septic infections generally is accompanied by the development of any bactericidal power in the blood fluids.

Phagocytic Theory.

An entirely different conception of immunity is that familiarly known as Metchnikoff's *theory of phagocytosis*. In the humoral theory the problem of immunity is envisaged from the point of view of the bacteriological specialist and to some extent also from the standpoint of the chemist. Metchnikoff for his part approached the problem from that of the morphologist who takes the whole field of animal life as his province. His theory of immunity therefore starts with the generalisation that the protozoa feed upon the lower forms of life such as microbes. It further regards the fact that the higher animals have been evolved out of colonial aggregations of protozoa. In every such colonial aggregation certain of the associated cells instead of becoming specialised persist in the condition of wandering cells.

With regard to these free-living members of the protozoal colony Metchnikoff divined that they function as defensive cells. And he showed in connexion with sponges and other invertebrates that these wandering cells collect round invading microbes and other foreign intrusions and that they therefore proceed to ingest and digest them or to aid otherwise in their elimination. Lastly Metchnikoff pointed out that the leucocytes of the vertebrate were homologous to those wandering cells and performed exactly the same defensive offices. In particular when leucocytes emigrate from the capillaries into a focus of bacterial infection, in the normal case, they follow this up by ingesting the intruding micro-organisms, and killing them intracellularly. *Natural* or *native immunity* was thus, in the conception of Metchnikoff, due to efficient leucocytic functioning ; and *acquired immunity* to the leucocytes having by a *process of training* (as he called it) acquired a power of more effectively confronting, ingesting and destroying microbes.

Eclectic Theory.

The central tenet of the Metchnikoff theory—the tenet that the leucocytes play a very important rôle in the defence of the body against infection—has now found universal acceptance, but at the same time the doctrine that resistance to infection depends in every case on the action of the leucocytes has proved untenable. In connexion with the defence of the organism we must distinguish between (1) defence against infection by serophytic microbes, that is against microbes which like the streptococcus and staphylococcus proliferate in the normal serum, (2) defence against infection by microbes which are incapable of multiplying in the antitryptic normal serum but are not directly killed by it, and (3) defence against microbes which are directly killed by the serum.

(*a*) In connexion with the first kind of microbe the fact that although these grow freely in the serum and plasma, these are killed in large numbers in the blood and in the serum, when living leucocytes are added, shows that the cellular elements of the blood here do the work of destruction.

(*b*) In connexion with the successful killing of these microbes, the leucocytes must, however, in all cases have free mechanical access to the microbes. There would for example be default in this respect if, as would happen in dealing with infected defibrinated or infected centrifuged blood *in vitro*, the leucocytes settled

to the bottom and the microbes were buoyed up out of their reach in the serum. The same would of course happen *in vivo* in all serous effusions.

(*c*) In connexion with those microbes which are not directly poisoned but fail to proliferate in the serum, the most important restraining influence is the native antitryptic power of the blood, but once the antitryptic inhibitory action of the blood fluids and serous effusions has been neutralised the leucocytes, even when everything else favours them, will be impotent to inhibit microbic growth. On the contrary as soon as the leucocytes degenerate they will furnish a further quantum of trypsin and in this way directly conduce to the pullulation of all manner of microbes.

Lastly, the leucocytes do not seem to contribute to the defence of the organism against those microbes which, like the typhoid bacillus and the cholera vibrio, are directly poisoned by the serum. When we implant such microbes into defibrinated blood and then incubate and make microscopic preparations those microbes which are quickly ingested are found intact within the phagocytes while those which are left exposed to the action of the serum are distorted and dissolved. And again, when living emigrated leucocytes are brought to bear upon typhoid bacilli it would seem that few of the microbes are killed. Precisely similar results are obtained with extracts made from leucocytes. It was shown by Schattenfroh that such extracts exert bactericidal action upon the staphylococcus and streptococcus while they exert no such action upon the typhoid bacillus and the cholera vibrio. And again it was shown by Colebrook that the products of inflammation derived from foci of inflammation set up by incorporating into rabbits lint soaked in typhoid vaccine are powerfully bactericidal for the staphylococcus and streptococcus while for the microbes of typhoid and cholera they are not more bactericidal than ordinary serum.

This review of the facts shows that only in the case of serophytic microbes does the defence of the body depend upon the leucocytes and that when it is a question of defence against the other two classes of microbes (those whose growth is entirely inhibited and those which are directly poisoned by the serum) the leucocytes are either impotent or directly harmful.

Properties of the Leucocytes Concerned in Immunisation.

Metchnikoff originally taught that the leucocytes were attracted to microbes and induced to phagocytose them by the toxin secreted by the microbes ; and that there was here only an interaction between leucocytes and microbes and that the blood fluids could be left entirely out of the story. That that doctrine cannot be sustained can be demonstrated by a very simple experiment.

We begin by receiving a sample of blood taken direct from the vessels into normal salt solution. We then centrifuge, and recentrifuge in further volumes of this normal salt solution, so as to wash the cellular elements free from all traces of the blood fluids. We then make two so-called *phagocytic mixtures* combining in the one case (*a*) one volume of a bacterial suspension with (*b*) one volume of washed leucocytes and (*c*) one volume of 0·85 per cent. *salt solution* ; and in the other case (*a*) one volume of washed leucocytes, (*b*) one volume of the same bacterial

suspension and (*e*) one volume of *normal serum*. These mixtures are then placed in the incubator and after a suitable lapse of time a sample of each is examined under the microscope. In the first phagocytic mixture—that in which no serum was employed—the leucocytes have failed to ingest any microbes. In the specimen in which serum has been employed, the microbes have been plentifully ingested. This result depends not upon any stimulating effect exerted upon the leucocytes, but upon the fact that the serum has effected a chemical change—a so-called *opsonic change*—in the microbes. That this *opsonic change* does not in any sense affect the vitality of the microbe is shown in the case of serophytic microbes by the fact that the serum that exerts an opsonic effect furnished culture medium in which the affected microbes will all grow out into colonies. The blood fluids intervene further in the destruction of microbes in the interior of the phagocyte by combining with them in such a way as to favour their intracellular digestion. This action, which was first described by Douglas, is known as the protryptic action of the blood fluids.

The living leucocyte can also kill microbes apart from phagocytosis. The following experiment is instructive. A shallow receptacle is filled with a solid nutrient medium whose surface has been uniformly implanted with staphylococcus or streptococcus. This done, the three cover glasses are thickly carpeted with living leucocytes obtained direct from the blood. We now, after different treatment, impose these cover glasses side by side upon the implanted nutrient surface. In the case of the *first* cover glass the adhering leucocytes are brought into application in conjunction with the adhering serum ; in the case of the *second* they have been washed free from every trace of serum ; and in the case of the *third* cover glass the adhering leucocytes, instead of being employed living, have been killed by drying.

The whole preparation is now incubated at blood heat for 12 hours or more. The microbes will then be found to have grown out forming an uninterrupted sheet of colonies over the whole surface of the nutrient medium except under those areas of cover glasses 1 and 2 which are carpeted with living leucocytes. Here the implanted microbes have not proliferated, and microscopic examination of the cover glasses shows that where the leucocytes came into action in conjunction with serum the microbes are all lying intracellularly ; while where the leucocytes were washed free from serum—the microbes are all lying extracellularly. The experiment thus shows that microbes can be killed by leucocytes both intra- and extra-cellularly, i.e., both by phagocytosis and also apart from phagocytosis. This holds true both under the conditions obtaining in this particular experiment, and also in numerous other conditions. That destruction of microbes by leucocytes without the intervention of phagocytosis occurs also very frequently *in vivo* is practically certain.

Results of Experiments.

Research has thus shown that the destruction of microbes in the body does not proceed only along the lines laid down in the humoral and phagocytic theories. Instead of there being, as was assumed in those theories, only one physiological device by which microbes without distinction are killed in the organism, nature

would appear to provide different distinctive devices for different microbes. Some pathogenetic microbes are combated by the bactericidal action of the serum unassisted by the leucocytes, others again are prevented from proliferating by special agencies, such for example as the antitryptic power of the blood; and again a third description of microbe, the serophytic microbes, are destroyed intracellularly and extracellularly by the leucocytes aided, or as the case may be, unaided, by the blood fluids.

The above deals only with the normal protective machinery of the body as distinguished from that which comes into play in artificial immunity. In connexion with the latter Metchnikoff, as will be remembered, taught that acquired resistance was due not to any changes in the blood fluids, but to the leucocytes having been subjected to a process of training which gave to them a greater capacity for confronting and ingesting microbes. This doctrine had to go by the board when it was shown in numberless cases that increased phagocytosis goes hand in hand with increasing opsonic power in the blood fluids.

From this it was incautiously assumed—though this tenet was never definitely formulated—that the leucocytes constitute in artificial immunity an invariable, and the blood fluids the only variable, factor. That the phagocytic efficiency of the leucocytes was also a variable factor was first shown by Shattock and Dudgeon, who observed that the phagocytic efficiency of the patient's leucocytes is in many cases of pyrexial infection greater than that of the normal man. The phagocytic efficiency of a patient's leucocytes may also, as was further shown by Shattock and Dudgeon, be less than normal

These observations have a direct bearing upon Metchnikoff's doctrine with respect to acquired immunity, since, in all infections which are associated with constitutional disturbances, antigens from the foci of infection are being brought into operation—in other words the organism is experiencing and is reacting to *auto-inoculations*. It follows that, conformably with the doctrine of Metchnikoff, the leucocytes should in every case of pyrexial infection be conducted by successive degrees to a condition of continually increased phagocytic efficiency. Instead of that these cellular elements are, sometimes in localised infection, and practically always in fatal streptococcus septicaemia, reduced to a condition of diminished efficiency.

Artificial Immunisation.

We have to consider next how to increase the effectiveness of the anti-bacterial machinery, i.e., how to produce artificial immunisation against infection. The original point of departure was the observation that those patients who had contracted and recovered from an infectious disease were thereby rendered proof against reinfection. That observation led, in connexion with smallpox, to the adoption of a procedure for the warding off of the disease. The procedure adopted in various parts of the world and introduced to Europe from Turkey, consisted in the implantation into the susceptible individual of material obtained from a patient suffering from mild cases of smallpox. This procedure, which was denoted inoculation because it resembled the grafting of an *eye* or *bud* into a new stock, is in

reality the parent from which all other procedures of artificial immunisation are derived. What required still to be done was to purge inoculation of its dangers and to regulate the immunising stimulus. This was successfully done in Jennerian vaccination. But the achievement of Jenner was in point of fact purely empirical.

Pasteur's Work.

After Jenner came Pasteur and with Pasteur scientific methods are for the first time brought into application in connexion with prophylactic inoculation. His initial achievement was to recognise that the essential in Jennerian vaccination was that for an infective organism obtained from actual cases of smallpox there has been substituted an infective organism which by the operations of nature—to wit by transfer to the calf—had been attenuated in such a manner as to render it non-lethal for man. By the exploitation of that general principle, by the employment of pure cultures and by a technique of artificial attenuation adapted with infinite resource to each separate case, the whole series of Pasteurian successes in the field of artificial immunisation were one after another achieved. Artificial immunisation was not, however, purged from all its risks by the procedures of Pasteur.

It had not yet been transformed into a scientifically regulated procedure. The Pasteurian vaccines were in point of fact standardised only thus far that recourse to attenuation placed in each case a certain limit upon the proliferation of the vaccinating material in the organism of a normally resistant man or animal.

Standardisation.

A great step in advance was taken when it was established in connexion with anti-typhoid inoculation that the antigen required for the setting in motion of the machinery of immunisation can be furnished by the incorporation of sterilised microbic cultures. And further, important steps to the achievement of a standardisation of bacterial vaccines were made when a technique for the enumeration of the microbes in bacterial suspensions was devised, and when it was recognised that weighed quanta of desiccated and powdered bacterial substance could be employed in cases where, owing to the felting together of the microbes, enumeration of the microbial suspension was impracticable. The counting of the microbes or the weighing of the bacterial substance is, however, only a means to an end—the standardisation of a vaccine, i.e., the determination of the doses which will give the best ' curve of immunisation '.

The Curve of Immunisation.

The expression *curve of immunisation* calls attention to certain fundamentally important points in connexion with the reaction of the body to the incorporation of vaccines. In the pre-Pasteurian and Pasteurian periods, when ideas about the nature of immunising response were still vague, it was assumed with regard to vaccines that they produced their effects only after a certain incubation period (ordinarily only after 10 days). And it was further taken for granted that the curve of immunisation would from the beginning move always in the upward

direction. When, however, immunisation curves came to be constructed (and this was done first in connexion with anti-typhoid inoculation) unanticipated features revealed themselves and in connexion with these also time-relations which did not conform with expectation.

Negative and Positive Phases and Practical Importance of These.

In what may be called the normal case—i.e., in the case where the dose of vaccine inoculated is sufficient to produce an appreciable constitutional disturbance, a bi-phasic curve of immunisation is obtained. For 24 or more hours after the inoculation the anti-bacterial power of the blood is reduced. This—the so-called *negative phase*—is followed by a phase of increased anti-bacterial power—the so-called *positive phase*—which may last for one or two or a number of days as the case may be. After this the anti-bacterial power falls away gradually to a level only slightly higher than the original normal. But despite this there would seem to persist in the organism (and this would seem to be the chief profit from inoculation) a power of making more rapid and more ample immunising response to any subsequent incorporation of antigen whether in the form of a subsequent inoculation of a similar vaccine, or actual infection.

When, instead of a quantum of vaccine which produces a constitutional disturbance, a smaller dose is inoculated, the negative phase is elided and a positive phase is well developed already 24 hours after the incorporation of the vaccine. And, finally, when excessive doses of vaccine, such as produce very severe constitutional disturbances, are incorporated, the negative phase may be correspondingly intense and may persist for many weeks. These facts have an important bearing upon prophylactic operations; they must also, as reflection will show, have an importance in connexion with the immunisation procedure to which horses are subjected with a view to their producing anti-bacterial and antitoxic sera. Again substantially the same relations as between the quantum of vaccine inoculated and the type of response elicited, obtain in the case where vaccines are inoculated into patients who are the subjects of infection. But in that case the conditions are so far different that we have to consider in each case two quanta of antigen: that administered in the vaccine, and that already contained in the patient's organism. Where a patient is the subject of only a minimal infection, we may employ doses of vaccine nearly as great as those employed for the prophylaxis of healthy men. Where a patient is heavily infected, we are restricted to the employment of minimal doses, and finally, when the patient is already labouring under an excessive infection, the injection of vaccines can only do harm.

The principle that the kind of response, and the amplitude of the reaction, and the time-relations of the phases are in each case a function of the quantum of antigen brought into application is found to apply also to the case where the vaccine is added to the extravascular blood. We obtain *in vitro* every variety of effect according to the dose of vaccine brought into application and the time for which it operates upon the blood—the effects varying from an instantaneous increase of bactericidal power to a loss of most of that destructive power. And further different effects are obtained according as we select now one and now another method of testing. We

obtain for example one result when we measure the bactericidal power of the whole blood ; another when we measure the opsonic power of the serum ; a third when we measure the phagocytic efficiency of the leucocytes. It must remain for the future to unravel these complications. For the moment the essential point to note is that the machinery of immunisation which was supposed to be constructed upon a very simple—and one may add foolproof—plan is in reality built up of very delicate and complicated elements.

The lessons which can be drawn from immunisation curves may now be summarised. The first of these is that excessive doses of vaccine may delay and possibly interfere with the prophylactic response, and further that such excessive doses of vaccine administered to patients suffering from an infection may definitely aggravate their condition. Other important lessons are that when appropriate doses of vaccine (i.e., doses which are not followed by a negative phase) are administered, prophylactic effects may be obtained almost immediately after inoculation. And further it should be possible to arrest a general infection by inoculating in the incubation period and then employing a reduced dose of vaccine.

In connexion with this attention may be directed to the statistical records of Haffkine's anti-plague inoculations in the Byculla Jail in the city of Bombay, and to the cases put on record by Miss Corthorn. The records of the Byculla inoculations make it probable that a prophylactic effect was here exerted already 24 hours after the vaccine had been given. And the cases recorded by Miss Corthorn seem to make good that attacks of the plague were not unfrequently aborted by the inoculation of the vaccine.

Further evidence showing that immunisation develops very rapidly after the inoculation of an antigen is furnished in the work of von Pirquet. If a first implantation of vaccinia is followed by similar implantations on successive days, the response to these latter differs from the response made to the first. This altered clinical response—which would appear to indicate a more rapid destruction of the later implanted infective material—was described under the name of *allergy*. It was further shown by von Pirquet in connexion with the inoculation of foreign serum that the supervening clinical reaction—which appears to indicate the throwing out of that foreign serum from the blood—is accelerated when the injection of serum is made into an organism which has been before subjected to that procedure. The interval between the injection of the foreign serum and the clinical manifestations associated with its elimination from the blood (normally 10–14 days) may be reduced to a very few minutes.

Further Questions in Connexion with Artificial Immunisation.

A series of further questions in relation to artificial immunity have to be considered. (1) Are there agencies other than vaccines proper—in other words, other than living or dead microbes of substances derived from the bodies of microbes—which will affect the bactericidal power of the blood and leucocytes ? (2) Again, are the anti-bacterial substances in the serum specific in the sense of operating only upon one particular variety of microbe or are they non-specific ? (3) Further, will leucocytes which have acquired increased phagocytic efficiency, ingest more actively

only one particular species of microbe or all microbes without distinction ? (4) Finally, what are the cells in the body which elaborate the anti-bacterial substances ? All these questions are intimately linked up.

Non-bacterial Vaccines.

In connexion with the question as to whether there are agencies other than vaccines proper which can increase or, as the case may be, diminish the bactericidal power of the blood, it has been shown that the infliction of burns increases the bactericidal power of the serum for the anthrax bacillus. This increased bactericidal power is no doubt referable to an absorption into the blood of disintegration products derived from the burnt tissues. Again, it has recently been ascertained that increased bactericidal power can be developed in the blood *in vitro* by adding to it foreign sera and also non-foreign sera which have been artificially altered by heating to 60° C. Lastly, it has been shown in connexion with the irradiation of the skin with ultra-violet light, the light of the electric arc and sunlight, that by these agencies also the bactericidal power of the blood is increased, such increased bactericidal power depending upon an increased phagocytic power of the leucocytes and also an increased anti-bacterial power in the serum. It is not yet known how irradiation produces these results, but the consideration that radiations such as are here in question are much more likely to act by breaking down than by building up albuminous substances, taken together with the fact that increased bactericidal power is achieved only with a certain quantum of irradiation and that the blood suffers deterioration when larger doses are employed, would seem to point to the conclusion that we are here, in each case, dealing with effects produced by the absorption into the blood of an antigen in the form of disintegration products generated by the irradiation.

Specificity.

The non-bacterial antigens which have been under discussion above occupy at present a position apart in the respect that it would generally be held with regard to these that they might quite likely evoke a non-specific immunising response ; whereas it would be generally held that bacterial vaccines would produce only specific immunising response. But against this there is conclusive evidence to show that bacterial vaccines also evoke non-specific immunising response in the form of increased bactericidal power in the blood, increased phagocytic efficiency in the leucocytes and sometimes also increased anti-bacterial power in the serum. Thus, for example, by the incorporation of staphylococcus the blood can be rendered more bactericidal for streptococcus. This can be achieved also by the addition of staphylococcus vaccine to the blood *in vitro*. Similarly an addition of tuberculin to the extravascular blood will increase the bactericidal power of the blood to staphylococcus, increasing at the same time the phagocytic efficiency of the leucocytes.

These laboratory experiments are in consonance with the statistical results obtained in connexion with anti-pneumococcus inoculations at the Premier Mine in the Transvaal. Here, in 1912, in addition to a striking reduction in the incidence and death-rate of pneumonia, there was achieved a striking reduction in the incidence and death-rate from ' other diseases '.

Derivation of the Products of Immunisation.

The discussion of the deeper problems as to where anti-bacterial substances are elaborated in the body, and as to how their production is to be explained, may be deferred for a moment. For the facts relating to immunisation against bacterial toxins must first be taken into consideration, by bringing out the following points: (1) The machinery of immunisation is in reality a machinery for neutralising or otherwise disposing of poisonous substances—poisonous substances being by definition those which enter into crippling or lethal chemical combination with the blood fluids and tissues. (2) The machinery of immunisation achieves its ends by furnishing substances which enter, as the case may be, into neutralising or precipitating or destructive union with the poisonous substances above spoken of. (3) The machinery of immunisation is brought into operation only by a particular class of poisons—to wit, by those which enter into crippling but not immediately lethal chemical combination with the cellular protoplasm—those which, to use the expressions of Ehrlich, intrude themselves into the 'side-chains' and not into the 'vital ring' of that protoplasm. Of such poisons four kinds specially invite attention. These are: (*a*) the poisonous constituents of the bacterial protoplasm, (*b*) the albuminous substances contained in foreign sera and certain other foreign albuminous substances, (*c*) bacterial toxins such as those which can be filtered off from cultures of diphtheria and tetanus and (*d*) vegetable and animal toxalbumens such as abrin, ricin and the various snake venoms. With respect to the first the body responds to their inoculation by a production of *bacterio-tropic substances*, i.e., substances which enter into detrimental or lethal chemical combination with bacteria. The inoculation of sera is followed by the elaboration and delivery into the blood of *sero-tropic substances* which neutralise and precipitate these sera. The inoculation of bacterial toxins in like manner—and this sovereign discovery was made by Behring—leads to the production and delivery into the blood of toxi-tropic substances. These, known as bacterial *antitoxins*, neutralise and precipitate the corresponding toxins. And, finally, the inoculation of vegetable and animal toxalbumens leads to the delivery into the blood of the appropriate neutralising substances.

Practical Results.

The discovery of bacterial antitoxins had led to important practical applications in connexion with the treatment and prophylaxis of, in particular, diphtheria and tetanus. By virtue of the fact that toxins and antitoxins lend themselves to accurate quantitative study, it has contributed much to our knowledge of the machinery of immunisation. It was through the study of the curves of immunising response made to the inoculation of tetanus toxin that the negative phase first became known. Again it was by the study of antitoxin production that it was for the first time unequivocally established that the organism which has made previous response to even a minimal quantum of a toxin is thereafter capable of responding to any further quantum of that poison with a prompter and ampler elaboration of antitoxins.

Further, by the study of the antitoxin content of the blood in its relation to

diphtheria toxin, it has been shown that the presence of even a small quantum of antitoxin in the blood protects against infection, and further that the insusceptibility of the majority of adults to diphtheritic infection is correlated with the possession of a minute quantum of antitoxin derived, as it would seem, from repeated minimal diphtheritic infections contracted in their earlier life. The same would appear to hold also of the scarlet fever streptococcus. We have here, as reflection will show, facts which illuminate the epidemiology of diphtheria and scarlatina and show that it is possible and may under circumstances be advisable to substitute for a prophylactic inoculation of a bacterial vaccine, an injection of antitoxin, or alternatively an injection of such quantum of toxin as will evoke an antitoxic response.

Two further points about antitoxins have important bearings upon the problem as to where and how products of immunisation are produced in the organism. (1) Antitoxins are quite rigidly specific—each antitoxin neutralising only the particular kind of toxin in response to which it was engendered. (2) After a first inoculation of toxins antitoxins are only very slowly produced. Ordinarily an interval of 10 to 20 days elapses before they make their appearance in the blood.

These properties are not, let it be noted, differential properties of antitoxins ; they characterise also certain kinds of bacteriotropic substances. Specificity and comparatively tardy appearance in the blood characterise for example agglutinins, and the so-called ' thermostable immune bodies '.

Since there are two kinds of products of immunisation : one kind that are eminently non-specific and which are produced immediately (and can, as we have seen, be produced in the blood *in vitro*) ; and another kind which are rigidly specific and are elaborated only after a considerable lapse of time and are so far as appears produced only *in vivo* ; we may now seek for an answer to the problem as to how and where these various products of immunisation are engendered. Since it may be taken as certain that these two kinds cannot well originate in the same cells and be engendered by the same kind of metabolic operation, we may divide up the problem and consider first by what cells and by what kind of metabolic operation the non-specific anti-bacterial substances are produced and then take up the question as to where and how antitoxins and such anti-bacterial substances as are specific are generated.

Production of Non-specific Anti-bacterial Substances.

The former question presents no difficulty. The facts set forth above make it clear that non-specific anti-bacterial substances are elaborated by the leucocytes. And further the facts suggest that the leucocytes are secretory cells which produce these substances when bacterial disintegration products are brought to bear upon them in suitable concentration.

Further the facts comport with the idea that leucocytes which have elaborated anti-bacterial secretions but have not as yet excreted these into the environing blood fluids will, by virtue of their increased content in anti-bacterial substances, possess increased anti-bacterial efficiency, while the blood fluids will not have gained anything in anti-bacterial power.

Conversely leucocytes that have excreted their anti-bacterial substances will

exhibit diminished anti-bacterial power and the blood fluids which have received these secretions will have received an accretion of anti-bacterial power.

Production of Specific Products of Immunisation: Ehrlich's Side-chain Theory.

The problem as to how these products of immunisation which indenture chemically with one specific counterpart substance are engendered in the body, is of quite another order of difficulty, and it is one of the memorable achievements of Ehrlich to have conceived how the furnishing of such specific products of immunisation could be accounted for. The problem presented itself to Ehrlich's mind in the following vivid manner: 'If,' it was thus that Ehrlich communed with himself, 'If I take a guinea-pig—that is to say, a creature whose country of origin is South America—and administer to it abrin—a poison derived exclusively from Africa (and thus one which neither the tame guinea-pig nor its ancestry can ever have encountered) and if I now find that my guinea-pig furnishes me with an antidotal substance which indentures with the abrin as does a key with the wards of the lock for which it is made, is there then for me any way of escape from the conclusion that the organism of my guinea-pig has specially constructed an antidotal substance to fit the particular kind of poison I have administered—performing in this a feat of chemical analysis and synthesis which would balk the ablest chemist?' From the intellectual *impasse* into which this interrogatory seemed to conduct there was, Ehrlich discerned, a possible way of escape. He reflected that there must of necessity exist in the organism of any animal which is affected by a given poison a counterpart substance (or to use his technical term) *a receptor* which enters into chemical combination with that poison. In other words there must exist already preformed in the organism of susceptible animals, substances which have a chemical constitution such as would admit of their functioning as antitoxins.

At the same time these counterpart substances differ fundamentally from the non-specific anti-bacterial substances which were considered above. First of all they are not, as are the substances last mentioned, available in the form of secretory products produced only with a view to their being ejected from the cell. Instead of that, specific counterpart substances are integral elements of the cellular protoplasm, and elements of which it may be assumed with certainty that they subserve special functions in the internal economy of the cells of which they are constituents. Further, the specific counterpart substances we are here considering differ from the non-specific anti-bacterial substances in the respect that while these latter are elaborated only in one particular variety of cell (to wit, in the leucocyte) the former are widely distributed in the organism, being located in each case in a different assortment of cells. Thus, for example, the counterpart substances to which the diphtheritic toxin would anchor itself would be located in a different assortment of cells than the counterpart substances with which the tetanus toxin would combine. And again the counterpart substances which would combine with abrin would be different. This would hold true also of all the counterpart substances which would unite with the poisons derived from each particular variety of bacterial protoplasm.

So far it has been shown only that susceptible animals must by the very nature of things contain in their cell-protoplasm constituent elements which are the exact

chemical counterparts of poisons. We are still very far from the solution of the mystery (*a*) of the organism furnishing antitoxins in the circulating blood; (*b*) of its furnishing antitoxins only to special classes of poisons; and (*c*) of its furnishing these in quantities far in excess of the quantum of counterpart substances originally contained in the organism. Ehrlich in exploring for some way of exit from the labyrinth constituted by these questions oriented himself by the aid of a ground-plan, in which there was set out his general conception of the stereo-chemistry of protoplasm. In the plan in question the protoplasm is a structure made up of side-chains assembled round a central ring—the continued life of the protoplasm depending upon the integrity of the central (or as we may call it *vital*) ring; while the side-chains consist of elements which are integrated into the protoplasm for its nourishing and vital functioning. To this original ground plan there was now added by Ehrlich a new feature. The conception which he now added was that the side-chains of the protoplasm would, as soon as they became redundant, be cast forth from the cell into the circulating blood, forming there what he called *free receptors*.

Interpreted in the light of this so-called *side-chain* theory, the incorporation of poisons which make a chemical attack upon the vital ring of the cell protoplasm would extinguish the life of the cell and would therefore be incompatible with an elaboration of antitoxins. The situation is entirely different when the poison, instead of directing its attack to the vital ring, anchors itself on to one of the side-chains. After a temporary putting out of action of those functions which are discharged by the particular side-chains in question, this would lead to the replacement of the crippled side-chains, and thereafter to a hyper-replacement and to such redundancy of these in the protoplasm as would involve casting forth these side-chain receptors into the circulating blood. This theory, which is quite as applicable to the production of specific anti-bacterial substances as to the production of antitoxins—would seem to lie open to critical assault in that the hyper-replacement of side-chains does not necessarily conduct to an excretion of these into the circulating blood. In point of fact, in the case of muscle, on which Ehrlich here relies, it leads to something very different, to a hypertrophy of this tissue.

Finally, the side-chain theory has met with hostile criticism more especially on the ground that it would compel us to believe that the noble tissues, such as those of the central nervous tissue which are poisoned by the toxins of diphtheria and tetanus, can be converted into secretory organs so prolific as to furnish in the blood tens and hundreds of thousands of units of the corresponding antitoxins. In point of fact the side-chain theory does not in any way require us to believe this. It would do so only if it had been established that poisons such as diphtheria and tetanus toxins attack only the central nervous system. But in point of fact Ehrlich assumed that the poisons which are responded to by a production of antitoxins are all *polytropic*—in other words they turn towards and combine chemically with a number of different tissues. So far therefore as the side-chain theory is concerned, we are thus authorised to assume that, not the cells of the central nervous system, but all or any of the other and less noble tissues which are affected by the toxins, are those which produce the harvest of antitoxins.

ON THE NEED FOR ABANDONING MUCH IN IMMUNOLOGY THAT HAS BEEN REGARDED AS ASSURED

BY THE AUTHOR

(*Based on a Lecture delivered at a General Meeting of the Royal Society of Medicine, 12th December, 1941*)

PART I

Of the six new technical methods described in Part I of this paper as originally published the first five will be dealt with in their place in Volume V of these Collected Researches—and the only one which is essential for the understanding of the findings reported in Part II is here produced.

That procedure is one which makes it possible to discriminate from each other (*a*) the Opsonic Action which is exerted by the serum upon the microbes; (*b*) the Phago-incitor (Stimulin) action exerted by the serum on the leucocytes; and (*c*) the Phago-incitor action exerted upon the leucocytes by the microbes of the Phagocytic mixture which have up to the present been supposed to function only as so much inert phagocytic pabulum.

The traditional procedure for measuring phagocytic power does not distinguish between those three chemical activities. It does not even contemplate the possibility of any except an opsonic chemical action taking place in phagocytic mixtures.

Much can be learned on this question by carrying out the phagocytic tests in two operations instead of in one.

In the original phagocytic procedure equal parts of washed normal corpuscles, of the patient's serum, and of the microbic suspension were taken, and note was taken of the compendial result achieved.

In the procedure which I propose to substitute for purposes of exploring the triple action just referred to (I propose to call it the *Caesuric* Procedure) we—*this constitutes our First Operation*—mix equal volumes of (*a*) serum and microbic suspension, or (*b*) serum and washed corpuscles, or (*c*) microbic suspension and washed corpuscles. Let us call these pre-phagocytic mixtures.

We then, after incubating the above pre-phagocytic mixtures for fifteen minutes, pass to our *Second Operation*. If we are dealing with a '*sero-opsonic*' *pre-phagocytic mixture*, we add to 2 volumes of it, 1 volume of washed corpuscles. If we are dealing with a '*sero-stimulin*' *pre-phagocytic mixture* we add to 2 volumes of it, 1 volume of microbes. And if we are dealing with a *microbi-leucocytic pre-phagocytic mixture* we add to 2 volumes of it 1 volume of serum. We then reincubate for fifteen minutes, thus bringing those chemical agents which have been operating in our pre-phagocytic mixtures a *second time* into action in completed phagocytic mixtures.

Tables V and IX show that the caesuric procedure furnishes valuable information.

Obviously the method would be more valuable if the adventitious chemical action which comes into operation in the secondary incubation could be eliminated.

There are two methods by which this might be done :

The *first* would be to centrifuge the phagocytic mixture, as soon as the third of the triad of operative factors have been added. By thus avoiding second incubation we should cut out most, if not all, the adventitious chemical action.

The *second* device—which could be combined with the first—would be to employ when testing a serum for stimulins, a bacterial pabulum of pre-opsonised microbes.

My experience is that we gain little, if indeed anything, by these devices ; and for two reasons, *first* because allowing as we do in the first case, less time for opsonisation and stimulin action, we greatly reduce our phagocytic count.

And, if our second device gives us no better results, it is because we, by furnishing the leucocytes with a bacterial pabulum of pre-opsonised microbes, obtain an all-round increase in phagocytosis.

And that, like the all-round decrease of phagocytosis obtained by the first device, largely obliterates phagocytic differences.

PART II

THE EVIDENCE AGAINST THE PRESENT UNIVERSALLY ACCEPTED DOCTRINES OF PHAGOCYTOSIS AND IMMUNISATION

Introductory

Douglas and I showed in our original paper on the opsonic power of the serum that the phagocytosis which occurred when washed corpuscles, microbes and serum were brought together was due to the serum acting upon the microbes in such a way as to prepare them for ingestion.

After establishing the fact that the blood fluids converted the microbes into an attractive pabulum for the leucocytes (whence the word ' opsonin '), and further showing that this property is abolished, or all but abolished, by heating the serum to 60° C., we went on to test by what we called the ' chiastic procedure ' the serum and washed corpuscles of two patients, whom we had successfully treated with staphylococcus vaccine, against the serum and corpuscles of normal men.[1]

In the chiastic procedure here in question four separate phagocytic mixtures are made all containing a volume of one and the same staphylococcus suspension. To the volume of staphylococcus suspension employed in capillary *Tube 1* there are added equal volumes of *Normal Serum*, and of *Normal Washed Corpuscles* ; to a similar volume of suspension there are added in *Tube 2*, equal volumes of *Normal Serum* and of the *Patient's Washed Corpuscles* ; in *Tube 3*, equal volumes of the

[1] *Proc. Roy. Soc.*, 1903, 72; *ibid.*, 1904, 73.

Patient's Serum and of *Normal Washed Corpuscles*; and in *Tube 4*, equal volumes of the *Patient's Serum* and of his *Washed Corpuscles*.

It emerged in experiments done in this way on the bloods of our two patients that there was no improvement in their corpuscles but a distinct improvement in their sera. And we drew from these and from similar experiments in which the serum and corpuscles of tubercular patients were compared with those of normal men, the conclusion that epiphylactic responses such as those which are induced by therapeutic inoculations that do not cause constitutional disturbances, and by the auto-inoculations of apyrexic patients, increase the opsonic power of the serum, and leave the leucocytes unchanged.

It ought, of course, to have suggested itself to us that what we had found in patients examined ten days after inoculation might not hold true of such patients examined earlier; and further that what we had found to hold true of apyrexic patients would not necessarily hold true of those suffering from pyrexic infections.

Also it ought to have suggested itself to us *as possible* that the blood of our inoculated or auto-inoculating patients might, or at any rate might in certain cases, contain in addition to bacteriotropic substances (opsonins) leucocytotropic substances which would, acting as stimulins or depressants, raise or lower the efficiency of the phagocytes.

Again, it never even occurred to us that the epiphylactic response of the blood which follows upon the inoculation of vaccines and upon auto-inoculations, could affect any but the particular variety of microbe which was evoking the response.

And finally, it never dawned upon us that the microbes which were employed to serve as a bacterial pabulum and to furnish a measure of phagocytic power, could play any active part in the phagocytic reaction.

All these *a priori* inferences were mistaken, and we were, as has only recently emerged, just as much out of our depth with regard to what was happening in our phagocytic mixtures, as to what was happening in our patients.

I do not make light of these errors. 'Errors', Goethe tell us, 'always matter; but how very much they matter becomes clear to a man only at the end of his way.' But I realize that if I were put back into the blinkers which confined my outlook and that of my contemporaries forty years ago, *I*—I do not want to shelter myself by saying *we*—should almost certainly fall again into the same pitfalls.

It had, at that time, been recently demonstrated by Ehrlich that epiphylactic response was *antithetic chemical response*. And it seemed to follow from this that inoculations of bacterial vaccines (and I would add the engendering of auto-inoculations) would produce only bacteriotropic as distinguished from leucocytotropic substances. And again it seemed to follow by analogy from the fact that the antitoxins produced by toxins are strictly specific that the same would hold also of bacterial inoculations.

Again, forty years ago, it had not occurred to anyone that epiphylactic response could be evoked *in vitro*. And much less had it suggested itself that epiphylactic response *in vitro* would come under regular observation in all phagocytic, and (as is evidenced in the 'Caput and Cauda phenomenon') in all haemo-bactericidal tests.

And to-day I often ask myself how much water will flow under the bridges before that fundamental fact that epiphylactic response occurs in the blood *in vitro* succeeds in getting itself into bacteriological textbooks.

Let me now proceed to deal in detail with some of the more important points which have been incidentally referred to.

Thermolability of Opsonins.

Only about a year after Douglas and I had shown that opsonins of normal serum are largely thermolabile, Neufeld and Rimpau [1] announced that the heated serum of 'immune animals' contained phago-incitor substances, which rendered the pneumococci and streptococci with which he had inoculated his animals, phagocytable or, as the case may be, more phagocytable.

There were in the ratiocinative operations of these authors two main false assumptions.

The *first* was the assumption that animals which have been heavily and repeatedly inoculated may without further question be called 'immune'; and that their sera may properly be described as 'immune'; or 'anti-sera'.

I pointed out thirty-five years ago [2] that these so-called 'immune sera' might in lieu of, or along with, epiphylactic substances produced in the organism, contain antigenic substances remaining over in the blood from the original inoculations or auto-inoculations.

I have nothing to alter in that except that where I, thirty-five years ago, wrote antigens I should now probably write 'leucocytic stimulins'. And whichever of these descriptive terms I applied to such bacterial substances as might still be circulating in the blood, it would be clear that these would, when transferred to another animal organism, confer on it instead of passive, *active* immunisation.

A conspicuous, but now forgotten, practical exemplification of active immunisation misinterpreted as a passive one was furnished by the beneficial results which Chantemesse (this was twenty-five or thirty years ago) claimed in typhoid fever from the inoculation of an 'antityphoid serum'. Of this so-called anti-serum he administered in heavily infected cases of typhoid fever reduced, and in still more heavily infected cases absolutely minimal doses—doses of not more than a fraction of a cubic centimetre. (I was unable to procure from him a sample of the serum he was using in order to see whether it contained antigens or antibodies.)

These considerations apply just as much to the sera of Neufeld and Rimpau's laboratory animals as to the sera of horses employed for the treatment of human patients.

A *second fallacious assumption* made by Neufeld and Rimpau was that convincing proof of a phago-incitor serum operating as an opsonin is furnished when a serum ceases, after it has been digested with heavy doses of the antigenic microbes, to exert its previous phago-incitor effect.

[1] Neufeld and Rimpau, *Deutsche med. Wchnschr.*, Sept. 1904.

[2] 'A Criticism of the Foundations of Serum Therapy', Vol. III of these *Collected Researches*, pp. 259 *et seq.*

It has to be kept in view in connexion with all such *absorption experiments* that when a serum is digested with a bacterial culture, and the microbes are afterwards removed by centrifugalisation, there may remain in the serum such a charge of bacterial toxins as would, even with opsonins present, paralyse phagocytosis.

In view of that consideration ' absorption tests ' can never furnish convincing proof that a serum contained, before it was operated upon, opsonic substances.

A year after the publication of Neufeld and Rimpau's work Reid and I,[1] following up the work of these observers, found phago-incitor substances in the heated serum of auto-inoculating tubercular patients. These ' phago-incitor substances ' I—avoiding the term ' bacteriotropins ' [2] which Neufeld and Rimpau had applied to them—called ' thermostable opsonins '.

We were, in calling the phago-incitor substances we were dealing with ' thermostable opsonins ', beguiled by the self-same fallacies which had misled Neufeld and Rimpau. In other words we had no better warranty for concluding that the heated sera of our auto-inoculating tuberculous patients contained opsonins, than the fact that they induced phagocytosis ; and the further fact that the phagocytic power of these sera was lost, when they were digested with heavy doses of tubercle bacilli.

But when I look back on the fact that many of our tuberculous patients were in a very unwholesome condition I cannot help thinking that their blood may not infrequently have contained, instead of, or along with, opsonins, tuberculin derived from the tubercle bacilli in their system. And such tuberculin would, as will be shown presently, operate as a phagocytic stimulin.

Further points—and these are new points—which invalidate Neufeld and Rimpau's argument that they had in hand a thermo-stable opsonic serum are referred to below.

I have in view the point that a serum may (as is shown in Table V) be converted into a leucocyte-stimulating serum by heating ; and also the point that heating may (as has been shown in Table I) convert sera which do not agglutinate staphylococci into sera which do, with the result that both the percentage of phagocytes and the number of microbes phagocyted may be greatly increased—this being explained by the fact that leucocytes are not attracted by chemotaxis to individual staphylococci to anything like the same degree as they are to agglomerated staphylococci.

The next important happening in the field of phagocytic research was that Shattock and Dudgeon [3]—employing the same chiastic method that Douglas and I had done, but conducting their experiments with a ' phagocytic pabulum ' consisting of melanin particles—found that the leucocytes of patients suffering from

[1] *Vide* these *Collected Researches*, Vol. III, pp. 151 *et seq.*

[2] I eschewed the term ' bacteriotropins ' because I had already five years before (*Lancet*, Dec. 23, 1899, 1st paragraph) employed that term in its proper generic signification, i.e. as a name for *every and any substance which enters into chemical combination with microbes*. And the use to which Neufeld and Rimpau put this term is also, quite apart from the question of priority of usage, a perfectly improper use. This will be seen when we regard the fact that the limitation of the term *bacteriotropins* to one special variety of ' bacterio-tropic ' substances is as wrong in principle as would be the limitation of a zoological class—let us say the class ' *Mammalia* ' to one particular species of mammals.

[3] Shattock and Dudgeon, *Proc. Roy. Soc. B.*, 1908, 80.

febrile infections differ from those of normal men in that they have usually a *supra-*—but occasionally an *infra-normal* phagocytic efficiency.

And Shattock and Dudgeon pointed out that no one could, in view of their having worked with a phagocytic pabulum of melanin particles, come to any other conclusion than that the increased leucocytic response obtained by auto-inoculations was non-specific.

We shall return to this question of non-specific response to bacterial infections later. In the meantime let us note that the results obtained by Shattock and Dudgeon are irreconcilable with Erhlich's theory that immunising response consists *exclusively* in the production of antithetic chemical substances.

For a long time after the date of Shattock and Dudgeon's publication—an interval during which I was working in succession on the study of therapeutic inoculation applied to tuberculosis, prophylactic inoculation against pneumonia, and the treatment of war wounds—I was turning over in my mind not only Shattock and Dudgeon's findings, but also the many new facts about epiphylactic response which my work was bringing to light. And the conviction was steadily growing in my mind that much of the currently accepted doctrine of immunisation was erroneous.

Finally, after much tentative work, I obtained convincing proof of non-specific (i.e. *homo-* and *hetero-*) bactericidal response to the implantation of vaccines into the blood *in vitro*.

It was shown in the papers cited below [1] that separate implantations of staphylococcus and streptococcus vaccines into the blood *in vitro* resulted in a notable increase in the bactericidal power exerted by serum upon both these microbes.

And let me here remind the reader, though the recent advances in chemotherapy have overshadowed this, that in both those papers I drew attention to the fact that the power of inducing epiphylactic response in extravascular blood had put into our hands a method of transfusion—I called it *immuno-transfusion*—from which good results were to be expected in the treatment of septicaemia.

Also I pointed out in this connexion that the blood which was used for immuno-transfusion might be either normal blood subjected to vaccine treatment *in vitro*; or, if this was preferred, blood obtained from a donor who had been inoculated a few hours before with a staphylococcus or streptococcus vaccine [2]—the optimum dosage of the vaccine used having in each case been ascertained by what I call ' a vaccine response test '.

About the same time I obtained clear evidence of leucocytes killing microbes extracellularly.

[1] Wright, ' Sur la production de substances bactéricides non spécifiques au moyen des vaccins *in vivo* et *in vitro* '. *Vide supra*, pp. 50 *et seq.*

Wright, ' A Lecture on the Lessons of the War '. *Vide supra*, pp. 55 *et seq.*

[2] Immuno-transfusion in this latter form has, as I gather from the French medical literature of the twenty-two years 1918–1940, given very striking results. In England the method has as good as never come into application.

And I myself have only twice administered to a patient blood inoculated *in vitro*. The first was the case reported *supra* on pp. 67–68; the other was the case Example 2, p. 113.

EXPERIMENTAL EVIDENCE SHOWING THAT LEUCOCYTES KILL MICROBES EXTRACELLULARLY

The procedure by which it was first established that leucocytes can kill extra-cellularly was described and figured in my paper 'On the Physiology of Wounds'.[1]

The procedure consists in taking leucocytes which have emigrated on to glass laths from blood centrifuged before it clots and disposing these laths—one directly as it comes out of the serum, and the other after the serum has been washed off—upon an agar plate inseminated with staphylococcus or streptococcus. When the implanted plates are then incubated the staphylococcus grows out all over the surface of the agar, except under these central strips of each lath where we have a thick belt of emigrated leucocytes. There the agar surface, both in the case where the leucocytes are operating in serum and in the case where they are operating in salt solution, remains absolutely bare.

When the laths are now stained and microscopically examined, it is found that in the case where the leucocytes were operating in serum every leucocyte is full of staphylococci, and these have (we may assume) been inhibited in their growth or killed intracellularly.

On the other hand, in the specimen where the leucocytes were operating in salt solution the leucocytes are empty of microbes, and here the staphylococci have manifestly been inhibited in their growth or killed extracellularly.

I have confirmed the fact that the leucocytes exert their bactericidal power extra- as well as intra-cellularly by five other experimental methods.

I shall label them Procedures 2, 3, 4, 5, and 6.

Procedure 2.—Staphylococci in moderate numbers are implanted into blood drawn off from the finger. (Moderate numbers would in this case mean some thousands or tens of thousands of staphylococci per cubic centimetre.) This blood is immediately centrifuged in flat 'emigration tubes'[2] and is thus divided into an upper portion consisting of plasma, which is presently converted into a *white clot*, and a lower portion consisting of red blood corpuscles surmounted by a cap of leucocytes—this portion being presently converted into a *red clot*. The centrifuged blood is now, after the emigration tube has been very tenderly capped with plasticine, placed upright in a plasticine slide in the incubator: and incubation is continued for twenty-four hours.

When the clot is now microscopically examined, it is found that many of the implanted staphylococci (but not by any means all of them, for a large proportion

[1] *Vide* these *Collected Researches*, Vol. I, pp. 140–141.

[2] The method of making these is described in *Technique of the Teat and Capillary Tube*, Wright and Colebrook, pp. 22–23.

are killed instantaneously in the blood) have grown out into colonies in the white clot.

But it is the distribution of the colonies, as distinct from the bactericidal power of the blood, which is of special interest. The colonies are found to have grown out in considerable numbers in the distal portion of the white clot ; but that none (this will of course depend upon our having hit off the proper measure of *in vitro* infection) will have grown out in the leucocyte invaded proximal area of the white clot or—this is the relevant point—in the area which lies immediately distal to that into which the leucocytes have penetrated. In that ' no man's land ' there are, as microscopic examination shows, neither leucocytes nor microbes. The microbes which should by rights have grown out there must have been killed by bactericidal substances excreted by the leucocytes.

Procedure 3.—Here we make ' extra- ' and ' intra-coagular implantations ' of staphylococcus into blood in capillary tubes. An *intra-coagular implantation* is made by inoculating staphylococcus into blood drawn from the finger, aspirating this inoculated blood into capillary tubes and allowing it to clot undisturbed.

In an *extra-coagular implantation* a similar volume of *unimplanted* blood is drawn up into a capillary tube, and we then, after the clot has contracted and the serum has exuded, implant into this serum the same volume of staphylococcus suspension as was employed for the intra-coagular implantation—let us say 5 c.mm. of a 10,000-fold dilution of a twenty-four-hour broth culture. We make the implantation with the aid of a capillary pipette drawn out into a hair-fine extremity.

Along with the two foregoing implantations a similar quantum of staphylococcus suspension is implanted into another capillary tube which contains only serum. These tubes—let me for convenience number them 1, 2, and 3—are, after sealing up, fixed down upon a plasticine slide, and are then incubated horizontally (horizontally to prevent the colonies running together).

After twenty-four hours' incubation the staphylococcic colonies in Tube 3, which contains only serum, are seen to be many times more numerous than those in the extracoagular serum in Tube 2. These are so few that they can quite easily be counted by the naked eye.

Dealing now with Tube 1, the colonies which have grown out in the interior of the clot are enumerated as follows : The clot is blown out into a test tube of water. This is then placed in a water bath standing at 60° C., and the clot is left there until it is fully decolorised. After that it is transferred to a slide; is fixed down upon this by drying, and is then lightly stained with methylene blue. This done the colonies are counted under the low power of the microscope.

These, instead of being, as one might have expected, definitely fewer than those in the ex-coagular serum, are generally a little more numerous.

With regard to the interpretation of the findings, those in Tube 2 point to stimulins deriving from the microbes implanted into the serum operating upon the leucocytes of the clot, and causing these to pour out bactericidal elements. These now diffuse into the serum, rendering this bactericidal. And the fact that the colonies which grow out in the clot are, despite the presumably better opportunity for phagocytosis there afforded, fewer than those which have grown out in

the serum in Tube 2, is no doubt due to the bactericidal substances which have been excreted by the leucocytes coming less effectively into operation upon the microbes which are lodged in the clot than on the microbes which are floating free in the serum.

Procedure 4.—We draw up some melted agar into the stem of a capillary tube and then immediately blow it out. We leave by this procedure a coating of agar upon the wall of our capillary tube. We then draw up into this a volume of blood from the finger and set this aside to clot and contract. By this contraction the agar lining is pulled off from the walls of the tube and the serum now exudes through the agar capsule into the extracoagular space. Into this space we introduce by the same procedure which was employed for extra-coagular implantations, a small measured quantum of staphylococcus suspension.

At the same time we—to provide ourselves with a control—make a similar implantation of staphylococci into two samples of serum and draw these up the one into a naked and the other into an agar-lined tube.

After twenty-four hours' incubation we find that the colonies which have grown out in the control tubes containing only serum are much more numerous than those which have grown in the serum in the tube containing the agar-enveloped clot. And we must, as in Procedure 3, assume that the leucocytes of the clot have (under the impulsion of bacterial stimulins which have traversed the agar capsule) secreted bactericidal substances which have killed the microbes which are implanted into the serum outside.

Procedure 5.—Here we prepare one or more *leucocyte-lined capillary tubes.* This is done by drawing up into our capillary tube first a very small quantum of normal salt solution and then about sufficient blood to occupy about one-half of the stem, and after this another very small quantum of normal salt solution. The purpose of flanking the blood above and below with salt solution is to prevent the clot drying and fixing itself on to the walls of the tube.

After filling in our pipette as explained we seal up its distal end, mark off with a blue pencil the limits of our blood-clot, and then incubate. After some twenty to thirty minutes we blow out the clot and run enough normal salt solution through the tube to carry away loose leucocytes and red blood corpuscles. We then aspirate into our tube some serum lightly implanted with staphylococcus, disposing this so that the serum shall fill up the space between our two blue pencil marks, and extend on each side a little distance beyond them. We then take exactly the same amount of our staphylococcus implanted serum and draw this up into an unlined capillary tube. This done we seal up the distal ends of our pipettes in the flame, and provide against evaporation from the proximal ends by blocking the neck of our capillary pipette either with a little mercury, or with a little salt solution. We then place our pipettes in the incubator, fixing them down horizontally upon a plasticine slide.

Comparison of the tubes twenty-four hours after shows that a great number of staphylococci have been killed in the leucocyte-lined tube, and it will be noticed that the bactericidal effect exerted by the leucocytes extends some little distance on each side beyond the blue pencil marks.

The experiment can be repeated with even more striking results with capillary tubes which have been furnished with a double lining of leucocytes—such a double lining being obtained by refilling the tube after Clot No. 1 has been blown out with a new lot of blood and then carrying out the rest of the technique exactly as in the single leucocyte-lined tube.

Procedure 6.—We here first prepare some defibrinated blood and then add to 100 c.mm. of this blood one-tenth of its volume of approximately a 30,000-fold dilution of a twenty-four-hour broth culture of staphylococcus. 10 c.mm. of such a 30,000-fold dilution will represent an implantation of about 100 staphylococci per cubic centimetre of blood.

We now take three to six capillary tubes ; draw up into each 100 c.mm. of the implanted blood ; amputate the barrels of the pipettes ; and then centrifuge until the corpuscles have been carried down completely. That done, and there will now be just as many microbes in the upper as in the lower layers of the serum, we block the proximal ends of the tubes with plasticine and then incubate them upright in plasticine slides for one and a half to two hours.

We now take a series of evacuating pipettes (one for each tube) and draw out the distal end of each stem into a throttle—taking care to make this sufficiently wide. This done we mark off in each tube the middle point of the contained serum with a blue pencil, and then, working very cautiously, draw up into our evacuating pipette, first the upper, and then, after interposing an air-bubble, the lower half of the serum.

This done, we seal up the stems of our evacuating pipettes above and below, and arrange them horizontally upon a plasticine slide ; and now incubate for twenty-four hours. After that we count the colonies which have developed in each portion of the serum (Table I).

TABLE I

	Number of staphylococcus colonies which developed in centrifuged serum taken from	
	the distal half of the tube	the proximal half of the tube
Defibrinated blood heavily implanted with staphylococci	Uncountable	60
Defibrinated blood implanted with half as many staphylococci	100, 100 } 101	0, 11 } 5·5
Defibrinated blood implanted with roughly a quarter as many staphylococci	41, 23 } 32	8, 10 } 9

The point that leucocytes can be killed quite as surely extracellularly as intracellularly settled, let me now take up the question as to whether the doctrine of Shattock and Dudgeon that the leucocytes of pyrexic, i.e., auto-inoculating patients have generally acquired a leucocytic efficiency superior to that of the leucocytes of

TABLE II.—*Showing the Effect of Chiastic Experiments in which the Washed Leucocytes and Serum of Normal Defibrinated Blood were Compared with the Washed Leucocytes and Serum of the Same Defibrinated Blood treated with Tuberculin B.E.*

The test-microbes employed were staphylococci, and the interpretation of the Initials employed is given at the foot of the table.

Amount of Tuberculin B.E.	Period for which the inoculated and control bloods were incubated	Constitution of the phagocytic mixtures	Phagocytic count	Summary of results
$\frac{1}{1000000}$ of a suspension containing 1 mg. of dried tubercle powder in 1 c.c.	25 minutes	NS + NC + Staph. NS + IC + Staph. IS + NC + Staph. IS + IC + Staph.	3·29 6·70 3·96 8·01	Phagocytic efficiency of my blood increased as 1 : 2·4 ; that of my leucocytes as 1 : 2 ; and the phago-incitor power of my serum as 1 : 1·2.
Ditto	20 minutes	NS + NC + Staph. NS + IC + Staph. IS + NC + Staph. IS + IC + Staph.	1·09 2·16 1·04 2·06	Phagocytic efficiency of my blood increased as 1 : 2 ; that of my leucocytes as 1 : 2 ; the phago-incitor power of my serum remaining as 1 : 1.
Ditto	Not noted (Probably between 20 and 40 minutes)	NS + NC + Staph. NS + IC + Staph. IS + NC + Staph. IS + IC + Staph.	3·24 6·72 5·20 5·60	Phagocytic efficiency of my blood increased as 1 : 1·7 ; that of my leucocytes as 1 : 1·4 ; the phago-incitor power of my serum remaining as 1 : 1, nearly.
Ditto	Not noted (Probably between 20 and 40 minutes)	NS + NC + Staph. NS + IC + Staph. IS + NC + Staph. IS + IC + Staph.	1·34 1·70 2·38 2·80	Phagocytic efficiency of my blood increased as 1 : 2·1 ; and that of my leucocytes as 1 : 1·2 ; and the phago-incitor power of my serum increased as 1 : 1·7.
Ditto	40 minutes	NS + NC + Staph. NS + IC + Staph. IS + NC + Staph. IS + IC + Staph.	7·1 9·7 6·4 8·1	Phagocytic efficiency of my blood increased as 1 : 1·1, and that of my leucocytes as 1 : 1·3 ; and the phago-incitor power of my serum reduced as 1 : 0·9, nearly.
$\frac{1}{500000}$ mg.	40 minutes	NS + NC + Staph. NS + IC + Staph. IS + NC + Staph. IS + IC + Staph.	3·9 5·1 6·4 6·0	Phagocytic efficiency of my blood increased as 1 : 1·5 ; that of my leucocytes remains as 1 : 1, nearly ; and the phago-incitor power of my serum is increased as 1 : 1·4.

NS = Normal Serum ; NC = Normal Corpuscles ; IS = Immune Serum ; IC = Immune Corpuscles

the normal blood, should prevail over the doctrine of Douglas and myself that the leucocytes are unaffected by inoculation. Table II throws light on that question.

I, employing as before the chiastic procedure, here compared *my own defibrinated blood untreated* with *my defibrinated blood inoculated in vitro* with doess of $\frac{1}{1000000}$ and in one case with $\frac{1}{500000}$ of its volume of a Tuberculin B.E.[1]

The inoculated and the control bloods were then incubated side by side for longer or shorter periods. After that they were centrifuged ; the serum pipetted off, and the corpuscles washed in normal salt solution. Chiastic phagocytic mixtures were then made of the washed corpuscles and the sera in the manner particularised in Table II *supra*.

Epitome of Results of Table II.—Phagocytic efficiency of the whole blood improved on the average as 1 : 1·7 ; *Phagocytic efficiency of the leucocytes* improved on the average as 1 : 1·5 ; *Phago-incitor power of the serum* improved on the average as 1 : 1·2 ; and in two of the experiments as 1 : 1·7 and 1 : 1·4 respectively.

TABLE III.—*Showing the Non-specific Response of Leucocytes to Septicaemic Infections.*

Here the Washed Corpuscles of Infected Patients and of Normal Controls were incubated with 1 volume of normal serum and 1 volume of a suspension of 2, 3, or 4 different strains or species of microbes.

The results of the phagocytic counts so made are shown in the table below in the form of ratios—the phagocytic intake of the normal man being in each case represented by 1.

Name of patient	Strain 1	Streptococcus Strain 2	Strain 3	Staphylococcus
Boon (mild streptococcus infection)	1·8	1·8		1·7
Ellis (fatal streptococcal septicaemia) ..	0·73	0·76		0·8
Same patient (a week later)	0·2	0·3	0·17	0·1
Hatcher (Whitlow)	0·49	0·51		0·47
Barker (streptococcal infection)	1·3			0·8
Allan (acute fatal streptococcus septicaemia)	1·9	2·5		2·3

These results show that Douglas and I were wrong in concluding that the leucocytes are not affected by bacterial vaccines, and that Shattock and Dudgeon were, on the whole, right in concluding that they were.

Further, the results set forth in the above table show—and this is in agreement with the inferences of Shattock and Dudgeon—that the increase of phagocytic efficiency which is obtained by bacterial vaccines is non-specific.

[1] The Tuberculin B.E. which was employed here and in all the other experiments reported in this paper contained 1 mg. of dried and ground up tubercle bacilli per cubic centimetre of fluid.

More direct confirmation of Shattock and Dudgeon's inferences has been obtained by my sometime pupil—Dr. Ronald Hare of Toronto—by the phagocytic testing of the leucocytes of septicaemic patients with different kinds and strains of microbes. These unpublished results, which have been very generously placed at my disposal, are set out in the Table III above.

THE EFFECT EXERTED BY HEATING THE SERUM FOR TEN MINUTES TO 60° C.

The problem of the nature of the thermostable phago-incitor substances found by Neufeld and Rimpau in the heated serum of their 'immune animals' and by Reid and myself in the heated sera of tuberculous patients, calling for further investigation, my choice of methods lay between testing the heated sera by the 'Absorptional' and by the 'Caesuric Method'. And inasmuch as the former method is, as has been indicated above, very fallacious when employed as a prelude to phagocytic tests, I naturally employed the latter.

I took a series of samples of my serum and tested these unheated and heated, both by the 'sero-microbic', and by the 'sero-corpuscular pre-phagocytic' procedure. Table IV *infra* shows the results yielded by these two different methods.

In the first section of Table IV it will be seen that the opsonic power of the serum is, as was previously found by Douglas and myself, very much reduced by heating. The figures set forth in the second section of the table throw, however, fresh light upon the question, for they show that heating does a great deal besides reducing the opsonic index of the serum. It, as the figures show, converts the serum into a powerful leucocytic stimulin. It is therefore probable that *some* (one does not know how much) of the phago-incitor action, which was attributed by Neufeld and Rimpau to a '*bacteriotropin*' and by Reid and myself to '*thermostable opsonins*' may have been attributable to '*thermo-generated stimulins*'.

I leave that question for a moment to consider another fundamental point which I and my fellow-workers (Douglas and Reid) elicited with regard to phagocytosis in our original researches on that subject—researches which we may, using an apt expression of Francis Bacon, call our *Vindemiatio Prima* or '*First Grape-harvesting*'.

We had concluded in that Vindemiatio Prima that leucocytes did not ingest microbes in the absence of serum; in other words that leucocytes could not be credited with the faculty of spontaneously phagocyting microbes.

That question obviously required to be re-examined. For our original findings might quite well hold of normal leucocytes, and yet not hold of the leucocytes of auto-inoculating patients. And that, as the results displayed in Table V (Columns 2 and 3), VI, and VII show, is what we do actually find.

In view of these data it is obvious that we should, if we had not already been compelled to do so by Shattock and Dudgeon's work and by the data set out in

TABLE IV.—*Section 1 Sets Out the Results of Phagocytic Tests Obtained by Making, to begin with, 'Sero-microbic, Pre-phagocytic', and Section 2, the Results by Making, to begin with, 'Sero-corpuscular, Pre-phagocytic Mixtures'; and then afterwards Completing these Mixtures.*

	Phagocytic counts obtained in consecutive experiments Serial No. of the Phagocytic Test									Average
	1	2	3	4	5	6	7	8	9	
SECTION 1										
Sero-microbic Pre-phagocytic method										
Here the UNHEATED SERUM was incubated first for 15′ with a suspension of staphylococci; and then, after leucocytes had been added, the completed phagocytic mixtures were incubated for another 15′.	1·75	3·9	6·0	3·5	6·6	1·83	2·1	1·7	6·7	3·8
Here the same procedure was repeated with HEATED SERUM.	0·12	1·15	1·6	1·5	0·85	0·42	0·38	1·3	3·2	1·3
SECTION 2										
Sero-corpuscular Pre-phagocytic method.										
Here the UNHEATED SERUM was incubated first for 15′ with a suspension of washed corpuscles; and then after staphylococci had been added the completed phagocytic mixtures were incubated for another 15′.	1·61	1·38	4·7	1·8	0·64	3·0	1·2	0·21	2·0	1·83
Here the same procedure was repeated with HEATED SERUM.	1·85	3·4	7·3	2·0	2·9	5·0	1·95	1·9	2·85	3·1

TABLE V.—*Spontaneous Phagocytosis to Staphylococci—Comparison of Normal Leucocytes with Those of Auto-Inoculating Patients.*

Technique.—The washed corpuscles derived from defibrinated bloods are mixed in each case with an equal volume of an 8 to 12-fold dilution of a twenty-four-hour broth culture of staphylococcus. These mixtures are then centrifuged for forty-five seconds in one direction and then for forty-five seconds in the opposite direction. The supernatant fluid is then amputated and film preparations made from the corpuscular deposit.

About 200 leucocytes are then counted in each specimen and the average staphylococci intake of the individual leucocytes is determined in the ordinary way by dividing the total number of microbes ingested by the number of leucocytes counted.

Single observations of normal men		Single observations of febrile and non-febrile patients suffering from :			
A. S.	0·04	Gonorrhoea	0·86	Strept. septicaemia	0·25
D. S.	0·03	Gonococcal arthritis ..	0·88	Puerperal fever—Case 1 ..	0·25
C. W. M.	0·01	Rheumatism—Case 1	0·25	Puerperal fever—Case 2 ..	0·22
F. H.	0·02	Rheumatism—Case 2	0·48	Puerperal fever—Case 3 ..	0·15
A. E. W.	0·02	Rheumatism—Case 3	0·11	Puerperal cellulitis	0·20
A. H.	0·02	Chancre	0·60	Septic pneumonia	0·19
E. A. B.	0·05	Secondary syphilis ..	0·22	Febrile cellulitis	0·20
K. B. R.	0·05	Endocarditis—Case 1	0·26	Mastoid infection	0·55
		Endocarditis—Case 2	0·70	Appendicitis with peritonitis ..	0·27
		Endocarditis—Case 3	0·70	Cervical abscess	1·00
		Tubercular peritonitis	0·35	Haemorrhagic colitis	0·16
Average 0·03		Average 0·4 (nearly)			

TABLE VI.—*Spontaneous Phagocytosis in (for the most part Febrile) Phthisis.*

The technique here used was the same as that employed above

Average result of the observations made with the leucocytes of normal men	Results of single observations made upon febrile and non-febrile phthisical patients			
0·03	Case 1	1·59	Case 10	0·29
	,, 2	0·54	,, 11	0·37
	,, 3	0·21	,, 12	0·37
	,, 4	0·54	,, 13	0·10
	,, 5	1·80	,, 14	0·54
	,, 6	0·50	,, 15	1·50
	,, 7	0·13	,, 16	0·74
	,, 8	0·10	,, 17	1·20
	,, 9	0·06	,, 18	0·86
Average 0·03	Average 0·65			

Table II, have had to delete from our immunological code the pronouncement that leucocytes are unaffected by inoculation or auto-inoculation.

And it emerges from comparative observations which now run into hundreds that we get on the whole much better information about immunising response by testing the leucocytes of an infected patient than by testing his serum. Also we get our results with much less trouble.

An example of epiphylactic response to an inoculation of Tuberculin B.E. is furnished in the table below.

TABLE VII.—*Spontaneous Phagocytosis of Staphylococcus by the Washed Corpuscles of a Patient in the Last Stages of Phthisis; Before and After the Inoculation of Tuberculin* B.E.

25.10.32	Before inoculation	1·58
	Inoculation of $\frac{\text{B.E.}}{3000}$ mgm.	
25.10.32	Two hours after inoculation	0·63
27.10.32		3·26
31.10.32		2·03
9.12.32	Six hours before death	0·32

The point of superiority which this method has over all others is that incubation is avoided, and with that, almost all the possibility of auto-inoculation *in vitro*.

Another point of very practical interest which emerges from consideration of the data set forth in the tables above is that we have here an unfallacious method of determining what dose of vaccine to administer to a patient to evoke the best epiphylactic response.

STIMULINS

The term '*stimulins*' was introduced by Metchnikoff; and what he had in view when he spoke of a 'stimulin' was a product of active immunisation which circulated in the blood of immune animals and men and which operated by 'training' the leucocytes to attack the species of microbes which had supplied the immunising ictus. In other words a 'stimulin' as conceived by Metchnikoff was a kind of hormone engendered in the animal body in response to an inoculation or auto-inoculation.

It will be appreciated that thermo-generated stimulins of serum which have been considered above belong to a category of stimulins which have nothing in common with those which Metchnikoff had in mind, except only the fact that they

are leucocytotropic and not bacteriotropic—that is to say they operate on the leucocytes instead of combining chemically with the microbes. But they differ from Metchnikoff's notion of a stimulin in not being generated in response to an immunising ictus. And this holds true even more emphatically of the bacterial antigens which come under consideration in the next table.

That bacteria and bacterial derivatives function as leucocytic stimulins may be said to be *a priori* probable, and experimental confirmation of this deduction is as a matter of fact very easily obtained.

One of the simplest ways of doing this is to make caesuric phagocytic experiments such as those set out in Table VIII. Here there was made (A) an ordinary compendial phagocytic mixture; and (B) a phagocytic mixture made by adding 1 volume of serum to 2 volumes of a microbi-corpuscular pre-phagocytic mixture which had been incubated for fifteen minutes.

TABLE VIII

	Phagocytic counts obtained in experiments No.					
	1	2	3	4	5	Average
A. Here the microbes, leucocytes and serum were incubated, all of them together for 15 minutes	3·9	1·4	0·63	0·44	1·4	1·55
B. Here the microbes and leucocytes were incubated first for 15 minutes by themselves; and this microbi-corpuscular pre-phagocytic mixture was, after it had been by the addition of serum, converted into a phagocytic mixture, reincubated for another 15 minutes	6·2	2·58	2·1	1·35	2·58	2·96

We here have evidence that the microbes which are added to a phagocytic mixture to provide pabulum for the leucocytes operate upon these as vaccines. If further evidence of vaccines acting as leucocytic stimulins is required we have it in the subjoined table (Table IX). And this table further shows that the stimulin action exerted by vaccines is non-specific.

We have still to consider more than one other class of stimulins. Among these there have to be considered drugs. And the drugs which especially invite examinations are the sulphanilamides. I have made experiments with one of these—M & B 693.

TABLE IX.—*Comparison of the Phagocytic Intake of (A) Leucocytes Incubated in Normal Salt Solution; and (B) of Leucocytes Incubated in Normal Salt Solution Containing Graduated Quanta of Tuberculin B.E.*

The stimulin effect was judged of by the number of microbes ingested when the two kinds of leucocytes in question were incubated or centrifuged with a suspension of staphylococcus.

The figures for the different samples are expressed below as the ratios, the figure for the Control being arbitrarily represented as 1.

No. of Expt.	Length of incubation	Phagocytic efficiency of the leucocytes operating in plain normal saline taken as	Phagocytic efficiency of the leucocytes operating in normal saline containing B.E. in the following quantities :					
			$\frac{1}{2M}$ to $\frac{1}{1{\cdot}5M}$	$\frac{1}{M}$ to $\frac{1}{0{\cdot}8M}$	$\frac{1}{600T}$ to $\frac{1}{400T}$	$\frac{1}{300T}$ to $\frac{1}{100T}$	$\frac{1}{100T}$ to $\frac{1}{10T}$	above $\frac{1}{10T}$
1	9 minutes	1	—	—	—	—	17	—
2	35 minutes	1	1·5 ; 5·6	—	—	8·5 ; 1	—	1
3	60 minutes	1	2	—	3·1	3·7	—	—
4	Not noted	1	—	—	—	12	24 ; 24	—
5	20 minutes	1	4·6 ; 4	10	3	1	—	—
6	60 minutes	1	—	—	2·5	—	—	—
7	immediate centrifuging	1	11	17	—	—	—	—
8	1 hr. 45 min.	1	—	—	—	3	2	5 ; 10
9	30 minutes	1	5 ; 6	15	18	—	—	—

M = 1 millionth, T = 1 thousandth of a tuberculin suspension containing 1 mg. per c.c. of powdered tubercle bacilli.

Where the two separate figures appear in a single column of the table these indicate that I was here dealing with two specimens of blood which fell within the particular rubric.

The results obtained are set out in Table X—four different experimental procedures being, as will be seen from the last column in the table, here employed.

Procedure 1 : The separated washed corpuscles of the different samples of blood were incubated for fifteen minutes with normal serum and staphylococci suspended in normal saline.

Procedure 2 : The separated sera of the bloods were incubated for fifteen minutes with normal washed corpuscles and staphylococci.

Procedure 3 : The sera were incubated for fifteen minutes with staphylococci suspended in normal saline, and then after normal washed corpuscles had been added to the sero-microbic mixture, the completed phagocytic mixtures were incubated for another fifteen minutes.

Procedure 4 : The sera were incubated for fifteen minutes with washed normal corpuscles and after staphylococci had been added to the sero-corpuscular mixture the completed phagocytic mixture was incubated for another fifteen minutes.

TABLE X.—*Showing the Phagocytic Counts Obtained with Normal Blood, Serum, and Corpuscles, and Those Obtained with the Same Blood, Serum or Corpuscles, Incubated with '693'; together with (These Being Added for the Sake of Comparison) the Phagocytic Counts Obtained with Serum, Blood or Corpuscles Treated with Vaccines.*

Serial No. of Experiment	Length of time during which the drug or vaccine operated upon the blood	Amount of the drug or of the vaccine which was added to the different blood specimens			Phagocytic indices of blood specimens			Procedures by which the figures here recorded were obtained
		No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	
Expt. 1	75 minutes	Nil	$\frac{693}{10000}$	$\frac{B.E.}{500000}$	1	1·3	1·4	Procedure 1
Expt. 2	30 minutes	,,	,,	—	1	1·8	—	,, 1
		,,	,,	—	1	2·5	—	,, 2
		,,	,,	—	1	0·7	—	,, 3
		,,	,,	—	1	2	—	,, 4
Expt. 3	40 minutes	Nil	,,	,,	1	1	1·6	,, 1
		,,	,,	,,	1	1·4	1·7	,, 3
		,,	,,	,,	1	1·4	1·2	,, 4
Expt. 4	30 minutes	,,	,,	3,300 Staph. per c.c.	1	1·2	1·4	,, 1
		,,	,,	,,	1	1·6	1·5	,, 3
		,,	,,	,,	1	2·1	1·1	,, 4
Expt. 5	33 minutes	,,	,,	10,000 Staph. per c.c.	1	1·2	1·4	,, 1
		,,	,,	,,	1	1·6	1·7	,, 3
		,,	,,	,,	1	1·3	2·4	,, 4
Expt. 6	30 minutes	,,	,,	$\frac{B.E.}{500000}$	1	0·9	1·3	,, 1
		,,	,,	,,	1	0·8	0·6	,, 2
		,,	,,	,,	1	1	—	,, 3
		,,	,,	,,	1	1·3	1·1	,, 4

Epitome of the data set out in the foregoing table.

It will be seen on study of the table that $\frac{1}{10000}$ of M & B 693 operates upon the leucocytes as a stimulin—we may call such stimulins '*pharmaco-stimulins*'—and that it increases their phagocytic avidity in the ratio of 1 : 1·4 (this being the average of six experiments); and sometimes as much as 1 : 2·5. This result compares with an average stimulin effect of 1 : 1·27 obtained under the same conditions with blood inoculated with $\frac{1}{500000}$ B.E.; and 1·3 for blood inoculated with 3300 staphylococci per c.c.

Going back now to Tables IV, VIII, IX and X it will be seen that we have had demonstration in these of three different kinds of stimulins: in IV of *stimulins produced by heating serum*; in VIII and IX of *bacterial stimulins*; and in X of both these and of *pharmaco-stimulins*. To these may now be added a fourth kind of stimulins—those which can be extracted from leucocytes by incubating them in normal salt solution. These may be conveniently called *eco-leucocytic stimulins*.

I shall discuss these on another occasion for there are certain points more relevant to our subject matter which still require to be considered. The first of these is :

POSSIBLE BEARING OF THE FACTS IN TABLES IV, VIII, IX AND X ON THE TREATMENT OF BACTERIAL INFECTIONS IN WOUNDS

In the researches on the infections of granulating wounds which were carried out during the last war by my fellow-worker Prof. A. Fleming, striking results were obtained by making impression preparations from wound surfaces which had been untreated for some hours. The impression preparations obtained were in each case immediately turned down upon agar plates, and were then incubated.[1]

Here the ordinary result was for fairly numerous colonies—for the most part colonies of staphylococci and streptococci—to grow out under cover-glasses wherever there were any vacant spaces between the blobs of pus.

And when impression preparations were taken from a wound after it had been carefully washed, leucocytes were here, as is natural, entirely wanting ; but the bacterial colonies were now more numerous than before for the microbes could now, in the absence of leucocytes, grow out unrestrained.

When specimens were taken a little later, and freshly emigrated leucocytes had occupied the surface of the wound there were only few, and in many cases no surviving microbes.

And finally after a considerable interval of time microbic colonies again grew out in the impression preparations in the interspaces between the blobs of pus—this showing that the sterilisation of the wound by the leucocytes had been somewhere or other left incomplete.

The important point is that when the corrupted discharges have been carefully removed from the wound surface, and this is maintained in a condition favourable to the emigration of leucocytes and to their bactericidal action, the wound is so nearly sterilized that only a slight reinforcement of the bactericidal forces already in action would seem to be required to effect complete sterilisation.

Now that reinforcement might possibly, as the results set out in Tables VIII, IX, and X show, be supplied by a local application of a leucocytic stimulin either in the form of a sulphanilamide or a bacterial vaccine.

The local application of sulphanilamide has of course become the routine treatment of bacterial infections of wounds.

But we do not know whether the drug should be employed in the strength which gives the most effective bactericidal result, or in the strength which gives the best results as a non-specific leucocytic stimulin.

That vaccines might also be employed in the treatment of bacterial infections of wounds would seem clear not only from the results set out in the tables just referred to, but also from *in vitro* experiments carried out as follows :

Two filter paper discs are taken and soaked—one in a very high dilution of a broth culture of staphylococcus (a watery dilution containing 1000 to 3000 staphy-

[1] *Vide* these *Collected Researches*, Vol. I, p. 108.

lococci per c.c.) ; and the other in the same dilution of staphylococcus culture to which an appropriate dose of vaccine has been added.[1] Both discs are drained of all superfluous fluid and are then flooded with the same amount of defibrinated blood, or blood taken direct from the finger, and the discs are then covered in with cover-glasses and incubated for not less than 3 hours.

After that the paper discs are taken from under the cover-glasses ; the red blood corpuscles are dissolved in hot water ; and the now decolorised discs are lightly stained with methylene blue. A comparison now shows that always fewer —and generally very many fewer—staphylococcus colonies have grown out in the vaccinated than in the control disc.

There is nothing fundamentally new in this result. For exactly the same result is obtained when an appropriate dose of vaccine is added to the fluid blood instead of its being imposed upon the paper disc.

In the case where we put the vaccine into the paper disc we are doing the same experiment as we should be doing if we put vaccine into a patient's wound ; and when we put the vaccine into surrounding blood we are doing the same experiment as we should be if we were inoculating the vaccine in the ordinary way.

Let me now as a preface to what I want to say about vaccines dwell for a moment on the, at first sight, paradoxical fact that the blood, when it is tested under conditions in which epi-phylactic response is excluded, has no staphylophylactic power ; that is to say it kills either no staphylococci or at best a minimal number of these.

I have dealt with the facts which lead up to this conclusion (I think it is a conclusion which applies generally to all serophytes) in a series of three papers, the first two of which were published in 1902 [2] and in 1915 [3].

In the *first* paper I showed that the *serum* exerts absolutely no bactericidal power upon the staphylococcus or streptococcus or the *M. melitensis* or the plague bacillus.

In the *second* I emphasised the fact that the *serum* furnishes an ideal cultivation medium for the whole of septicaemia-producing or as I prefer to call them, *serophytic microbes.*

And finally I showed in a paper published in 1923 [4] that many normal *whole bloods*, brought into application upon minimal numbers, exert upon these practically no bactericidal power ; and that we can never, as we do when we are speaking of the typho-bactericidal power of the normal serum, say of the non-epiphylactic blood that it will kill a definite number of staphylococci or other serophytes.

My whole blood, for example, exerts no bactericidal power when it is implanted with minimal numbers of staphylococci. It *begins* to develop bactericidal power only when more than 200 staphylococci per cubic centimetre are implanted. And it develops its maximum bactericidal power when it is implanted with between 4000 and 5000 staphylococci per cubic centimetre.

[1] I have always used a vaccine consisting of $\frac{1}{1000000}$ dilution of B.E. suspension which contained 1 mg. of desiccated tubercle per cc.

[2] *Vide* Vol. III of these *Collected Researches*, pp. 58 and 59.

[3] *Vide* Vol. I of these *Collected Researches*, pp. 3 and 34.

[4] *Vide supra*, p. 79.

I have called that the *Caput and Cauda phenomenon* because when the figures for bactericidal power achieved by graduated doses of vaccine are set out in the form of a table we find that the percentage of microbes killed in the more heavily implanted bloods which occupy the head or caput of the table is always much greater than those for the lightly implanted bloods which occupy the whole tail of the cauda of the table.

This fact that the blood, which had originally none, develops a bactericidal power to staphylococcus under the stimulus of a sufficient bacterial implantation, furnishes, as we shall see later, a possible explanation of the fact that the effects of prophylactic inoculation persist long after the manifest blood changes have passed off.

From that I pass to put before you certain general considerations about vaccines. Let me deal first with the question of dosage.

DOSAGE OF BACTERIAL VACCINES

An all-important first principle to appreciate in connexion with the testing of the phagocytic power developed in the blood by vaccines applied therapeutically *in vivo* or *in vitro* is that the phagocytic intake is never a simple function of the strength of the vaccine brought into application. Epiphylactic response is powerfully affected (this comes out with special clearness when we measure the phagocytic response to a vaccine implanted into blood *in vitro*) by the larger or smaller number of microbes we employ as '*phagocytic pabulum*'.

The smaller the number of microbes employed as 'pabulum' the larger is the dose of vaccine required to give a maximal epiphylactic response. And the larger the number of microbes employed as phagocytic pabulum the smaller is the quantum of vaccine required to give the same measure of response. In other words the principle of dosage which has to be borne in mind is that the epiphylactic ictus brought to bear upon the leucocytes is always composed of two or three factors—two in the case of blood inoculated *in vitro* (*one* being the microbes supplied to serve as a phagocytic pabulum, and *the other* the amount of vaccine superadded); and three in the case where an infected patient is inoculated (the *first* being the number of microbes harboured in his body; the *second* being the dose of vaccine administered; and the *third* being, when his phagocytosis is being tested, the phagocytic pabulum employed in the test).

The influence of the different amounts of staphylococcic pabulum employed in the phagocytic tests comes out clearly in Table IX. For the quantitatively different epiphylactic responses there recorded in different experiments in which one and the same dose of tubercle vaccine, and one and the same blood, were employed would seem to be due to the different strengths of bacterial pabulum used in the different experiments.

Experiments made *ad hoc* bring confirmation of the principle in question. In the experiments set out below equal samples of my defibrinated blood were incu-

bated with two different doses of Tuberculin B.E. vaccine, there being, in the two sets of experiments, added to the phagocytic mixtures by way of pabulum the same quanta of staphylococcus culture.

We have in this, and the previous tables, the answer to the question of dosage which should, when treating a patient by vaccine therapy, be kept constantly in mind. These tables show that the initial dose of vaccine to be administered to an

TABLE XI

Serial No. of expt.	Composition of Phagocytic mixtures employed	Phagocytic index of the vaccinated blood
1	2 vols. blood inoculated with $\frac{\text{B.E.}}{1\text{M.}}$ per c.c. + 1 vol. $\frac{\text{Staph.}}{5}$	1·7
	2 vols. blood inoculated with $\frac{\text{B.E.}}{1\text{M.}}$ per c.c. + 1 vol. $\frac{\text{Staph.}}{4}$	1·8
	2 vols. blood inoculated with $\frac{\text{B.E.}}{1\text{M.}}$ per c.c. + 1 vol. $\frac{\text{Staph.}}{3}$	1·1
	2 vols. blood inoculated with $\frac{\text{B.E.}}{1\text{M.}}$ per c.c. + 1 vol. $\frac{\text{Staph.}}{2}$	1·2
2	2 vols. blood inoculated with $\frac{\text{B.E.}}{2\text{M.}}$ per c.c. + 1 vol. $\frac{\text{Staph.}}{16}$	1·0
	2 vols. blood inoculated with $\frac{\text{B.E.}}{2\text{M.}}$ per c.c. + 1 vol. $\frac{\text{Staph.}}{8}$	1·1
	2 vols. blood inoculated with $\frac{\text{B.E.}}{2\text{M.}}$ per c.c. + 1 vol. $\frac{\text{Staph.}}{4}$	1·1
	2 vols. blood inoculated with $\frac{\text{B.E.}}{2\text{M.}}$ per c.c. + 1 vol. $\frac{\text{Staph.}}{2}$	1·5

infected patient must always be a matter of clinical judgment—the heavier the patient's infection the smaller should be the amount of vaccine given for a first inoculation. And the rule for subsequent inoculations is this : When the first dose is adjudged to have been operative and to have exerted a favourable result, and when we have good reason to think (but never before) that the population of microbes in the patient's body has been reduced, we may correspondingly increase the dose of vaccine administered. And when the infection is very nearly extinguished we may use a dose of vaccine little less than that which would be administered for prophylaxis to a healthy man.

QUESTION AS TO WHETHER BACTERIAL VACCINES OPERATE SPECIFICALLY OR NON-SPECIFICALLY

As the terms ' specific ' and ' non-specific ' carry different meanings for different persons it will be necessary to begin by saying something about the terminology.

If only the single strain of microbes out of which the vaccine has been manufactured is killed or impeded in its growth by the vaccine inoculated the effect of the inoculation would be *specific in the narrowest sense*, i.e., type or strain-specific. If on the other hand the bacterial vaccine operates upon all microbes which are ' *homonymous* ' with that from which the vaccine was made the effect would be *non-specific in the sense in which the term is customarily used*. And if, in addition to homonymic, heteronymic microbes are killed or are impeded in their growth by the vaccine inoculated, the immunising effect exerted would be *non-specific in the widest sense.*

Homonymous microbes would be those which are called by the same name because they have similar cultural characters ; produce similar clinical symptoms ; manufacture similar chemical products ; generate, when used as vaccines, similar epiphylactic elements ; and have, subject to the qualification below, similar chemical susceptibilities.

It is necessary to appreciate that when microbes which are called by the same name are found to differ in any cultural, clinical or immunological character ; and also when they have been found to produce different chemical products ; and to have different chemical susceptibilities ; they can by the aforesaid criteria be distinguished into *sub-species*, or *types*, or *variants* or strains which finally receive distinguishing appellations of some sort.

There have in this way been differentiated—and this concerns the specific action of vaccines—the human, bovine, avian and reptilian strains of tubercle bacillus ; and the sub-species or variants of the typhoid bacillus which are known as paratyphoid A and paratyphoid B.

Heteronymous microbes would be microbes which are called by different names because they have different cultural characters, produce different clinical symptoms, and different immunological responses ; and have—this being subject to the reservation now to be made—different chemical susceptibilities.

Susceptibility or insusceptibility to one particular reagent cannot be regarded as a trustworthy criterion of generic affinity or lack of such affinity. This comes out clearly in the fact that M & B 693 operates with conspicuous effect only upon one particular strain of streptococci, while it operates also upon microbes as genetically distinct as the meningococcus and the pneumococcus.

And further proof that the susceptibility or insusceptibility to one particular reagent is not of classificatory importance, is furnished by the fact that it is possible to breed out so-called ' arsenic-fast ' types of trypanosomes which do not as far as it is known differ from unmodified trypanosomes except only in the matter of being resistant to one particular arsenical.

These points of terminology having been made clear it will be seen that the

question as to whether bacterial vaccines operate specifically or non-specifically resolves itself into three different questions.

(1) Do the bacteriotropic substances which are produced by and any stimulins which are set free in the body in response to the inoculation of a bacterial vaccine give protection only against microbes which are culturally, clinically, chemically and immunologically indistinguishable ?

(2) Do these substances protect not only against the microbes which constitute the prime target of inoculation, but in addition against microbes which have slightly different cultural, clinical and immunological properties, but would be included under one and the same microbic species ?

(3) Do the substances in question operate not only upon *homonymous* but also upon *heteronymous* microbes ?

To the *first question* a definite answer is given in the fact that prophylactic inoculation—I limit myself for the moment to anti-typhoid and anti-cholera vaccination—would seem to be everywhere effective, i.e., effective upon all the different strains or varieties of typhoid and cholera which exist. And we have striking confirmation of this applying generally in the fact that vaccine therapy with stock vaccines is—at any rate in the majority of cases—as effective as treatment with autogenous vaccines.

With regard to the *second question*, a unique opportunity for deciding whether a bacterial inoculation is specific in the narrowest sense, or whether it protects also against homonymous microbes of different kinds, presented itself in the 1914–18 war. For there then offered themselves for observation : a large population of soldiers who were inoculated (in the early years of the war) with a simple typhoid vaccine ; and side by side with these a large population of soldiers who were (in the later years of the war) inoculated with a typhoid vaccine in conjunction with a paratyphoid A and a paratyphoid B vaccine.

That statistical opportunity was missed because it was thought necessary to add to the typhoid vaccine, without reducing the dose of that vaccine, half as much paratyphoid A vaccine and half as much paratyphoid B vaccine—this of course resulting in the second population of soldiers being inoculated with double the number of microbes given to the first population.

And at any rate the question under investigation remains unresolved for the statistics which were collected showed that there was no significant difference between the number of cases of paratyphoid A and B in the two populations.

And further evidence pointing to non ' type-specific ' immunisation is furnished by the favourable prophylactic results against pneumococcus which were obtained by myself and my fellow-workers in the Transvaal with vaccines made with untyped pneumococcus.

The very striking results in question are set forth in a series of papers which were published in the *Lancet* in 1912 and were republished in book form.[1]

Facts which would seem to conduct to a directly contradictory conclusion have been put on record by many—chiefly American—investigators.

[1] *Vide supra*, pp. 31–50.

The experiments of these investigators would seem to indicate that animals which are inoculated with one particular strain of pneumococcus are protected only against that particular strain. But these, being purely statistical experiments have, like all such experiments, the vice of taking cognisance of only one particular effect—in this case *survival or non-survival after inoculation with supra-lethal doses.* And when this is borne in mind it becomes plain that all that the statistical results here in question really establish is that better protection is given against the strain of pneumococcus which was employed as a vaccine than against other strains.

And what we have to settle before we call a pneumococcus type-specific or non-type-specific is *how much* protection is afforded against ' homologous ' and how much against ' heterologous ' strains.

Again most of the facts which are relied upon as proof of non-specific response to pneumococci are results obtained by serum therapy on animals and man. And there is, as we shall see in a moment, great difficulty in referring the favourable results obtained by pneumococcic serum-therapy to the administration of epiphylactic substances elaborated in the organism of the donor animal.

The *third issue* as to whether bacterial vaccines can, or cannot, afford protection against heteronymous microbes, can be put to the test in a number of different ways.

We have already in this connexion considered the observations which were made by Shattock and Dudgeon on the one hand, and by Dr. Ronald Hare on the other. (*Vide* Table III, p. 194 *supra.*)

And what looks like clear evidence of the protective effect exerted by a vaccine on heteronymous microbes is that set out in the statistical tables which show the results obtained by pneumococcal vaccination in the Premier Mines in the Transvaal in the years 1911 to 1913.[1]

Further, convincing clinical evidence of the non-specific effect of vaccines is available in the fact that the intra-vascular inoculation of typhoid and coliform vaccines has proved itself an effective treatment for many different infections.

In particular it is credited with having given strikingly favourable results in gonococcal infections.

That these results find their most natural explanation in a non-specific immunising response and not in a newly-invented type of reaction which has been christened ' Protein Shock ', will not be doubtful to anyone who has, from laboratory experimentation, learned to expect non-specific immunisation *in vitro.*

Passing now from clinical to clinico-laboratory experiment I may lay stress on an experiment I published ten years ago. This showed that an intravenous inoculation of coliform vaccine first markedly diminished the staphylo-bactericidal power of the patient's blood and then formidably increased it. (*Vide supra*, p. 157, Fig. 3).

[1] The tables here in question are those given *supra* pp. 48 and 49.

HAS CONVINCING PROOF OF ANY PURELY SPECIFIC IMMUNISATION EVER BEEN ADDUCED ?

I shall confine myself to my own work and that of my fellow-workers. To test the specificity of the response to typhoid vaccine, I took the *sera* of six laboratory workers who had been inoculated against typhoid and tested their bactericidal power both to cholera and typhoid. I found in these experiments that the *sera* I was dealing with showed a greatly increased bactericidal effect to typhoid but no increased bactericidal power to cholera. I inferred from that that bacterial vaccines produce only specific epiphylactic effects. But in coming to this conclusion I had neglected to think of the leucocytes.

I may instance the further fact that when my fellow-worker, Dr. John Freeman, massaged a gonococcal knee with a view to determining whether the auto-inoculation produced would have a specific or non-specific effect, he found [1] that it markedly increased the phago-incitor power of the serum to gonococcus, but left the phago-incitor power of the serum to the tubercle bacillus quite uninfluenced.

Here again we all in my laboratory, quite forgetting that a different result might have been obtained if the leucocytes had been tested, rashly assumed that auto-inoculations operate only upon homonymous microbes.

Finally coming back to the fact that we get, as pointed out above, better results by testing leucocytic than by testing serum response, I may draw attention to the fact that when my colleagues and I were working on the pneumonia which was then epidemic in the Transvaal mines, we very laboriously searched for evidence of immunising response to pneumococcus in the sera of ourselves, of healthy natives, and of natives who were suffering with pneumonia, and also of healthy natives inoculated with pneumococcus vaccine. We did this in order to test the effects of vaccine therapy, and also to choose a proper dose of vaccine for prophylactic inoculation. And the upshot of all this work was that satisfying evidence of the development of either increased bactericidal or phago-incitor power in the serum was conspicuously lacking.

The results obtained in our thousands of phago-incitor tests are too complicated to set forth here but the bactericidal results of our large numbers of serum tests are shown in Table XII.

And the very startling negative or practically negative results here recorded may be usefully contrasted with the fact that when at a later date the bloods of normal Europeans were tested by haemo-bactericidal methods (i.e., slide-cell or capillary-clot methods) they gave instead of the small figures recorded below, figures of 50,000 and over of pneumococci killed per 50 c.mm. of blood.

The data in Table XII suggest that the current interpretation of the favourable results of pneumococcal serum therapy may not be the right one. For if the sera of auto-inoculating pneumonic patients, and healthy men inoculated with pneumococcus vaccine, contain no bactericidal and seemingly no increased phago-incitor

[1] *Vide* these *Collected Researches*, Vol. III, p. 368, chart 44.

substances, it becomes difficult to believe that the sera of inoculated animals contain such substances.

TABLE XII.[1]—*Showing the Results Obtained by Testing the Pneumo-bactericidal Power of the Sera of Four Large Groups of Persons.*

Source from which the sera were derived	Total number of observations	Number of observations in which no microbes were killed by the serum	Cases in which 10–30 pneumococci were killed by 50 c.mm. of serum	Cases in which 30–90 pneumococci were killed by 50 c.mm. of serum	Average number of pneumococci killed by 50 c.mm. of serum from the group
Healthy Europeans	41	36	4	1	2 (approx.)
Healthy Tropical Natives ..	27	19	8	0	4 (approx.)
Tropical natives after inoculation with pneumococcus vaccine	176	161	15	0	1 (approx.)
Natives suffering from pneumonia	20	11	9	0	5 (approx.)

IS THE ACCREDITED INTERPRETATION OF THE GOOD RESULTS OBTAINED WITH SERUM-THERAPY OF PNEUMONIA THE ONLY POSSIBLE ONE ?

There are two other possible interpretations of the recorded facts. The first is that antigens, possibly antigens which act as stimulins, may be contained in the pneumococcal serum administered. This general possibility has already been considered in connexion with Neufeld and Rimpau's reported thermostable phagoincitor substances.

And the second possible explanation of the favourable results obtained in human serum therapy is that the processes of concentration and re-solution which have been applied to the serum administered to man may have converted these sera into stimulins. Many of the results which I have obtained with re-constituted sera seem to suggest that.

One is driven to this desperate resource of guessing at the explanation of the favourable results of pneumonic serum therapy by the fact there would seem never to have been any proper evaluation of the epiphylactic power of the anti-pneumococcus sera administered, or any proper evaluation of the leucocytes and sera of the patients before and after administration of the serum.

PERSISTENCE OF THE PROTECTIVE EFFECTS OF PROPHYLACTIC VACCINATION

Everyone who has considered the evanescence of the increased bactericidal power of the serum which is achieved by antityphoid inoculation is agreed that the persistent specific immunisation which is achieved must be due to some of the cells

[1] Reprinted from the Author's *Drugs and Vaccines in Pneumonia*, p. 63 (Constable, London, 1914).

of the body remaining, long after all constitutional effects of inoculation have passed off, sensitive to re-immunisation by such minimal typhoid inoculations as would inevitably occur in patients who were living in places where they are from time to time exposed to typhoid infection.

What cells in the body perform this work of re-immunisation to typhoid is one of the important unresolved questions of bacteriology.

It may quite well be that it is the leucocytes which respond to infection ; for that is not precluded by the fact that it is confidently asserted that that function is performed either by fixed cells distributed all over the body, or by fixed cells closely associated with the blood, and the ascription of this function to the leucocytes would at any rate harmonise with the fact that extravascular blood that possesses either none or only minimal staphylo-bactericidal power acquires when inoculated plentiful bactericidal power.

Further, it looks as if the question could easily be settled by direct experiment. It would be perfectly easy to take a series of samples of blood of patients who have been inoculated with typhoid (the taking of the first of these samples being deferred until the constitutional disturbance produced by the inoculation has died down) and to compare (using for this purpose very small doses of typhoid vaccine) the sensitiveness of the leucocytes of these bloods with that of the leucocytes of normal bloods. By the same method the question as to how long anti-typhoid inoculation lasts could I think be settled.

If I were not too far ' declined into the vale of years ', and if I had not more important matters to investigate, I should myself undertake such a series of experiments. As things are I commend the suggested procedure to younger research workers.

Finally I subjoin, by way of a key to the reasoning in this paper, the following tables :

TABLE A.—*Pasteurian Doctrine of Immunisation.*

(This was based on what was known or believed to hold true of Jennerian vaccination ; and it constituted the foundation of all the Pasteurian extensions of that system of immunisation.)

(1) Immunisation can be undertaken whenever the pathogenic microbe of a disease has been isolated, or when material which contains that microbe is available.

(2) It is a *sine qua non* for successful vaccination that it should be carried out with living microbes.

(3) Immunity is established only after the expiration of ten days.

(4) Immunisation is practicable only in advance of the infection—with a possible exception in the case of any disease in which the incubation period lasts sufficiently long for immunisation to be completed before the symptoms of the disease become manifest.

(5) The amount of vaccine which is administered need not be minutely regulated. All that is required is to make sure that the microbe which is employed has been adequately and permanently attenuated.

(6) Success or failure of an immunisation procedure can be judged only by statistical methods.

That involves in the case of animals, inoculating them and their controls with a virulent culture of the specific microbe of the disease. And it involves in the case of man the registering of the incidence- and death-rates of formidable numbers of vaccinated persons and controls who have been equally exposed to the infection against which it is hoped to give protection.

RECTIFYING GENERALIZATIONS ENUNCIATED BY THE AUTHOR IN PAPERS PUBLISHED BETWEEN 1897 AND 1901

(1) Inoculations can be successfully carried out with sterilised vaccines.

The fact that agglutinins are elaborated in response to bacterial inoculations has been previously shown by Richard Pfeiffer.[1]

(2) An increase in the bactericidal powers of the blood can be registered very shortly; to wit, within one day or less after vaccination.[2]

(3) Vaccines can be employed not only prophylactically but also therapeutically in the treatment of infected patients; and here the phagocytic power of the blood is very rapidly increased.[3]

(4) The measure of immunity conferred by an inoculation procedure can be gauged by measuring the changes effected in the bactericidal and phagocytic power of the blood.

[1] Wright and Semple: 'On Vaccination against Typhoid Fever', *Brit. M. J.*, Jan. 30, 1897.

Wright and Leishman: 'On the Results which have been Obtained by the Anti-typhoid Inoculations, and on the Methods which have been Employed in the Preparation of the Vaccine', *Brit. M. J.*, Jan. 20, 1900.

[2] Wright: 'On the Changes Effected by Anti-typhoid Inoculations in the Bactericidal Power of the Blood, with Remarks on the Probable Significance of these Changes', *Lancet*, Sept. 14, 1901.

[3] Wright: 'Notes on the Treatment of Furunculosis, Sycosis and Acne by the Inoculation of a Staphylococcus Vaccine, and Generally on the Treatment of Localised Bacterial Invasions by Therapeutic Inoculations of the Corresponding Bacterial Vaccines', Vol. III of these *Collected Researches*, p. 168 *et seq.*

TABLE B.—*Dealing with the Machinery of Immunisation.*

Rival Doctrines dating from 1890 or before.

Humoral Theory.—Immunisation consists in the elaboration of bactericidal substances and in the conveyance of these into the blood-stream.

Phagocytic Doctrine of Metchnikoff.—Immunisation consists in training the leucocytes to confront and phagocytose the specific pathogenic microbes against which the animal has been inoculated.

Doctrine of the author with regard to the machinery of immunisation set out in papers published between 1897 and 1908	Logical grounds which were felt to justify the conclusions set out in column 1	Revision in the light of new data of the doctrine set out in column 1
(1) Phagocytosis of microbes takes place only in the presence of serum.	When leucocytes have been washed free from serum phagocytosis of microbes is abolished.	*Vide* new data set out in Tables V, VI, and VII, pp. 197 and 198.
(2) The chemical reaction which takes place in a phagocytic mixture (i.e. mixture of corpuscles, serum and microbes) is an opsonic action.	Of the three *a priori* possibilities (1) that the serum acts opsonically on the microbes; (2) that the serum may function as a leucocytic stimulin; and (3) that the microbes which are added as phagocytic pabulum may vaccinate the leucocytes; only the first appears to be admissible.	*Vide* new data showing that the microbes which are contained in a phagocytic mixture operate as a vaccine on the leucocytes of that mixture (*vide* Table VIII, p. 199) and that a serum may exert a stimulin effect on the leucocytes. *Vide* Table IV, Sects. 1 and 2, p. 196.
(3) Serum is rendered inert by heating to 60° C.—this result being due to the destruction of the opsonins.	Comparison of the effects of heated serum with that of unheated serum (*Vide* Table IV, Sect. 1, p. 196).	*Vide* new data set out in Table IV, Sect. 2, p. 196.
(4) Immunising response of the patient to the inoculation of bacterial vaccines affects his serum and leaves his leucocytes unchanged.	*Vide* Vol. III of these *Collected Researches*, p. 87, pp. 89 to 91 and p. 119.	*Vide* new data set out in Chiastic Table II, p. 193.
(5) The whole of the increased phagocytosis which is obtained by the inoculation of bacterial vaccines is to be ascribed to an elaboration of opsonins in the serum and not to an elaboration of leucocytic stimulins.	This was inferred from Ehrlich's axiom that inoculations lead always to the elaboration of antithetic chemical substances, and as a corollary from that that bacterial vaccines lead to the elaboration of *bacteriotropic* as distinguished from *leucocytotropic* substances.	*Vide* Tables IX and X for evidence of stimulin action exerted on leucocytes by bacterial vaccines, and also by M & B 693, pp. 220 and 201.
(6) Leucocytes kill microbes only intracellularly.	*A priori* assumptions.	Leucocytes can kill microbes extracellularly. *Vide* p. 189 *et seq.*
(7) The blood *in vitro* will not respond to inoculation.	*A priori* assumptions.	The blood does, when inoculated *in vitro*, develop increased bactericidal and phagocytic power, and does this often instantaneously; often in the course of a few minutes; and it would seem always in the course of two hours.
(8) The anti-bacterial substances which are elaborated in the body in response to bacterial vaccines operate only on the kind or sort of microbes which have been inoculated.	*Vide* Immunisation Curve No. 44.	*Vide* direct laboratory experiments: Shattock and Dudgeon's results p. 194, and Dr. R. Hare's results, Table III, p. 194, also inoculations on blood *in vitro*. Table II, p. 193; Table IX, p. 200; Fig. 3, p. 157. *Vide* also results of prophylactic inoculation against pneumonia in the Transvaal, pp. 31–49 *supra*.

APPENDIX I

ON ANTI-TYPHOID INOCULATION

(Being an Excerpt from the Author's 'Short Treatise on Anti-Typhoid Inoculation', Constable, London, 1904)

Question as to what form of Culture, or what Bacterial Derivative, will induce the Organism to furnish the Typhotropic Substances which are required for the Prevention and Mitigation of Typhoid Fever.

In order to induce the organism to furnish the bacteriotropic substances which it will require when it is confronted by the typhoid bacillus, we must, as consideration will show, introduce into the body constituents of the protoplasm of the typhoid bacillus, as distinguished from the metabolic products which may have been elaborated by the micro-organism in the course of its cultivation.

Having recognised that the vaccine employed for anti-typhoid inoculation must contain constituents of the bacterial protoplasm, we have still to decide in what particular form these shall be administered.

In making our election between the different varieties of vaccines which might be brought into application we may take as our guide either tradition and *a priori* considerations, or, emancipating ourselves from these, we may take as our guide the data furnished by a qualitative and quantitative determination of the antitropins which are elaborated in response to each particular variety of vaccine.

If conformity to the practice adopted in the earliest preventive inoculations were exacted, we should have to employ, as in the case of the Pasteurian inoculations against anthrax, and in the case of the anti-cholera inoculations of Haffkine—which were modelled upon the Pasteurian pattern—cultures of living attenuated micro-organisms. It is unnecessary to point out that such a course would in the case of the typhoid bacilli which we are here considering entail grave risks :—the risk of disseminating the germ of the disease, and that of communicating the disease in a serious form in any case where by mischance the inoculated patient happened to be characterised by an abnormal susceptibility to typhoid infection.

If, while discounting the Pasteurian principle of the necessity of employing living micro-organisms, we were still to cling to the view that every method which involves a chemical alteration of the bacterial proplasm is inadmissible, it would be necessary to resort to devices for devitalising our bacteria without exposing them to the action of heat or antiseptics.

Such a restriction would involve us in the grave inconveniences and risks which are associated with the proposed method of Macfadyen.[1] It would, on the one hand, necessitate resort to the complicated apparatus which is required for the

[1] *Proc. Roy. Soc.*, 1903.

trituration of bacteria ; and, on the other hand, it would deprive us of the security against contamination and against the risk of communicating the typhoid infection, which is obtained by the employment of cultures which have been devitalised by heat.[1]

If, lastly, we are prepared to accept evidence of the production of the desired antitropic substances as proof of the efficacy of the vaccine, we shall find ourselves, as the reader will realise at the end of the next section, and again when we come to deal with the statistics of anti-typhoid inoculation, free to employ for our inoculations cultures which have been sterilised by exposure to a temperature of 60° C.

It will be unnecessary to point out to anyone who has experience of such matters the manifold practical advantages which result from the employment of such sterilised cultures.

Question as to whether the Inoculation of a Typhoid Culture which has been Sterilised by Exposure to Temperatures of 60° C. is capable of inducing in the Organism the Elaboration of the Typhotropic Substances which are required.

I may appropriately open the consideration of this question by pointing out that the suggestion that preventive inoculations should be undertaken against typhoid fever upon the Pasteurian system—a suggestion which was originally made to me by Mr. Haffkine—was, considering the risk which seemed to me to be involved in such a process, destined, so far as I was concerned, to remain indefinitely inoperative. The whole aspect of this suggestion was immediately changed as soon as I learned in the course of conversation with Professor R. Pfeiffer that he had in man obtained the specific agglutination-reaction to typhoid by the subcutaneous inoculation of a heated typhoid culture. This observation, since it pointed to the continued presence of effective vaccinating elements in the heated culture, immediately supplied the basis for the system of anti-typhoid inoculation which I have employed.[2]

In the course of the researches which have been carried on by me, so far as time and opportunity have allowed, during the last six and a half years, further evidence has accumulated in my hands with regard to the integrity of the vaccinating elements in typhoid cultures which have been subjected to the action of heat. The facts may be grouped under two headings :—(*a*) facts showing that the typhoid culture is unaltered so far as its immunising properties are concerned by exposure to the temperature which is required for the devitalisation of the bacteria ; and (*b*) facts showing that the chemical relations which obtain between the protective

[1] It may be pointed out that the method of preparing typhoid vaccine which has been suggested by Macfadyen is essentially the same as the method previously employed by Koch in the preparation of his tubercle vaccine (new tuberculin). What has been said with regard to the inconveniences and risks of Macfadyen's method applies therefore, *mutatis mutandis*, also to Koch's method of preparing tubercle vaccine.

[2] It may be observed that Professor Pfeiffer also recognised that his observation with regard to the production of agglutinins by inoculation of sterilised cultures had opened the way to anti-typhoid inoculation. The results of two experimental anti-typhoid inoculations were published by him in conjunction with Kolle (*Deutsche Medic. Wochenschft.*, 12th Nov., 1896), shortly after the publication of my first two anti-typhoid inoculations (*Lancet*, 9th Sep., 1896).

substances of the blood and the unheated typhoid bacillus, obtain also between these protective substances and the typhoid bacillus after it has been devitalised by exposure to a temperature of 60° C.

Observations which show that the Typhoid Culture preserves its Vaccinating Efficacy after Exposure to Temperatures of 60°–65° C.

The proposition that the typhoid culture preserves its vaccinating efficacy after exposure to a temperature of 60° C. is established :—

(1) by the fact that the bactericidal power of the blood is increased—sometimes as much as one-thousandfold—as the result of a single inoculation of a suitable quantum of a sterilised typhoid culture ;[1]

(2) by the fact that an increased bacteriolytic power is developed in the blood of patients who have been inoculated with a suitable quantum of such sterilised typhoid cultures ;[2]

(3) by the fact that a patient who has recovered from a first inoculation of a sterilised typhoid culture does not upon second inoculation suffer from the very severe constitutional intoxication which would supervene in the case of an uninoculated person inoculated with this dose of typhoid vaccine ;

(4) by the fact that an increased opsonic power is developed in the blood of patients who have been inoculated with a suitable quantum of sterilised typhoid culture.

We have in (1) and (2) evidence of the elaboration of antitropins which exert a destructive effect on the typhoid bacillus ; in (3) evidence of the elaboration of antitropins which discharge the office of antitoxins, and in (4) evidence of the elaboration of opsonic antitropins.

Summarising the above, we see that inoculation of cultures of typhoid bacilli which have been sterilised by exposure to a temperature of 60° C. induces in the organism an elaboration of :—

(*a*) Agglutinating antitropins ;
(*b*) Bactericidal antitropins ;
(*c*) Bacteriolytic antitropins ;
(*d*) Antitoxic antitropins ;
(*e*) Opsonic antitropins.

[1] Author's paper, *Lancet*, 14th September, 1901.

[2] This may be demonstrated by making a series of progressive dilutions of the serum of a normal and of a vaccinated person, and mixing in each case the successive dilutions of serum with equivalent volumes of culture in a capillary tube. On examining microscopically films made with the contents of the capillary tube after the serum has been allowed to act upon the bacteria for half an hour at blood-heat, it will be found that the bacteriolytic effect has manifested itself in a much higher dilution in the case of the serum obtained from the inoculated patient, than in the case of the serum obtained from a normal person who acts as control.

APPENDIX II

ON THE PHARMACO-THERAPY OF PNEUMONIA

(Excerpt from the Author's 'Drugs and Vaccines in Pneumonia'. Constable, London, 1914)

Introductory

The Experiments reported in this Appendix would appear to be the first carried out on man which established that all the ordinary antiseptics are, when applied in blood, bactericidally ineffective; and that Morgenroth had given us in Optochin the first drug which operated bactericidally in full strength in the blood. Further, the experiments here reported show, I think, for the first time that Optochin renders the blood of animals and patients bactericidal to the Pneumococcus.

(I) Measurement of the Bactericidal Effect exerted upon the Pneumococcus by Graduated Dilutions of *Ordinary Antiseptics* and *Optochin* in Water and Human Serum respectively.

The object of experiments upon the Pneumococcus such as are here in question is to determine (*a*) whether the chemical energy of the drug is expended wastefully upon the blood fluids; (*b*) what concentration of the drug will be required to exert a bactericidal effect in the blood; and (*c*) whether the introduction of the antiseptic into the blood interferes with phagocytosis or any other of the antibacterial properties of the blood.

The experiments which are here subjoined, and which were conducted with a modification of the technique which I have elsewhere [1] described, bring out very clearly that, while our ordinary antiseptics are polytropic and expend their energy wastefully upon the blood-fluids, we have in aethylhydrocupreinhydrochlorate a chemical agent which exerts its effect practically undiminished in serum. The experiments bring out that dilutions of 1 part of lysol in 500 parts of serum, dilutions of 1 part of creosote in 2,500 to 12,500 parts of serum, and dilutions of 1 part of guaiacol in 2,500 parts of serum fail to kill the pneumococcus; while dilutions of 1 part of lysol in 62,500 parts of water, dilutions of 1 part of creosote in 300,000 of water, and dilutions of 1 part of guaiacol in 1,500,000 of water, all kill the pneumococcus. They bring out further that dilutions of 1 part of aethylhydrocupreinhydrochlorate in 400,000 parts of serum kill the pneumococcus, and that dilutions of 1 in 800,000 inhibit the growth; and that the antiseptic values of the serum dilutions of the drug do not differ appreciably from the values obtained for watery solutions. Finally they show that aethylhydrocupreinhydrochlorate exerts its bactericidal effect specifically upon the pneumococcus.

[1] For the general method see the Author's *Technique of the Teat and the Capillary Glass Tube* (Constable, London, 1912), pp. 108–113. The modification consisted in using, instead of a unit volume of the bactericidal agent and a unit volume of microbial suspension, a 5 c.mm. volume of the bactericidal agent, and only so much of the bacterial suspension as was obtained by filling this in up to a 5 c.mm. mark placed upon the stem of the capillary tube, and then blowing it out again. The number of microbes which this proceeding furnished was elicited in each case by cultivating a series of eight or more control tubes filled in with serum broth after wetting the walls up to the 5 c.mm. division mark with the same bacterial suspension as was employed in the companion series of tubes.

Measurement of the bactericidal effect exerted upon the pneumococcus by lysol in graduated dilutions made with water and human serum [1] *respectively.*

	Strength of the lysol dilutions which were brought in contact with the pneumococci (in each case 5 c.mm. of the lysol dilution was digested overnight with several thousands of pneumococci)					
	$\frac{1}{500}$	$\frac{1}{2500}$	$\frac{1}{12500}$	$\frac{1}{62500}$	$\frac{1}{312500}$	$\frac{1}{1565000}$
Dilution in water - -	O	O	O	O	X	X
,, human serum	X	X	X	X	X	X

The signs O and X respectively are employed to signify that a growth was not, or was, obtained on cultivating in serum broth the microbes which had been digested with the bactericidal agent.

Measurement of the bactericidal effect exerted upon the pneumococcus bycreosote in graduated dilutions made with 0·85 per cent. salt solution and human serum respectively.

	Strength of the creosote dilutions which were brought in contact with the pneumococci (in each case 5 c.mm. of the creosote dilution was digested overnight with about 625 pneumococci)				
	$\frac{1}{500}$	$\frac{1}{2500}$	$\frac{1}{12500}$	$\frac{1}{62500}$	$\frac{1}{312500}$
Dilution in NaCl 0·85 per cent. -	O	O	O	O	O
,, human serum - -	O	X	X	X	X

	Strength of the creosote dilutions which were brought in contact with the pneumococci (in each case 5 c.mm. of the creosote dilution was digested with about 45,000 pneumococci)				
	$\frac{1}{500}$	$\frac{1}{2500}$	$\frac{1}{12500}$	$\frac{1}{62500}$	$\frac{1}{312500}$
Dilution in water - - - -	O	O	O	O	O
,, human serum - -	O	O	X	X	X

Measurement of the bactericidal effect exerted upon the pneumococcus by guaiacol in graduated dilutions made with water and human serum respectively.

	Strength of the guaiacol dilutions which were brought in contact with the pneumococci (in each case 5 c.mm. of the guaiacol dilution was digested overnight with about 45,000 pneumococci)					
	$\frac{1}{500}$	$\frac{1}{2500}$	$\frac{1}{12500}$	$\frac{1}{62500}$	$\frac{1}{312500}$	$\frac{1}{1565000}$
Dilution in water - -	O	O	O	O	O	O
,, human serum	O	X	X	X	X	X

Measurement of the bactericidal effect exerted upon the pneumococcus by aethylhydrocupreinhydrochlorate in graduated dilutions made with 0·85 per cent. NaCl and human serum respectively.

	Strength of the aethylhydrocupreinhydrochlorate dilutions which were brought in contact with the pneumococci (in each case 5 c.mm. of the aethylhydrocuprein dilution was digested overnight with several thousands of pneumococci)						
	$\frac{1}{1000}$	$\frac{1}{10000}$	$\frac{1}{50000}$	$\frac{1}{100000}$	$\frac{1}{200000}$	$\frac{1}{400000}$	$\frac{1}{800000}$
Dilution in NaCl 0·85 per cent.	O	O	O	O	O	O	O
Dilution in human serum	O	O	O	O	O	O	X [2]

Note.—A control in which the same number of pneumococci were introduced intc 5 c.mm. of untreated normal human serum gave a plentiful culture of pneumococci.

[1] The human sera which were employed in this and the following experiments exerted no bactericidal effect on the pneumococcus.

[2] Very sparse culture after thirty-six hours.

Measurement of the comparative bactericidal effects of serum dilutions of aethylhydrocupreinhydrochlorate upon pneumococcus and staphylococcus, and pneumococcus and Bacillus paratyphosus respectively.

	Strength of the dilutions of aethylhydrocupreinhydrochlorate in serum which were brought into contact with the bacteria (in each case 5 c.mm. of the aethylhydrocupreinhydrochlorate dilution was digested overnight with some thousands of microbes)							
	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{5000}$	$\frac{1}{10000}$	$\frac{1}{15000}$	$\frac{1}{20000}$	$\frac{1}{25000}$	$\frac{1}{40000}$
Staphylococcus - -	—	—	X	X	X	X	—	—
Pneumococcus - -	O	O	O	O	O	O	O	O
	$\frac{1}{200}$	$\frac{1}{1000}$	$\frac{1}{10000}$	$\frac{1}{20000}$	$\frac{1}{40000}$	$\frac{1}{60000}$	$\frac{1}{80000}$	$\frac{1}{100000}$
Bacillus paratyphosus -	X	X	X	X	X	X	X	X
Pneumococcus - -	—	—	O	O	O	O	O	O

Note.—In each case a control in which the same number of pneumococci were introduced into 5 c.mm. of untreated normal serum gave a plentiful culture of pneumococci.

It is clear upon the basis of these experiments that we may reasonably infer that aethylhydrocupreinhydrochlorate would be capable of exerting a bactericidal action upon the pneumococcus *in vivo*. Further, if we work upon the figures of these experiments, upon the assumption that we are dealing with a man of 70 kilos (of which 5 kilos would represent blood), and on the quite unrealisable assumption that when a drug is administered the whole quantum will be absorbed into the blood and come into operation at the same moment, the doses of the various antiseptics which would be requisite to produce a bactericidal effect upon the pneumococcus circulating in a patient's blood can be calculated. The dose in the case of lysol would be greater (experiment does not tell us how much greater) than 14 grm. ($\frac{1}{2}$ oz.) ; in the case of creosote and guaiacol greater than 2·8 grm. (over 45 minims) ; and of Optochin only 0·017 grm.

(II) Prophylactic and Therapeutic Experiments upon Animals inoculated with the Pneumococcus supplemented by *in vitro* Examinations of their Blood drawn off before and after the Administration of the Drug.

While *in vitro* experiments such as the above will tell us whether the chemical energy of a bactericidal agent is or is not wastefully expended upon the blood-fluids, and what is the minimum quantity of the drug that must be introduced into those fluids in order to achieve a bactericidal effect, they cannot tell us whether the drug will prevent or cure an infection, and whether the drug will exert a poisonous action upon some important element of the animal machinery. Experiments *in vivo* are here required.

But too high a value may be set upon statistical *in vivo* experiments. It is in the laboratory often forgotten that animal experiments, when not followed up by blood tests conducted *in vitro*, leave us quite in the dark as to the causes of the observed effects. For instance, in the case where infecting bacteria have been killed by the administration of a bacteriotropic drug, they leave unresolved the question whether

this result is to be ascribed wholly to the drug, or in part to the drug and in part to superadded immunising responses. Again it is often forgotten that the conditions in the human organism may be fundamentally different from those in the animal with respect to the form which the bacterial infection takes, the affect exerted by the blood-fluids on the infecting microbes, the absorption and excretion of the drug, and the susceptibility of the nobler tissues to its toxic action.

These points having been premised, we may turn first to the consideration of the results of the animal experiments which were carried out by Morgenroth and his fellow-workers. These experiments are destined to stand out as a landmark in the history of the pharmacotherapy, because they furnish the first demonstration of the possibility of preventing and curing a bacterial—as distinguished from a protozoal or spirochaetal—infection by the administration of a drug. All that it will be necessary to do here will be to give the general result of Morgenroth's experiments. These were conducted on mice inoculated with cultures of pneumococcus which killed without exception every untreated mouse. The aethylhydrocupreinhydrochlorate or sulphate was administered in some cases before, in others after, the inoculation. Employed thus the drug prevented the development of the infection in some 90 per cent. of the prophylactically treated ; and cured about 50 per cent. of the animals in which treatment was postponed till after inoculation. To the clear and complete evidence which Morgenroth's tabulated experiments furnish upon these points, there remained only to add bactericidal experiments conducted *in vitro* on the blood-fluids and urine of treated and untreated mice. Subjoined will be found details of such bactericidal experiments undertaken by us upon untreated mice and mice treated with the same doses of aethylhydrocupreinhydrochlorate as were employed by Morgenroth in his experiments.

Measurement of the bactericidal effect exerted upon an enumerated suspension of pneumococcus by the undiluted pooled serum of a group of mice treated with aethylhydrocupreinhydrochlorate (2 mgrm. to 10 grm. body-weight), and by the undiluted pooled serum of a control group of untreated mice.

							Bactericidal effect calculated out for 1 c.c. of serum
Dilutions of the pneumococcus suspension which were employed	$\frac{1}{1}$	$\frac{1}{5}$	$\frac{1}{15}$	$\frac{1}{45}$	$\frac{1}{135}$	$\frac{1}{305}$	
Pooled serum of five untreated mice -	X	X	X	X	X	X	1 c.c. serum kills 0 cocci
Pooled serum of five treated mice three hours after subcutaneous injection of the drug	X	X	X	O	O	O	1 c.c. serum kills 1,200 cocci
	$\frac{1}{1}$	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{64}$	$\frac{1}{256}$	$\frac{1}{512}$	
Pooled serum of four untreated normal mice	—	X	X	X	X	X	1 c.c. serum kills 0 cocci
Pooled serum of four treated mice three hours after injection of the drug	X	X	X	O	O	—	1 c.c. serum kills 1,600 cocci

Measurement of the bactericidal effect exerted upon an unenumerated suspension of pneumococcus by the undiluted pooled serum and pooled urine of a group of mice treated with aethylhydrocupreinhydrochlorate (2 mgrm. to 10 grm. body-weight), and by the undiluted pooled serum and the pooled urine of a control group of untreated mice.

Dilutions of the pneumococcus suspension which were employed	$\frac{1}{5}$	$\frac{1}{25}$	$\frac{1}{125}$	$\frac{1}{625}$	$\frac{1}{3125}$	
Pooled serum of four untreated mice	—	—	X	X	O	
Pooled serum of four treated mice killed five hours after subcutaneous injection of the drug	O	O	O	O	O	The serum of the treated killed more than twenty-five times as many pneumococci as the serum of the untreated mice
Pooled urine of four untreated mice	—	X	X	O	O	The urine of the treated killed more than five times as many microbes as the urine of the untreated mice
Pooled urine of four treated mice	O	O	O	O	O	

We have here, in the data with regard to the sera of mice treated with aethylhydrocupreinhydrochlorate, satisfactory explanation of the successful results obtained by Morgenroth in his experiments upon animals. Further, we have in the data which refer to the urine of the treated mice ; in those afterwards obtained in experiments made on the urine of man ; and also in the fact that the bactericidal power which the serum acquires after the exhibition of Morgenroth's drug is not diminished by heating to 60° C. satisfactory evidence that the bactericidal effects recorded above are due to the absorption of the drug into the blood-fluids and to its excretion in the urine.

(III) Preliminary Experiments on Uninfected or Infected Men supplemented by Experiments upon their Blood drawn off before and after the Administration of the Drug.

These experiments upon man were designed to test the inferences drawn from the *in vitro* experiments on human blood which have been described under (I), and from the *in vivo* and *in vitro* experiments on animals which have been described under (II). The ideal order of experimentation is to commence with experiments on normal men : *first*, because it would be to the advantage of the sick that we should, when dealing with them, have something to guide us in our dosage and the interspacing of our doses ; *secondly*, because we may reasonably hope to obtain from normal men timelier warning of any toxic effect that might be produced by our drug.

The experiments which are subjoined were carried out in part upon normal men and in part upon pneumonia patients ; and we may in this particular section confine ourselves to the issue as to whether, under the administration of the drug, the blood-fluids of the patient acquire a bactericidal power, or alter with respect to their opsonic power. And we may leave over to the next section the issue as to

whether the exhibition of the drug exerts a favourable influence on the course of pneumonia.

Measurement of the bactericidal and opsonic power exerted upon the pneumococcus by human serum drawn off before and after the administration of aethylhydrocuprein-hydrochlorate.

Experiment No. I.—(*a*) Three normal natives receive on 31st January, 1912, 0·5 grm. of Morgenroth's drug by the mouth.

Dilutions of the pneumococcus suspension which were employed	$\frac{1}{25}$	$\frac{1}{75}$	$\frac{1}{225}$	$\frac{1}{675}$	$\frac{1}{2025}$	Bactericidal effect exerted by 5 c.mm. of serum	Opsonic index
Approximate number of pneumococci in the quantum of suspension employed	54	18	6	2	$\frac{2}{3}$		
No. 106,603 — Before administration	X	X	O	O	O	Kills 6 pneumococci	0·27
No. 106,603 — Three hours after administration	O	O	O	O	O	Kills 54 pneumococci or more	0·36
No. 79,519 — Before administration	X	O	X	X	X	Kills 0 pneumococci	0·22
No. 79,519 — Three hours after administration	O	O	O	O	O	Kills 54 pneumococci or more	0·38
No. 106,717 — Before administration	X	X	X	X	O	Kills 0 pneumococci	0·30
No. 106,717 — Three hours after administration	O	O	O	O	O	Kills 54 pneumococci or more	0·42

(*b*) The same three men receive on 1st February, 1912, 1 grm. of Morgenroth's drug by the mouth, and blood specimens are taken three hours after.

Dilutions of the pneumococcus suspension which were employed	$\frac{1}{5}$	$\frac{1}{15}$	$\frac{1}{45}$	$\frac{1}{135}$	$\frac{1}{405}$	Bactericidal effect of 5 c.mm. of serum	Opsonic index
Approximate number of pneumococci in the quantum of suspension employed	54	18	6	2	$\frac{2}{3}$		
No. 106,603	X	X	X	X	X	Kills 0 pneumococci	0·34
No. 79,519	O	O	O	O	O	Kills 54 pneumococci or more	0·24
No. 106,717	X	X	O	O	O	Kills 6 pneumococci	0·37

Experiment No. II.—Normal natives Nos. 1 and 2 receive 1 grm. and Nos. 3 and 4 0·5 grm. of Morgenroth's drug by the mouth. Comparative determinations of the bactericidal power of their sera before, and three hours after the administration of the drug show, in the case of No. 1 : before administration, no inhibiting or bactericidal effect ; after administration, a bactericidal power of over 35 pneumococci per 5 c.mm. Similar estimations in the case of Nos. 2, 3 and 4 show : before administration, no inhibiting or bactericidal effect ; after administration, no bactericidal effect but inhibition of growth in all dilutions. Opsonic measurements carried out on these bloods gave the following results :

No.	Pneumococcus (*phagocytic counts*)		Staphylococcus (*phagocytic counts*)	
	Before administration	After administration	Before administration	After administration
1	2·12	1·94	1·02	1·29
2	3·34	2·94	1·51	1·78
3	2·0	2·14	1·9	1·93
4	1·76	2·36	1·59	1·24
Average	2·3	2·3	1·5	1·5

There was ground, both in this experiment and the next, for suspecting that one, or perhaps two, of the natives may have found opportunity to vomit the drug.

Experiment No. III.—Three normal natives receive 0·5 grm. of Morgenroth's drug by the mouth. Comparative determinations of the bactericidal power of their sera before and three hours after the administration of the drug show, in the case of Nos. 1, 2 and 3 : no bactericidal effect before administration ; in the case of Nos. 1 and 3 : no bactericidal effect after administration ; in the case of No. 2 : after administration, a bactericidal effect of 32 pneumococci per 5 c.mm.

Experiment No. IV.—A native with pneumonia receives 0·5 grm. of Morgenroth's drug hypodermically, and 0·5 grm. at the same time by the mouth, and samples of his blood are taken for bactericidal measurement before and three hours after the administration of the drug.

							Bactericidal effect of 5 c.mm. of serum
Dilutions of the pneumococcus suspension which were employed		$\frac{1}{5}$	$\frac{1}{25}$	$\frac{1}{125}$	$\frac{1}{625}$	$\frac{1}{3125}$	
Approximate number of pneumococci in the quantum of suspension employed		3,125	625	175	25	5 or more	—
Patient 60,847	Before administration	X	X	X	X	X	Kills 3,125 pneumococci or more
	After administration	O	O	O	X	O	

Experiment No. V.—Three natives with pneumonia receive Morgenroth's drug : No. 1 a dose of 1 grm. by the mouth ; No. 2 a dose of 1 grm. hypodermically, and No. 3 a dose of 0·5 grm. hypodermically.

Dilutions of the pneumococcus suspension which were employed		$\frac{1}{25}$	$\frac{1}{175}$	$\frac{1}{625}$	$\frac{1}{3125}$	Bactericidal effect exerted after administration of the drug
Patient 80,199	Before administration - -	X	X	X	X	Serum kills more than 125 times as many pneumococci as it did before
	Five hours after administration	O	O	O	O	
Patient 60,831	Before administration - -	X	X	X	O	Serum kills more than 25 times as many pneumococci as it did before
	Five hours after administration	O	O	O	O	
Patient 79,519	Before administration - -	X	X	X	O	Serum kills more than 5 times as many pneumococci as it did before
	Five hours after administration	X	O	O	O	

The experiments which have been set forth above make it clear that in man as in mice—but we found that the same did not hold in rabbits—the blood is rendered bactericidal to the pneumococcus by the administration of aethylhydrocupreinhydrochlorate. It is further brought out in Experiment No. 1 and more clearly in Experiment No. 2 that the *opsonic power* of the serum is uninfluenced by the exhibition of the drug.

The reason why, in connexion with the present inquiry, we are not at liberty to experiment upon and observe sufficient cases to build up trustworthy comparative statistics ; and the reason why we ourselves here desisted from bringing into application the statistical method, may be very briefly explained.

It very quickly emerged, as soon as experiments were undertaken upon man, that aethylhydrocupreinhydrochlorate does not come up to the ideal of being poisonous for the pneumococcus, and non-poisonous for the nobler tissues of the patient. The drug is in the human organism—and this holds true also in some measure of its congener : quinine—*ophthalmo-tropic*.

In the experience of Professor A. Fraenkel,[1] which, as soon as it became available, was cabled to me by Professor Morgenroth, three cases of amblyopia—all of which recovered—occurred among twenty-one pneumonic cases treated with the drug. The dose here appears to have ranged between 1 and 2 grm. daily.

In the meantime, in our experience in Johannesburg two cases,[2] one of which went on to amaurosis, occurred in eight pneumonic patients treated. The doses of aethylhydrocupreinhydrochlorate ranged here between 0·5 and 2 grm. daily. They

[1] *Berliner klin. Wochenschr.*, 1912, No. 14.

[2] In view of the fact that at this particular juncture two other cases of amblyopia occurred in the W.N.L.A. Hospital in which we were at work in patients not treated with optochin, there is just a possibility that those cases which occurred in our patient were not produced by the optochin.

were administered in some cases by the mouth, in others subcutaneously, and in others, again, both by the mouth and subcutaneously.

In a third series of nine pneumonic cases which were treated by Dr. John Parkinson at the London Hospital there were three cases in which the pupils became very widely dilated under the influence of the drug. The doses of aethylhydrocupreinhydrochlorate here ranged between 0·5 grm. or less to 1·5 grm. daily.

It will be appreciated that it was, in view of the two former experiences, out of question to apply the treatment to any large number of patients unless the contingent advantage from the bactericidal action of the drug proved to be such as altogether to outweigh this element of risk.

This, by the consent of all those who have worked with the drug, is not the case, and the very poor bactericidal results obtained on the repetition of the optochin in the case of the first and third patients in Experiment I[1]*b* look like a forewarning of this.

APPENDIX III

THE EFFECT OF SANOCRYSIN ON *B. TUBERCULOSIS*

By R. M. FRY, M.R.C.S., L.R.C.P.[2]

(*From the Laboratories of the Inoculation Department at St. Mary's Hospital, London*)

Möllgaard (1924), as a result of his experiments, states that sanocrysin in a concentration of 1 in 100,000 not only completely inhibited the growth of *B. tuberculosis* in cultures, but also prevented it from growing subsequently when removed from the influence of the drug. The technique used by him was as follows: Equal portions of the film from a glycerine broth culture of the bacillus were transferred to a series of tubes of glycerine broth to which varying concentrations of sanocrysin had been added. After six weeks' incubation there was no evidence of growth, and the films were then removed, washed, and transferred to glycerine broth containing no sanocrysin. After a further four weeks' incubation in this medium the films failed to show any growth.

It seemed advisable to put the microbes under more favourable conditions for growth, apart from the presence of the sanocrysin, and advantage was taken of the fact, demonstrated by Sir Almroth Wright[3] that human blood orp lasma forms an excellent medium for cultivation of the tubercle bacillus. This has the further advantage that, using the special technique described by him, growth may be recognised under the microscope after only a few days' incubation.

The technique used was as follows: Tubercle bacilli from a culture on a solid medium were ground up in a Hayden's mortar to break up clumps. They were then suspended in normal salt solution, and centrifuged rapidly for a few minutes to spin down any remaining clumps, and the supernatant emulsion was pipetted off and diluted to a suitable opacity, which had been roughly determined by previous

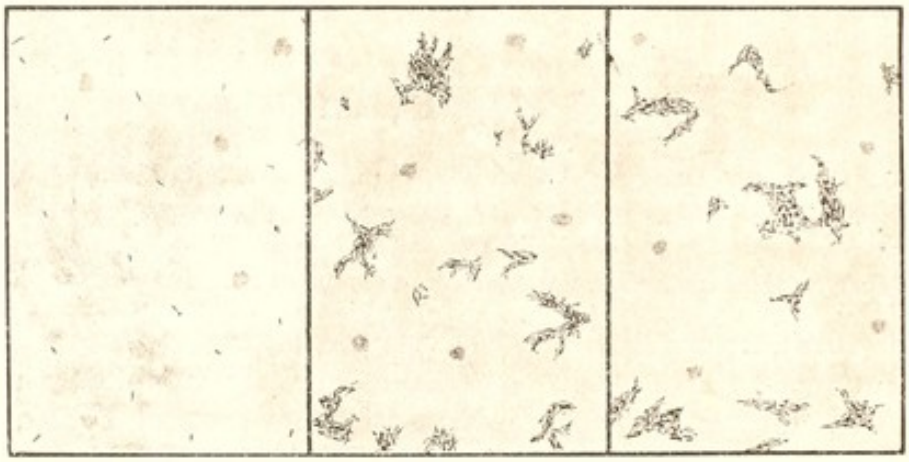
[1] *Vide* p. 222.

[2] Reprinted from the *British Journal of Experimental Pathology*, Vol. VII, 1926.

[3] *Vide supra* p. 121 *et seq.*

experiments. The emulsion was then examined unstained under the microscope for freedom from clumps. This emulsion, consisting of single bacilli only, was inoculated into unclotted blood or plasma, in a proportion of 1 to 20, the blood or plasma having been previously mixed with one-tenth of its volume of a solution of sanocrysin in normal salt solution. Small quantities of the mixture were then taken up into the stems of capillary pipettes, which were sealed and placed in the incubator.

Specimens were examined at intervals varying from three days to several weeks by cutting off the ends of the tubes and blowing out the cylindrical clots into water. The clot was washed to remove serum, and, in the case of blood-clots, haemoglobin, and was then laid out on a clean slide, dried, and stained. Examination with a microscope for clumps of bacilli then revealed the presence or absence of growth.



Unincubated control. Salt. Sanocrysin, 1/2500.

9 days' incubation.

FIG. 1.

Growth of *B. tuberculosis* in human blood.

One or more specimens in each experiment were examined soon after clotting had taken place, as a further control to confirm the absence of clumps from the original emulsion.

A large number of experiments of this sort were performed, with concentrations of sanocrysin varying from 1 in 50 to 1 in 100,000 in the blood. Normal human and ox bloods were used, with three different strains of *B. tuberculosis*, which were designated 'Lyons', 'Cockhorn', and '100', 'Lyons' and '100' being human strains, and 'Cockhorn' a bovine strain. The results obtained were fully constant. Concentrations of sanocrysin of 1 in 10,000, 1 in 5000 and 1 in 2500 had no inhibiting effect on the growth of the bacilli—the colonies were as numerous and as large as in the controls (Fig. 1). In higher concentrations than this, up to 1 in 250, there was sometimes no inhibition of growth, but on other occasions the colonies were definitely less numerous and smaller than in the controls. In every case, however, a large number of the bacilli had grown out into colonies. With concentrations as

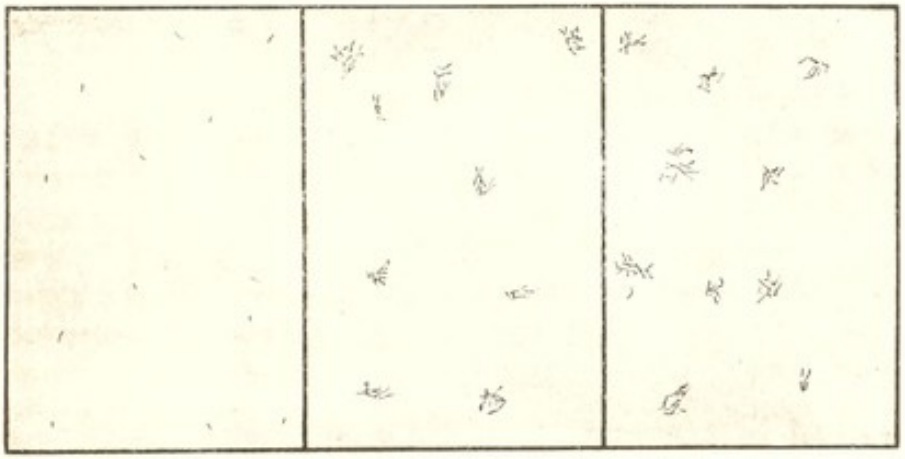
high as 1 in 100 there was not usually any growth of the bacilli, although in one case where a concentration of 1 in 50 was used, there were some well-developed colonies to be seen after 10 days' incubation.

Experiments were also undertaken to ascertain if there was, after a therapeutic dose of sanocrysin, any increased power of the blood to kill or inhibit the growth of the tubercle bacillus. These experiments were performed with blood from tuberculous patients who were undergoing treatment with sanocrysin, and also on rabbits. The technique adopted was similar to that in the previous experiments, except that the sanocrysin had been injected intravenously, instead of being mixed with the blood *in vitro*.

(a) *Experiments on Patients*

These experiments on man were performed on three different occasions, the blood being drawn immediately before, and again about ten minutes after the injection of one gramme of sanocrysin. In one experiment blood was also taken 48 hours after the injection. Normal human plasma was used as a control.

In each case specimens examined after 5 days or more showed many colonies in all the clots, there being no appreciable difference between the amount of growth in normal plasma and in the tubercular plasma before and after the injection (Fig. 2).



Unincubated control. Before. After.

7 days' incubation.

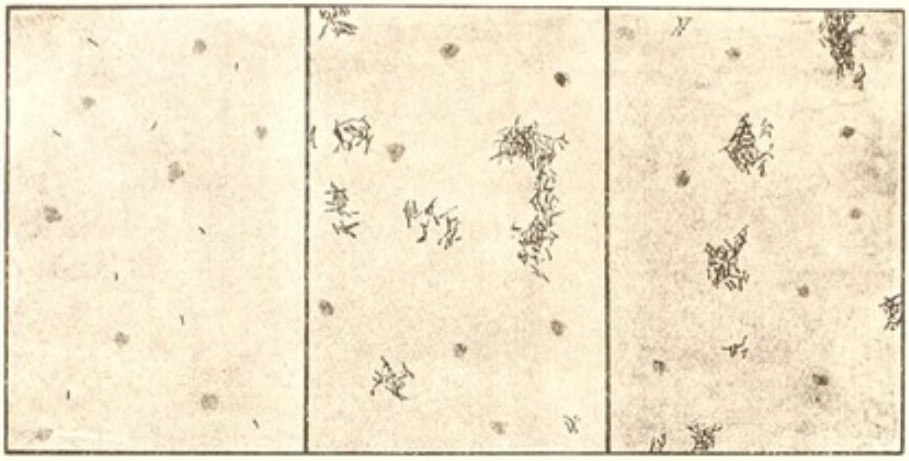
FIG. 2.

Growth of *B. tuberculosis* in plasma of a tuberculous patient before and after injection of 1 gm. sanocrysin.

(b) *Experiments on Rabbits*

In these experiments a rabbit was injected intravenously with a quantity of sanocrysin equivalent, weight for weight, to a dose of 3 gm. in a human being. Blood was drawn off before and after the injection, and specimens prepared in the manner described. After 21 days' incubation there were numerous large colonies

in specimens of both bloods, and no diminution of growth due to the sanocrysin could be detected (Fig. 3).



Unincubated control. Before. After.

21 days' incubation.

FIG. 3.

Growth of *B. tuberculosis* in the blood of a rabbit before and after injection of sanocrysin.

Summary and Conclusions.

(*a*) In normal human or ox blood or plasma mixed with sanocrysin *in vitro*, concentrations of sanocrysin up to 1 in 2500 had no effect on the growth of the tubercle bacillus. Above this concentration the results are rather variable, but in some cases good growth was obtained in concentrations up to 1 in 250, and in one case as high as 1 in 50.

(*b*) The bacillus grows as readily in the plasma of a tuberculous patient taken ten minutes or two days after a dose of 1 gm. of sanocrysin as in the plasma drawn before the dose, or in normal human plasma.

(*c*) The bacillus grows as readily in the plasma of a rabbit after a dose of sanocrysin equivalent to 3 gm. in a human being as in the plasma drawn before the injection.

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APPENDIX IV

ON INDUCTION IN GENERAL: AND IN PARTICULAR ON THOSE PROCEDURES (TRIAL-AND-ERROR AND CRUCIAL AND STATISTICAL EXPERIMENTATION) WHICH HAVE BEEN EMPLOYED IN THE EXPLORATION OF CAUSAL CONNEXION

(Based on a Lecture by the Author delivered in the Institute of Pathology and Research, St. Mary's Hospital, on 27th April, 1937)

It may be well, before concentrating upon the special subject matter of this lecture, to do a little mapping out of the ground.

The operations of reasoning have, ever since Aristotle, been distinguished into those of *Deduction* and those of *Induction—Deductive Reasoning* being defined as reasoning which starts from general propositions and draws from these either particular or at any rate less generalised conclusions; and *Inductive Reasoning* being defined by saying that 'it starts with particular propositions and ascends to more general conclusions'; or that 'it starts with the known and arrives at the unknown'.

No special study of logic is required to enable one to carry out, or sit as a competent arbiter upon, operations of deductive reasoning. For deductive logic is based on two absolutely common-sense principles—(*a*) That the premisses must be established as true; and (*b*) That if this or that is, in properly attested premisses, predicated of an entire class, it holds inexorably true of every member of that class; and if anything is predicated only of some of the class, this does not necessarily hold true of all or any specific member or members of the class under consideration.

No such simple principles hold of Induction. For there are, to begin with, a great number of different kinds of inductive operations. Further, many of these operations are carried out in the subconscious mind. And finally, quite irrespective of this, no inductions can be regarded as absolutely trustworthy—except, of course, those (not all logicians would call these Inductions) which are arrived at by exhaustive enumeration.

Occupying ourselves now with the different meanings of the term Induction, we may distinguish, *first*, that which Aristotle called *epagoge* (*epagoge* being the Greek for induction); *secondly*, Whewell's wider notion of induction; *thirdly*, that contended for by John Stewart Mill; and *lastly*, that which I propose to suggest to you in this lecture.

(1) Aristotle had in view when he introduced the term *epagoge* a ratiocinative operation in which that which holds of certain members of a Natural Class is inferred to hold of all members of the class.

(2) Whewell calls everything Induction which starts with particulars and leads up to 'a general concept which did not exist in the facts observed'.

(3) Mill uses the word 'induction' in quite a different sense to Aristotle and

Whewell. ' Induction ' means to him : ' ratiocinative operations which link up phenomena as cause and effect '.

(4) I here comprise under ' Induction ':—

(*a*) The operations which Aristotle had in view when he employed the word *epagoge*.

(*b*) Operations of Diagnosis—that is taxological operations which refer objects or ' trains of phenomena ' to their proper Natural Classes or Categories ; also operations in which we identify an object of thought by comparing it with a model, or a standard description, or by finding out the name of the person or thing.

(*c*) Operations of Sampling and Simple Enumeration.

(*d*) Operations of Counter-enumeration.

(*e*) The building up, by a procedure which I have called *Contesseration*, of a ' Conceptual Image ' out of a number of separate mental pictures.

(I think of these separate mental pictures as *tesserae* of a jig-saw puzzle—*tesserae* signifying : tiles or other tablets which when assembled make up a mosaic or tessellated pavement.)

(*f*) The linking up (I put this last because I shall consider it last) of phenomena as cause and effect.

Induction can, in accordance with the above—I slightly alter the order and the titles—be distinguished into :—

(1) Taxological (i.e., Eicono-ecdytic) Induction.
(2) 'Eido- (i.e., class) or 'Idio- (i.e., specifically individual) Diagnosis.
(3) Sampling and simple Enumerative Statistics.
(4) Counter-enumerational (and in particular Retrospective Statistical) Evaluation.
(5) Contesserative Induction.
(6) Experimental (Aetiological) Induction.

Let me now (as I have already indicated) first pass in review the first five kinds of Induction, reserving for more particular discussion Experimental Induction.

(1) **Taxological (i.e., Eicono-ecdytic) Induction.**

A little reflection will show that classificatory operations fall quite naturally under the definition of Inductional Operations. For when we classify, we start with *concrete* and *particular ideas*, and we arrive at *ideas which are more and more general and abstract* :—*Species*, *Genera*, *Orders*, and *Classes*.

The procedure by which these are arrived at is, of course, *Abstraction*—this being an operation of dismemberment which resolves *concrete ideas* into two sorts of cleavage products.

One set of these consists of ' *eiconic* ' (pictorial) *attributions*. And the other component is the ' *sub-eiconic* ' *residual product* which remains over when the idea has been divested of its attributions.

Let us take, as an example of the dismemberment of a *concrete idea* by *Abstraction*, the application of that procedure to our mental picture of a particular horse.

We begin our ' ecdysis ' (that is, our divesting operation) by taking away from

our mental picture of a horse its colour, its height, its age, and its sex. These attributes are, by common consent, called *Abstractions*, because they are 'abstracted' or 'carried away'. And all that remains of our original mental picture is a 'conceptual image' or notion of 'a horse in general'—this being an animal which possesses neither colour, nor size, nor age, nor sex. This 'concreto-abstract' or subabstract idea is again, to the great confusion of the terminology, called an '*Abstraction*'.

I would suggest that these contrasted kinds of ideas which are furnished by 'eicono-ecdysis' should have different names. And what I propose is that we should, when we refer to the attributes which have been dissevered from the concrete idea (I mean such attributes as colour, size, age, and sex), call these as we already do *abstractions*; and that the 'sub-eiconic ideas' which remain over after '*eicono-ecdysis*' should (this would be merely transposing 'abs-traction' into Greek) be renamed '*ap-agogues*'.

A further point which should be grasped is that '*ecdysis*' furnishes not only these two different kinds of ideas, but that it furnishes also two different kinds of classificatory schedules—those which go by the name of '*Artificial*', and those which go by the name of '*Natural Classes*'.

'*Artificial Classes*', as they are called, consist of '*abstractions*'; and the members of these classes (red and green objects might here serve as examples) have in *common only the one attribute* they are called after.

'*Natural Classes*' consist, on the contrary, of '*apagogues*', and the members of these Natural Classes ('horses in general' and 'birds in general' would serve as examples) have, in virtue of the fact that they are phylogenetically connected, *innumerable attributes in common*.

Under *classificatory operations* would be included not only the original defining operations which have resulted in the fashioning of 'Natural Classes' but also those accessory operations which suggested to Aristotle the name of *epagoge*. He had in mind when he employed that term operations which added new members to a class, or new items to its signification. An example of the former kind of operation would be the addition of *cetacea* to the class of the *mammalia*; and an example of the latter kind of operation would be the adding of new signs or symptoms to those which compose the definition of a disease.

The problem as to how a new attribution can be legitimately incorporated into the definition of a species appears never to have engaged the serious attention of Aristotle. He would seem to have assumed that such additions can be lawfully made only when an exhaustive examination of the members of a class has shown that the attribute in question is possessed by all.

Additions to the membership or to the definition of a biological class can, however, also be made by 'arguments of analogy'—'arguments from sound analogy' being distinguished from 'arguments from false analogy' by the fact that the former rest on co-membership of a 'natural', and the latter on co-membership of an 'artificial' class.

But even 'arguments from sound analogy' furnish, of course, only probable inferences—inferences which can never be absolutely relied upon.

(2) Diagnostic Induction.

The use of the term '*diagnosis*' is practically confined to Medicine. But '*diagnosis*' is, as a moment's reflection will show, a staple operation in every science which (and this is the first thing any science undertakes) has provided itself with a scheme of classification. '*Diagnosis*' and *Classification* are (since *diagnosis* signifies *referring items to their places in a Scheme of Classification*) indissociable procedures.

Mill, in treating of *Induction*, discusses (without discerning that he is dealing with '*Diagnosis*') two very typical operations of diagnostic induction. The one is the ratiocinative operation by which Kepler arrived at the generalisation that the planets circled round the sun in ellipses; and the other is the operation by which a circumnavigator comes to realise that the land he has sailed round is an island.

Mill refuses to regard either of these as 'inductive inferences' because 'no new general factual conclusion has here been inferred from the data which have come under observation'. And he feels confident that he has successfully made good his thesis when he contends that: had the planets left a visible trail behind them, no one would have thought of crediting Kepler with being the author of an 'induction'.

And Mill would no doubt, by parity of reasoning, have contended with regard to the recognition of a parcel of land or a country or continent as an island, that if it had been recognised as sea-girt by merely looking down upon it from a mountain-top or a travelling aeroplane, no one would ever have dreamed of calling that an induction.

But, in reality, inferences so arrived at would (except only in the respect that they would be much easier to make) be just as much 'diagnostic inductions' as the inferences drawn by Kepler, and as those made by the geographers, who concluded from the reports of voyagers who had each partially circumnavigated Australia that that continent was an island.

And as for Mill's protestation that nothing fundamentally new resulted when Kepler recognised that the planets moved in ellipses, or when a continent was pronounced to be an island, the whole stuffing is taken out of that contention when we realize that something which is far more illuminating than any isolated new fact is here in both cases arrived at.

That something consists in the assignment of a multitude of previously unrelated data to their places in a scheme of classification. And a man who achieves that has, to use the language of the Bible, done something much greater than 'he that taketh a City'.

That, in his narrower sphere, comes home very vividly to the medical man. It is not an uncommon experience for a medical man to feel, after taking stock of all the symptoms of a case, quite at a loss as to what the patient is suffering from. And then, often quite suddenly—it is a German saying—'a light may go up in his mind'; and with that the 'diagnosis'—in other words, the schedule to which the disease should be assigned—becomes clear.

The Author has often had this experience. And he has particularly in mind

an obscure case in which he suddenly, helped by the suggestion of a colleague, realised that he was dealing with a case of human glanders.

But it will, perhaps, be well to recognise about medical diagnoses that many of these are, because of the great complexity of the factors involved, very often uncertain. They can, at any rate, never (except perhaps where a diagnosis is established by the success of 'specific' treatment) be anything like as assured as the diagnostic inductions of astronomers and circumnavigators.

Identifying and Nuncupative Induction (or *Idio-diagnosis*).

There is also another kind of Statistical Induction. Let us call it 'Identifying Induction' or Idio-diagnosis.

An identifying induction is made when isolated finger-prints 'left in a house where a murder has been committed' are shown to resemble in all respects those of a complete set of finger-prints preserved in the registers of the police. And this is of course only one of the many methods of identification which are employed in criminal investigation.

Chemical analysis is another kind of '*Identifying Induction*'. This is of course a routine operation in every chemical laboratory. And every chemical student is, when he goes up for his examinations, given samples of chemical substances and told to analyse them, and say what they are.

Nuncupative Induction is practically the same procedure. For when a man inquires the name of a bird, or that of a plant or a mineral, what he is really keen to do is to read up its description in his text-books; or, if illustrated text-books are available, to look up its picture; and it is as a first step to doing this that he wants to know its name. And let us note that, to help in these identifications, the inquirer is often furnished with what is by botanists described as a *diagnostic key*, i.e., a key which will supply the names he is hunting for.

(3) Sampling and Simple Enumerational Induction.

There are three different kinds of this Induction.

(*A*) *Ordinary Sampling Induction.*

This would be a question of taking a random series of samples of any commodity, mixing them together, and then inferring that what holds of any portion of that mixture holds of the whole.

An example of this sort of induction would be the taking at random of a number of specimens of seed, or of a number of specimens of ore, and then testing the germinating power of a specimen of the mixed seed, or chemically analysing a specimen of the mixed ore.

(*B*) *Simple Enumerational Statistics.*

This consists in 'taking a Census', i.e., in collecting data from every household and arriving by the compilation of these at the conclusion that the total population of a country consists of so many persons; and that there are in the population so many men and so many women, and so many persons of different ages.

This was the original meaning of 'statistics'. And the method finds useful application in the drawing up of vital statistics; further in the enumeration of red blood corpuscles and leucocytes; and again in the compiling of cricket scores for batting averages; and above all in the counting up of one's money when it consists of all sorts of different coins and notes and cheques and a bank-balance.

But it has recently come to be believed that it is the office of the statistician to compare different sets of statistics with a view to determining whether there is any correlation between these, and whether or not the correlation in question is a causal correlation.

(4) **Counter-enumerational (and in particular Retrospective Statistical) Evaluation.**

We are here dealing with statistics which have been collected and brought together only because it occurred to someone, reasonably or unreasonably, that the facts and phenomena with which the statistics would deal might be in some way or other correlated.

Examples of the accoupled statistics here in question would be the following :—

(*a*) Statistics of the number of stars visible to the naked eye linked up with statistics of the number of stars visible with telescopes which magnify, and correspondingly approximate, 10, 20 and 100 times.

(*b*) Statistics of the total number and the different kinds of bees in a hive at a particular moment linked up with statistics of the same hive made at later periods.

(*c*) Records of changes in the weather and the coincident lunar phases.

(*d*) Statistics of the death rate of such and such a disease before and after the general adoption of a particular therapeutic agent. Let us call this *Retrospective Statistical Evaluation.*

(*e*) Statistics of the number of books published in a country in different years, and the birth-rates in those same years.

Let me now take the different kinds of accoupled statistics labelled above (*a*), (*b*), (*c*), (*d*) and (*e*), and see what inferences can be drawn from them, and let us then, when we find that there is a correlation between the figures, inquire what sort of correlation it is. It will be convenient to deal with the statistics referred to above in the order of their alphabetical labels.

(*a*) Examination of the stellar statistics here in question reveals, as we learn from Jeans, that the density of the star population diminishes as we prospect farther and farther out into space. And this, as he points out, is a significant correlation inasmuch as it shows that the universe of stars does not extend indefinitely.

(*b*) The apiary statistics tell us, as everybody understands, important things about the natural history of communities of bees.

(*c*) The accoupled lunar and meteorological statistics bring out the fact that there is no sort of correlation between the alterations in the weather and the phases of the moon.

(*d*) These statistics, when they show that there is a smaller death-rate in patients treated with a particular drug, bear witness to their being here a causal correlation.

(*e*) If statistical data showed that the number of books brought out in

different years had varied conformably with the birth-rate in those years, this would clearly be only a *freak correlation*.

The confident conclusions arrived at both in this last and in the preceding case bring up, as the reader will see, in an acute and insistent manner the question as to what it is that distinguishes between a ' freak ' or purely coincidental, and a causal correlation.

The first thing that has here to be regarded is that what we have in view when we speak of a cause is always an *operative agent* ; and of course it follows that neither location in space (for this is a *status*) nor location in time (for this also is a *status*) admit of being called causes.

There is here some confusion of thought ; for we sometimes speak of the *effects* of distance, and more often of the *effects* of time. But that is a mere misapplication of words. For when we talk of time producing certain *effects* all we really mean is that the passage of time brings out more and more clearly the results of all the various causes which are at work around us. But the last and most important point to appreciate in connexion with the discrimination of a purely coincidental and a causal statistical correlation, is that it is never anything in the statistics themselves which distinguishes mere coincidence from cause. It is always something extrinsic that does ; and when we say that statistics show that two phenomena are causally related, we mean only that they confirm an inference which was established by crucial experiments, or was suggested by *experientia vaga*, backed up in each case by ' contesserative reasoning ' which tells us that a causal correlation is in one case *likely* and in another *inconceivable*.

(5) **Contesserative Induction.**

In Contesserative Induction we arrive, just as we do in ' Classification ', ' Diagnosis ', and ' Sampling ', at a ' conceptual image ' which outweighs in importance the whole wilderness of loose facts from which it is derived.

It will be appreciated that the essential difference between ' *Contesserative* ' and ' *Diagnostic Induction* ' is that we have in the latter case at disposal a ready-made scheme of classification into which the object or ' train of events ' before us can be fitted. We have nothing like that, either in ' *Contesserative Induction* ' or in the jig-saw puzzle which is its physical prototype.

In ' Contesserative Induction ' we, in order to arrive at an *understanding* of a great multitude of uncomprehended data, try to fit them together, as we do in a jig-saw puzzle, *tessera* into *tessera*—not knowing, in advance, what sort of conceptual picture will emerge. Such conceptual pictures are called, when they are in the making, *hypotheses* and *theories*.

But there is this notable difference between the construction of a ' hypothesis ' and the building up of a jig-saw puzzle. The latter can (because every step is physically verified) hardly go wrong. Hypotheses, on the contrary, for the reason that we have here no such physical check upon them as is furnished by the dovetailing and mortising of the tesserae of the jig-saw puzzle, are eminently subject to error. And even ' *theories* ' (and we call a hypothesis a ' theory ' when it seems to provide a staunch ' framework of contesseration ') are often illusory. But though the very

best theories are fallible we require, in order to see things to scale, to bethink ourselves of this : that to understand is to visualise by the help of a theory, and that it is impossible to dignify loose facts—facts that are not held together by a theory —as either *knowledge* or *science*. They are merely *information*.

Familiar examples of ' contesserative inductions ' are the following :—

(*a*) The ' hypothetical reconstruction ' by archæologists of buildings of which ' not one stone has been left upon the other '.

(*b*) The ' hypothetical reconstruction ' by palæontologists of a ' prediluvial animal ' out of a single fossil bone.

(*c*) The filling in by ' conjecture ' (i.e. by contesseration from the context) of lacunae in ancient manuscripts ; and the rectification by that same procedure of clerical errors which have crept into texts.

(*d*) The establishment of a prisoner's guilt by circumstantial evidence.

While all these contesserative inductions are recognised as having an element of the speculative, there are some which seem to admit of no more doubt than do the final steps in the building of a jig-saw puzzle. I am thinking here of such contesserative inductions as were made by Mendeléeff in his so-called Periodical Table of Chemical Elements, and of the contesserative inductions which led up to the discovery of the planet Neptune.

(6) Experimental (i.e. Aetiological) Induction.

There are three methods by which we can put questions to Nature ; and the point about experimentation and putting questions to Nature is that unless we directly question her by experimentation she is invariably discretely silent about causal correlation.

There are as said three methods by which we can put interrogations to Nature. The *first* is *Random* or *Trial-and-Error Experimentation* ; the *second* is *Crucial Experimentation* ; and the *third* is the method of *Statistical Experimentation*.

There is also a fourth method of Aetiological Induction (it is not strictly speaking an experimental method) by which we evaluate the recent by the past. This is the method which was called above *Retrospective Statistical Evaluation*.

(*A*) *Random* or *Trial-and-Error Experimentation*.

The terms ' Random ' and ' Trial-and-Error ' are perhaps, as applied to experimentation, not self-explanatory.

Let me therefore explain that a Random or Trial-and-Error Experiment is one which is undertaken without any knowledge about what we are dealing with, and without any foresight of the result. It is in fact such an experiment as would be performed if a man came into a laboratory and poured, without the least knowledge of what would happen, some of the contents of the bottle which came first into his hand into the bottle which stood next to it.

Furthermore, Random or Trial-and-Error Experimentation is experimentation where there is no reasonable prospect of anything useful resulting. The chances against anything useful resulting from it are as a million to one.

None the less this method is in reality the mother of all arts and crafts, and every rule of thumb operation employed in the mechanical trades, in art, or in

medicine, has been arrived at by this process of random or trial-and-error experimentation.

We may think here, in connexion with the crafts, of cookery, of the art of the potter, and that of the blacksmith, and in the realm of art, we may think of poetry, prose-writing, painting and sculpture. And we may in connexion with medicine reflect how few of the procedures employed derive from anything else but trial and error and chance observations. Medicine advances by random experiments made upon patients with therapeutic agents recommended for use by trade advertisements and the experiences of other doctors. That is also the method by which every cook improves her cooking.

While, as will be seen from this, the method of trial and error has done humanity yeoman service, it is important to realise the following :—*First*, that it has provided men (I ought perhaps to say women who do the work of the house) with many silly ' rule of thumb ' procedures—procedures like putting a poker upright against the bars to make the fire burn up ; and pulling down a window blind to prevent the sun putting out the fire. And in the same way, the method of Trial and Error has added to the repertory of medicine many procedures which have nothing to recommend them.

Another important point to realise is that the technique which derives from ' rule of thumb ' procedures is nearly always from the very nature of the case capable of improvement. *The best* is not likely to be hit upon by chance.

Among the most glaring defects of the method of ' Trial and Error ' is that the experimenter has always to perform what I have called a *Saltus Empiricus*. In other words, he makes a flying jump over all the intermediary events, and concentrates his attention upon the overt effects which may eventually result from what he has done. He in point of fact casts his bread upon the waters, hoping to find it after many days.

(*B*) *Crucial Experimentation.*

It does not seem (though the idea is no doubt present in the germ in that ill-drafted prescription that ' a satisfactory experiment must contain only a single variable ') ever to have come home, even to men of science, that *Claustration* (that is operating in a favourable environment) is of the very essence of crucial experimentation.

Nor has, so far as I know, attention been directed to the important fact that there are two sorts of experiments: those which are carried out in a *Campus Apertus*—that is, in the open field of the world ; and those carried out in an artificial environment—that is, in a *Campus Clausus* or *Circumsaeptus*. All ' Trial and Error ' and ' Statistical Experiments ' are, as the reader will realise, carried out in a *Campus Apertus*. On the other hand, ' Crucial Experiments ' are not crucial experiments unless they are carried out either in a *Campus Clausus* or in a *Campus Apertus* which is for the moment doing duty as a *Campus Clausus*.

Let me explain in more detail what I mean by *Claustration*.

I mean by it : Taking steps to secure that no physical ' *Erg* ', other than the

one we propose to investigate, shall intrude into our experiment ; and that every '*Advect*' which might interfere with the '*eventus*' shall be excluded.

I would propose to denote by the term '*Erg*'—'any physical agent which might come into action'; and to denote by the term '*Advect*'—'any passive agent which might, by quenching or buffering, nullify the action of the erg whose action we are investigating'.

The decisive influence of *Claustration* comes out when we consider the following :

(*a*) Electrical experiments come off only when carried out in a *Campus Clausus* established by 'insulating' our apparatus and any connecting wires, and by that means cutting off contact between these and the immense electrical reservoir of the earth.

(*b*) Conformable chemical experiments (that is, regular results) can be obtained only in a *Campus Clausus*—in other words, only when we have eliminated at least most extraneous 'ergs' and 'advects' by operating in test-tubes made of insoluble or quasi-insoluble glass ; and by taking care to employ only reagents which are 'chemically pure'.

(*c*) Many physiological experiments cannot be carried out *in vivo*. And such of these experiments as admit of being carried out both *in vivo* and *in vitro* give always more regular results when carried out in *in vitro*.

The reason for that is, of course, that we, when we work *in vitro*, shut out a great number of interfering 'ergs' and 'advects'—in other words, we have gone some way towards the achievement of a *Campus Clausus*.

At the same time the mere carrying out of an experiment outside the body very rarely provides sufficient claustration. It is, for example, impossible to determine the respective anti-bacterial potencies of the blood fluids and the leucocytes without first isolating the one from the other.

The lesson which is to be learnt from facts of this kind is that *Claustration* is always a question of less or more. And an experiment may miscarry both through '*Hypo-*' and through '*Hyper-Claustration*'.

A notable example of the fallacies which derive from '*Hypo-Claustration*' is furnished by the older experiments on nutrition, which seemed to indicate that the supply of food to the organism was exactly on a par with the supply of fuel to an engine ; and that the only point which called for consideration in dietary was the number of calories supplied.

It was not, at the date when the experiments which conducted to these conclusions were undertaken, appreciated that there were being administered in the foodstuffs, along with the calories upon which the attention of the experimenters was focussed, also small quantities of those very effective 'ergs' which are now called '*Vitamins*'. And the experimenter was, because these vitamins had not been excluded, badly misled.

'*Hyper-Claustration*' requires to be specially guarded against in experimenting on antiseptics.

It was until recently common practice to judge of the value of antiseptics by making progressive dilutions of these in water, and seeing how far dilution can be

carried before the bactericidal power of the particular antiseptic is extinguished.

This procedure led to the attribution of fabulous bactericidal efficiency to antiseptics and to universal recourse to these agents in the case of local and often general infections.

I need hardly here explain that the testing of antiseptics in progressive dilutions made with water, is in reality 'hyper-claustrated' experimentation. For there are here fallaciously omitted from the experiments those albuminous substances which operate as quenching and buffering advects whenever antiseptics are employed in local treatment or are administered internally.

Up to the present, we have been considering mainly 'crucial experiments' which come off only when carried out in a *locus circumsaeptus*—that is, in an artificially constructed environment.

We have now to regard the question (reference has already been made to this above) that there are a good many experiments which come off in the open field of the world; and some of these nearly as regularly as in a *locus circumsaeptus*.

In the experiments in question, the operative '*erg*' is not interfered with by any competing '*erg*' and is unneutralised by any interfering advect. This is due either to specially favourable dispositions of Nature or to careful foresight on the part of the inventor. And reflection will show that the world would be a very inconvenient place to live in if we had to establish a *locus clausus* before we could strike a match, or receive or send a telephone message, or turn on the wireless.

Finally, I may come back to a point referred to in the opening paragraph of this section, to wit—to the fact that there is a fundamental distinction between non-experimental and experimental induction.

What comes to my mind here is Hunter's well-known piece of advice (it is contained in a letter to Edward Jenner): "Why think? Why not do the experiment?"

Let us see what that suggests. It suggests of course with regard to *thinking* (i.e., Contesserative Induction) that it is always a laborious and very often an inconclusive procedure; and it suggests with regard to *experimentation*, that it does not entail any of the intellectual labour which thinking does, and that it is always conclusive.

But these suggestions—though they are belauded by all who have no personal acquaintance with experimentation—are in point of fact much more wrong than right. They are wrong, first in the respect that nearly every experiment which has been worth doing has taken a great deal of labour to devise; and, secondly, in the respect that instead of all experiments being decisive, only very exceptional ones are.

A further point which has to be considered in connexion with what are ordinarily called 'crucial experiments' is that while there are such experiments in which we make a *Saltus Empiricus*, there are others in which we proceed step by step by the method which I call a *Processus* (one might also call it a *Progressus*) *Scientificus Tutus*.

In a *Saltus Empiricus* we, as I have already explained, make a *saltus* over the intermediate results, and take cognisance only of the final overt result.

In the case of a *Processus Scientificus Tutus*, our aim is to bring into view, by recourse to laboratory technique, the intermediate effects produced by the 'erg'—in other words, to follow out link by link a chain of causation until we have achieved clear vision of the whole sequence of events.

A striking example of a *Processus Scientificus Tutus*, as applied in Physics, was the following out of the successive changes in the emanation of radium.

And we have the biological parallel to this in the immunisation curves which are mapped out when we investigate the effects produced by inoculation with a vaccine.

Other and specially memorable examples of a *Processus Scientificus Tutus* are the following :—

(*a*) The classical work of Ronald Ross which showed the stages in the conveyance of malaria by mosquitoes from infected to uninfected animals.

(*b*) The work done by Liston, and the Second Indian Plague Commission, which traced the spread of plague through an Indian village by evacuating the population and replacing it by a population of guinea-pigs ; and then making daily examination of the fleas on the guinea-pigs — following the plague infection as it spread from the guinea-pigs in one house to those in another.

(*c*) The work of Charles Martin when he traced all the intermediate steps between the feeding of the fleas on plague-infected animals, and the implantation of the infection by these fleas.

(*C*) *Statistical Experimentation.*

There are, it may perhaps be well to remind the reader, three main kinds of Statistical Induction. Two of these—the procedure which I have called *Simple Statistical Enumeration* and that which I have called *Statistical Counter-enumeration* —have already engaged our attention.

The consideration of the third procedure—that of *Statistical Experimentation* —was deferred so that it might be dealt with here.

Let me, before I occupy myself with the procedures employed, say something about terminology in general, and about the terminology of Statistical Experimentation in particular.

As a first general point I would have the reader note that when, in any field of learning, we find ourselves provided with all the terms we require, we may feel sure that we are dealing with a study which has attracted to its service generations of men of pre-eminent intellectual ability.

Full vocabularies of that kind are those of Grammar, Formal Logic, Mathematics, Roman and English Law ; also those of Chemistry, Botany, Anatomy, Physiology and Medicine.

When, on the other hand, we, like an aphasic patient, or the ordinary man endeavouring to express himself in a foreign tongue, have to struggle with a terminology which fails to provide words for quite fundamental notions, it is safe to assume that we are dealing with a science which has attracted to its service only men of mediocre ability.

And let me say incidentally, that what applies to terminologies applies in a

measure also to languages. Languages which have a poor vocabulary are languages of ungifted, and those which have a rich vocabulary are languages of gifted people.

The vocabulary of *Statistical Experimentation* is, as the reader who has any acquaintance with it does not require to be told, distressingly defective.

Allow me to draw your attention to some of the notable lacunae in the vocabulary of Statistical Experimentation—lacunae which cannot have failed to arrest the attention of all who have occupied themselves with that vocabulary.

Fault can be found with it first on the ground that it, though it provides the name 'Controls' for those members of a class not experimented upon, fails to provide any generic name for those experimented upon.

These, when they have been experimented upon with vaccine, have to be denoted 'the vaccinated' or 'the inoculated'. And when they have been experimented upon with a particular diet, they have to be called 'the dieted'. And when they have been experimented upon with a drug, they have to be called (there could hardly be a less descriptive term) 'the treated'.

The reader will appreciate that a terminology of this sort—I mean a terminology which supplies *specific* but no *generic names*—has definitely barbaric affinities. I take the following from Jespersen[1] :—'The aborigines of Tasmania had a name for each variety of gum-tree or wattle-tree, but they had no word for *tree* ; . . . And the Zulus have words for a "red cow", a "white cow", and a "brown cow", but none for a "cow in general".'

It will, in view of the labour of expressing oneself with a terminology in which general ideas cannot be expressed, here be desirable to provide generic names for '*those experimented upon*' and '*those not experimented upon*'. We might perhaps call the former '*the observed*' and the latter the '*counter-observed*' ; or we might when lists are made of the observed and counter-observed call the former '*the inscripts*' and the latter '*the contra-scripts*'.

Another serious lacuna in the terminology of Statistical Experimentation comes into view when we regard the fact that there is not, as the terminology of *Statistical Experimentation* might lead us to suppose, only one kind of 'control'. There are definitely two sorts of 'controls'. There is to begin with the '*Se-ipsic*' or '*Auto-proteric*' control—that in which we compare the condition of a patient after treatment to his condition before. And there are also other '*Controls*'—I propose to call them the '*Allotrious Isochronous Controls*'—where we compare a group of 'inscripts' with a group of 'contra-scripts'.

It will be appreciated about '*Se-ipsic Controls*' that it is these, and not allotrious ones, which are used by every patient and every Medical Practitioner for gauging the efficacy of treatment. Another important point to note is that *Se-ipsic Controls* constitute, when we are dealing with hopelessly chronic cases or those which are steadily going from bad to worse, by far the most convincing controls. Let us remember from the New Testament the man who was born blind and was made to see, and note that he stood his ground against the Jewish sceptics by his simple statement, 'One thing I know, that, whereas I was blind, now I see.'

So far, I have been regarding only the lacunae in the vocabulary of Statistical

[1] *Growth and Structure of the English Language*, p. 51.

Experimentation. But there is, in connexion with every scientific vocabulary, this further point to be regarded : that there are in each inchoate science certain special technical terms which the enthusiasts for that method specially insist upon. And if the points which are so emphasised turn out to be dubious or unimportant, that of course does not add to the prestige of the science.

It will, to furnish illustration of this point, suffice to comment briefly on the words '*Significant*', '*Random Sampling*' and '*Probable Error*', which figure so prominently in the vocabulary of Statistical Experimentation.

First, with regard to the term '*Significant*', the Statistician is wont to lay it down as dogma that the results which emerge from a comparison of 'the inscripts' with 'the contra-scripts' must show a certain percentage difference before any value can be attributed to the statistical result.

It is customary to say that a 10 per cent. difference is the least which can be considered conclusive.

This pronouncement is obviously wrong. For if the 'working error' of a statistical experiment could be so reduced that only the 'error of chance' remained over, and if, in addition, the numbers of cases dealt with were sufficiently large to reduce that 'error of chance' to insignificance ; differences of much less than 10 per cent. would immediately become significant.

I pass to the term '*Random Sampling*'. This term has been used by Statisticians to call attention to the fact that the 'community' (the Statistician is wont to call it the 'population') operated upon must be equitably assorted into 'inscripts' and 'contra-scripts'. The term 'Random Sampling' altogether fails to bring that home to the mind, for since (we saw this in discussing 'Sampling Induction') all sampling worthy of the name is *random* sampling, the application of *random* to *sampling* does not, as a matter of fact, convey censure. If anything, it implies commendation.

Lastly, we have to consider the term '*Probable Error*'. This properly means the error which is imported into statistical results by chance *when this constitutes the only error.*

The term 'probable error' is, in accordance with that, applicable only to statistical experiments like those of Pascal and Bernoulli which were done by throwing dice, or tossing coins, or (I think) dealing out playing cards.

In such experiments there is, as will be realised from the following, no *operational error* which could vitiate the result. For the 'erg' or driving force here brought into operation—that is, the successive shakings of the dice-box and the tossing up of the coin and the shuffling and dealing out of cards—are, for all practical purposes, *replicative operations.* And with that the first of the requirements of Statistical Experimentation is definitely satisfied. Further, the dice and coins and cards which are employed in the succession of experiments considered may be said to be exact replicas the one of the other. And with that the second requirement of Statistical Experimentation is satisfied. Finally, the '*Eventus*' of the experiments which were carried out by Pascal and Bernoulli—that is to say the number of pips on the up-turned faces of the dice and cards, and the face upon which the coin falls—cannot possibly be misread.

That finally eliminates all operative fallacies which are incidental to statistical experiments, and leaves over only the Fallacy of Chance. And this—the so-called 'Probable Error'—is of course inversely as the number of times the experiment is repeated.

I pass from this to consider what I may call 'the rules of the game' when, as is bound to happen in Medicine, the requirements of a perfect statistical experiment cannot be complied with.

Let me deal first with 'the rules of the game' which are applicable to the testing of prophylactic agents such as vaccines.

(1) One should choose for experimentation a 'community' of persons who are fundamentally similar. I have in view here, in particular, identity of age and race.

(2) When '*Allotrious Controls*' are employed, '*the inscripts*' and '*the contra-scripts*' should be, in every important respect, similar.

(3) The number of '*Inscripts*' and '*Contra-scripts*' should be sufficient to bring down the 'error of chance' in each case to something inconsiderable—the 'error of chance' being, of course, in the inverse proportion to the number of cases included in each group.

There must, however, here be kept in mind this proviso:—that the more effective the method which comes into application, the larger is the probable error admissible. In other words, the smaller would be the number of cases required to establish the efficacy of a prophylactic agent. And the same applies, of course, also to all therapeutic agents.

(4) The '*erg*' which is employed in the whole sequence of experiments should (so far as that is possible) be qualitatively and quantitatively the same.

(5) The '*Eventus*' by which the results are adjudged should be so chosen as to admit of the question asked being answered in terms of an unqualified 'yes' or an unqualified 'no'.

(6) The '*Inscripts*' and '*Contra-scripts*' should be subjected to the same risks of infection for the same period; and the '*Contra-scripts*' should, if they are to furnish an accurate quantitative measure of the protection afforded, be subjected to just as great a risk of infection as they were before the statistical experiment was begun.

(7) Accurate book-keeping is required.

Let me now say something first about the difficulties and fallacies which the statistical experimenter has to look out for.

I may begin with the problem of dividing up the community chosen for our experiment into, so far as possible, equal and similar groups of '*Inscripts*' and '*Contra-scripts*'.

This presents no difficulty so long as the prophylactic method employed holds out any likelihood of success. For the inoculator will then, if he has any gift of persuasion, find it quite easy to get all the volunteers for inoculation he wants.

But when the therapeutic method has definitely won its spurs, and there is an epidemic in the near offing, everybody clamours to be vaccinated; and it may then be difficult to secure the desired number of Controls.

The remedy for such insufficiency of Controls is, as we shall see, recourse to the procedure of '*Retrospective Statistical Evaluation*'—that is to say, it consists in comparing the incidence and death-rate from the disease under consideration before and after the general adoption of the prophylactic method.

The next point which has to be attended to in the statistical game is that though the 'error of chance' (the so-called 'probable error') automatically diminishes as the number of cases increases, *the total working error steadily increases*. Moreover, the rate at which the total working error increases greatly exceeds that at which the 'probable error' diminishes.

It increases because, when the number of cases is in excess of that which skilful and conscientious medical men can cope with, inferior medical practitioners have to be called in. And when statistical work is confided to them, all the following enormities which happened in the South African War may happen again.

(*a*) One can no longer be sure that the prescribed dose of vaccine is administered.

For example, in the South African War, a considerable group of men in one transport were (to the confounding of the statistics) inoculated with ten-fold the prescribed dose of vaccine.

(*b*) There is always in war and epidemics increased risk of negligent book-keeping.

For example, the mortality from typhoid fever among the uninoculated in the Tintown Hospital at Ladysmith was, through a clerical error, recorded as 1·6 per cent., whereas it was, in reality, 16 per cent.

(*c*) Also there may, when supervision is slack, be a great deal of faked book-keeping.

Such faked book-keeping was (as shown in the Report of the Indian Plague Commission of 1898–1899) practised on a large scale in connexion with Haffkine's anti-plague inoculation.

And it was also practised in the South African War. For when a census was made of the numbers of inoculated and uninoculated among the patients in hospital, it was not uncommon for the uninoculated to register themselves as inoculated ; for the men suspected that steps would, if they gave themselves away as uninoculated, immediately be taken to inoculate them.

(*d*) Lastly, and perhaps most important of all, errors of 'Diagnosis' mount up formidably with every decline in professional competence.

And let me now say by way of comment upon statisticians :

1. Despite the fact that the untrustworthiness of statistics always increases *pari passu* with the number of cases, professional statisticians are so alarmed by the spectre of a considerable 'probable error' that they refuse to take any cognisance of statistics which don't run into hundreds and thousands.

2. Also statisticians (and they do this whether the figures are small or large) place an absolute taboo on the procedure which I have called *Retrospective Statistical Evaluation*.

Coming now to that prescription of the statistical game which tells us that we should focus our attention upon unequivocal criteria, it will not be necessary to enlarge on the fact that one can very seldom feel complete confidence in purely clinical results.

But it is, perhaps, just as well, since typhoid inoculation has been under discussion, to lay stress on the fact that not only is the clinical diagnosis of typhoid

definitely difficult, but that its diagnosis by laboratory methods is, in the case of the inoculated, hopelessly complicated by the fact that the agglutination test gives here only equivocal indications ; and that the negative results of the blood-culture test are, especially in the lighter cases of the disease such as occur among the inoculated, very inconclusive.

Both difficulties made themselves felt very sensibly in the Army in the War of 1914–1918. For it was (it will be remembered that nearly every man in the army was inoculated) nearly impossible to determine whether a man who suffered from fever of one or two days' duration ought, or ought not, to be diagnosed as a case of typhoid.

And it will be obvious from these considerations that the incidence rate of this disease furnishes at best a very unreliable criterion of prophylactic efficacy ; and that the only criterion on which reliance can be placed is the death-rate, when the cause of death is confirmed by post-mortem examinations.

The last rule of the statistical game which calls for critical consideration is the prescription that '*the inscripts*' and '*the contra-scripts*' should be herded together without thought of the fact that '*the contra-scripts*' should continue to be subjected to just as great risks of infection as they would have been if no inoculations had been carried out in the community.

Consideration shows that this requirement can, given that the vaccine is effective, never be realised if '*the inscripts*' and '*the contra-scripts*' are herded together. For if the incidence of the disease falls off among the inoculated, a *Circulus Felix* (a term which I have adopted from my colleague, Dr. John Freeman) is bound to be established. In other words, the 'Controls' are bound to benefit from the lessened chances of infection resulting from the diminished incidence of the disease in the inoculated with whom they consort.

And there would seem to be no way of avoiding this fallacy (I mean the fallacy of under-rating the efficiency of a vaccinating process) except by having recourse to the method of *Retrospective Statistical Evaluation*—that is, to comparing the total incidence-rate of the disease in the community before inoculation was resorted to with the incidence rate in the same community afterwards.

Statistical Experiments made to gauge the Efficacy of Therapeutic Procedures employed in the Treatment of Disease.

In connexion with this, I may begin by pointing out that accurate Statistical Evaluation is here almost out of the question.

Let me explain.

First, consideration will show that even patients who are suffering from one and the same infection cannot, because of the great differences which prevail among them, be equitably assorted, as healthy persons can for the purpose of prophylactic inoculation, into comparable groups of '*Inscripts*' and '*Contra-scripts*'. And only lay Statisticians would suggest that any equitable assortment of patients could be arrived at by assigning them, as they come into hospital, alternately to one or other of the above categories.

Again, if the patients in any hospital were assorted into two groups—a group of those who would receive treatment, and a group of others who were to serve only as fodder for statistics—those who were chosen to serve as 'Controls' would naturally refuse to be partners to that arrangement. And as a result, the whole experiment would come to grief.

And we should not be much better off if we substituted '*Auto-proteric*' for '*Allotrious Controls*'. For patients who differ so much that we can't compare the one who has just come into the hospital with the previous one or the next, cannot possibly be said to constitute a homogeneous statistical group.

It follows that if we insist on employing Statistical Methods, we should fall back upon the Method of *Retrospective Statistical Evaluation*, and that, except for the fact that it supplies us with numbers, comes to much the same as relying on the consentaneous verdict of capable clinicians.

Let me now try to resume, and to some degree amplify, what I have already said about these two statistical methods of judging of prophylactic and therapeutic procedures.

The former of these methods (I mean that which employs allotrious and isochronous controls), to wit the '*Classical Method of Statistical Experimentation*', has three particular pitfalls :—

(*a*) It is a principle of strictly limited application—one which can be used for testing the value of prophylactic, but not for testing that of therapeutic, measures.

(*b*) It is a method which involves, when it aims at arriving at results which can maintain themselves against criticism, a great deal of preliminary planning and preparation.

(*c*) It is a method which involves, when quantitatively accurate results are to be achieved, the taking of endless precautions ; and among them, one which it is impossible ever to carry out—to wit, the exposure of the '*Inscripts*' and '*Contra-scripts*' to the selfsame amount of infection ; while keeping the '*Contra-scripts*' exposed to the selfsame amount of infection as they were before the statistical experiment was begun.

The *second* method—that of *Retrospective Statistical Evaluation*—can be used not only for the gauging of prophylactic, but also for the gauging of therapeutic procedures. Further, it does not, like the Classical Method of Statistical Experimentation, involve the bringing together of any new material. All that is required is the compilation and collation of data already available.

But the method has this outstanding defect : it brings no conviction either to the uncritical or the prejudiced.

In particular, it fails to appeal to the following large classes of persons :—

(1) First, to those who 'have heard tell' that *post hoc propter hoc* induction is always fallacious.

This opinion, though it is very deep-rooted, is thoroughly erroneous; for all Crucial Experimentation is, as reflection will show, *post hoc propter hoc* induction.

But then this is experimentation carried out in a *locus clausus*, and the *caveat post hoc propter hoc* applies only to experiments carried out in a *locus apertus*.

(2) I have in view further those who have not considered the point that when retrospective evaluation is brought into application the experimenter has always presumptive grounds for concluding (those presumptive grounds may be either previous clinical or crucial experimentation) that the '*erg*' brought into application is effective; and further, he has no reason to think that any causal agent other than that which his thoughts are centred upon, is here playing any part.

(3) And the third class of persons who can never be persuaded by *Retrospective Statistical Evaluation* are all those who have a definite bias against therapeutic and prophylactic methods which derive, even indirectly, from experiments on animals.

These biased persons invariably assume that, when good results are established by Statistical Induction, these are due not to the agent experimented with, but to a concurrent reduction of the virulence of the microbes of the disease.

That belief would be tenable if only there were reasonable ground for believing that the virulence of every pathogenic microbe falls off the very moment that some research worker succeeds in finding an effective prophylactic method or a therapeutic agent which really works.

Having dealt with these general questions, we may now pass to consider whether the results which are obtained by *Retrospective Statistical Evaluation* are as valuable, or more valuable, than those obtained by the *Classical Method of Statistical Experimentation*.

Before dealing with this, let me define more precisely what I have in view when I speak of *valuable results*.

It is commonly asserted that 'Science is measurement'—*measurement* being understood to comprise the achievement of numerical results by *enumeration*, *weighing* and quantitative methods generally.

That is quite erroneous; and consideration will show that it is no particular good to anyone to be able to rehearse statistical or other figures. The all-important thing in Science is not *measurement* but *certainty*. Or, to be more precise, it is the achievement of an accurate picture of our environment. It is—I use here the nobler words of Bacon—"the building up in the human understanding of a true model of the world such as it is in fact".

Having now seen what it is we have to aim at, we may take the method of Jennerian vaccination and weigh it in the balances of these two (let us call them *competitive*) statistical methods.

With regard to this, let us have this clearly before us that smallpox was very rife all over Europe up to the end of the eighteenth century; and that it has since then, in the greater part of Europe, almost disappeared. And then let us meditate the fact which has been brought out by Professor Major Greenwood (and, I think, also by others) that there is not to be found in medical literature any satisfactory Statistical Proof of the efficacy of Jennerian vaccination.

Two contradictory interpretations can be placed upon this.

The one is that the efficacy of Jennerian vaccination is doubtful; and that the recorded decline of the incidence and case mortality of the disease was due, not to the introduction of Jennerian vaccination, but, as the anti-vaccinationists maintain, to a decline in the virulence of the disease.

The other is that Jennerian vaccination has proved itself very effective ; and that the default of statistical evidence of this is due to something quite wrong in the statistical procedures employed.

Hesitation as to which of these is the correct interpretation of the facts is completely set at rest when we see that it is the unanimous opinion of Public Health Officers who have had experience of smallpox epidemics imported from overseas that Jennerian vaccination, sufficiently widely applied, has invariably resulted in a stamping out of the infection.

And the testimony of all travellers who have had experience of Jennerian vaccination applied in case of smallpox breaking out in gangs of natives is likewise unanimous about the efficacy of the method.

And, for my own part, I cannot help laying great stress on the experience of a very intelligent fellow-traveller whom once I met when he was returning to Egypt. This traveller told me that when he had gone back to his estates in the Sudan immediately after the Mahdi had been driven out, he had not found there a single child alive. Every child in the place had been carried off by smallpox when Jennerian vaccination—which had under Anglo-Egyptian rule been practised—had, under the Mahdi, been discarded.

Results similar to those which *Retrospective Statistical Evaluation* furnishes of the efficacy of Jennerian vaccination are available also in connexion with other prophylactic inoculations.

For example, a comparison of the heavy mortality from typhoid in the British Army in the South African War of 1890–92 with the very light mortality from typhoid in the British Army in the war of 1914–18 furnishes good evidence of the efficacy of typhoid inoculation.

Confirmation of this is furnished by comparing the figures for typhoid in the U.S. troops in the Jacksonville camp in the Spanish-American War, with those of a similar body of troops in the San Antonio camp on the infected Mexican border.

In the former case, there were in the camp 11,000 soldiers, all uninoculated ; and there were 1729 cases of typhoid.

In the latter case, there were 13,000 United States troops, all inoculated ; and there was only one case of typhoid.

Other quite probative figures obtained by *Retrospective Statistical Evaluation* are those which relate to anti-pneumonic inoculations in the Premier Mine in Pretoria.

The figures in question have already been given (*vide supra*, pp. 48 and 49, Tables XII and XIII). They will probably have special interest for students of Statistics in virtue of the fact that they confirm the results of *Classical Statistical Experimentation* set out in Table XI. Further, it will be clear, on consideration of the figures in Table XIII, that recourse to the method of *Retrospective Statistical Evaluation* was necessitated when the percentage of inoculated in the mine amounted to something like 90 per cent. of the whole.

Again, very significant retrospective figures which refer to cases of tetanus were furnished in the last war by comparing the number of cases which occurred before and after the wounded were regularly inoculated with anti-tetanus serum.

Again, similar extremely expressive results are said to have been obtained in the present war by the inoculation of the English troops with tetanus toxoid—tetanus cases in the wounded having been rare, if not entirely absent.

And lastly, reference may be made to the fact that diphtheria has almost disappeared in large towns in Canada as a result of the preventive inoculation of children with diphtheria toxoid.

What holds of *Retrospective Statistical Evaluation* applied to the results of prophylactic inoculation holds also of its application to the results of treatment. And this is specially important, because isochronous, allotriously controlled, statistical experimentation, is (as we have seen) inapplicable to the testing of remedial agents.

And *Retrospective* (one might also call it Global) *Statistical Evaluation* has given important results in the case of diphtheria patients treated with diphtheria anti-toxin, in cases of pernicious anaemia treated with raw liver, and in cases of puerperal septicaemia treated with sulphanilamides by Dr. Leonard Colebrook.

It would seem from this consideration of the facts, and from the reviews of the fallacies of *Classical Statistical Experimentation* which preceded it, that the method of *Retrospective Global Statistical Evaluation* is bound to substitute itself everywhere for the method of *Classical Statistical Experimentation*.

Concluding Remarks.

A good deal of space has been devoted above to the consideration of the three different methods of *Experimentation*—to wit, '*Random Trial-and-Error Experimentation*', '*Crucial Experimentation*', and '*Statistical Experimentation*'. There remains to be considered the application of these methods to the resolution of the problems which have to be solved by Medical Research.

Those problems are : *first*, the discovery of new aetiological principles ; *secondly*, the testing of the utility of therapeutic methods which are in general use ; and *thirdly*, the refining upon scientific achievements which have been duly approved, and the giving of guidance in the treatment of individual cases.

The first thing we have to make clear to ourselves here is that 'Random' or mere 'Trial-and-Error Experiments' can be put aside as altogether useless. No one who is at work in a well-conducted laboratory ever dreams of performing a mere Trial-and-Error Experiment.

That leaves over only '*Statistical*' and '*Crucial*, or better, *Claustrated Experimentation*'. We may discuss them in order this.

Statistical Experimentation.

Reflexion will show that Statistical Experimentation has never revealed, nor will it ever reveal, any *aetiological principle*. Further, Statistical Experimentation can never be employed for what I may call '*Refining Experiments*'.

To find out what doses of a particular vaccine give the best protection it would, for example, be necessary to inoculate large batches of men with different doses and different kinds of vaccine ; and then to send them out into the world on the chance

of their all encountering there the same amount of infection. That would obviously be a quite hopeless method of experimentation.

And if we experimented on animals, we should arrive at results which, if they had any human significance at all, would at any rate have no quantitative application to man. For obviously the dose which would be the best for a mouse, or a guinea-pig, or a rabbit, could not be expected to be the best for a man.

Similarly, in the case of a therapeutic procedure, it would be clearly hopeless—since we are not, in the case of patients, dealing with homogeneous groups of sick men—to expect the Statistical Method to tell us what doses of a therapeutic agent should be applied in particular cases.

And as matters now stand (and this applies specially to the doses of quinine, diphtheria anti-toxin, and the sulphanilamides) dosage is in the main a matter of pious opinion—one man holding that the patient is given much too large doses, and an equally experienced Medical Man holding that the patient has been much under-dosed.

There remains, therefore, of the three possible applications of Statistical Experimentation, only that of validating or invalidating the efficacy of this or that selected dose of a prophylactic vaccine or chemo-therapeutic agent.

Let me now turn from *Statistical* to *Claustrated Experimentation*.

Claustrated Experimentation.

We must look to Claustrated Experimentation for the discovery of new aetiological principles; and further, for the validation of prophylactic and therapeutic procedures applied to man. And that, of course, will include the making of 'Refining Experiments' to determine the best dose of a prophylactic or therapeutic agent.

But it will perhaps, before proceeding further, be well to make clear to ourselves that the ordinary Medical Practitioner, like the ordinary layman, has absolutely no intellectual regard for, or appreciation of, what is usually called Crucial Experimentation.

For example, not so long ago, a Consultant who had 'blown in' to my laboratory delivered himself of this speech:—'No one in the Profession pays the least attention to those little games that you folks play in laboratories.'

And when one thinks over that speech, one sees a certain justification for it. At any rate one begins to understand it.

The Medical Practitioner and Consultant have no time to search the Scientific Journals for something that might be useful to them in their profession. All they have time for is to discover from the medical papers, and from their colleagues, what new methods have been successfully applied to patients.

Further—and this is a fundamental defect of all *Claustrated Experimentation*—the results arrived at are not the final and directly applicable data required by the practitioner, but only intermediate results.

And those results have consequently, before they can be accepted by the Profession, to be confirmed by experimentation undertaken upon patients.

In view of this, and despite the fact that I had crucial evidence that the bactericidal power of the blood to typhoid might be increased as much as a thousand times by typhoid inoculation, I had before the method had any chance of being taken into consideration to carry out anti-typhoid inoculations in India on many thousands of soldiers. This was in 1898.

And the same story repeats itself everywhere. Behring's discovery of diphtheria anti-toxin had, before it was taken up by the Medical Profession, to be confirmed by statistics obtained by the administration of diphtheria anti-toxin to children by Roux—Roux being a researcher and not a regular physician.

And anyone who happens to be interested in the point here in question will learn, from these *Collected Researches*, that I have, in connexion with all therapeutic suggestions I have put forward, furnished clinical evidence of their efficacy. And when I have not furnished sufficient clinical evidence to impress the mind of the Medical Reader, or have not repeated it sufficiently often, the method has not yet been adopted into practice.

Let me, after this long excursion which concerns the estimate in which Crucial Experimentation is held in the profession, come to the question of its uses in research.

Reflection will show that every new therapeutic principle derives from Crucial Experimentation.

A very conspicuous example is to be found in the fact that the therapeutic applications of calcium, citric acid, and the citrates which are reported in Vol. II of these *Collected Researches*, derive ultimately from the work of Arthus and Pagès, who showed that blood can be kept from clotting by decalcification.

And similarly, all anti-typhoid inoculation derives from the fact that Gruber and Durham showed that typhoid agglutinins are produced in the organism when animals are inoculated with bacterial cultures.

Further, Crucial Experimentation can confirm the efficacy of prophylactic and therapeutic procedures which are proposed for use.

Finally, it can be used for what I have called '*refining experiments*'. In other words, tests carried out on the blood of those to whom the therapeutic agent has been administered can determine the efficacy of every therapeutic or prophylactic measure without exception. And we can thus, by testing a series of different patients with different doses, arrive at the best dose of a vaccine or chemo-therapeutic agent.

In conclusion this may be said. When we realise the greater usefulness of Crucial Experimentation, it becomes a matter of wonder that the Statistical Method is in most laboratories of Medical Research preferred to the Method of Crucial Experimentation.

The reasons for this do not lie very deep down. Obviously what comes into consideration (it is perhaps doubtful whether it is, as Hamlet says, 'honest to have it thus set down') is that Statistical is infinitely easier than Crucial Experimentation.

In the *first* place (and this means a great deal of relief for the laboratory worker) Statistical Experimentation can be carried out on animals. Carrying out work on themselves or other men has always been uncongenial to the research workers.

Secondly, no sort of technical skill is required. Anyone can do a statistical experiment who knows how to fill and empty a syringe.

Thirdly, no (or very little) technical training is required for taking cognisance of the result of the experiment. Usually, all that has to be done is to count the proportion of animals which survive, and to *post-mortem* those which are found dead.

In short, the methods of statistical enquiry are so simple that any intelligent laboratory assistant can carry them out.

Crucial Experimentation is much more difficult. The laboratory worker who wants to carry out a crucial experiment must submit himself to a severe apprenticeship in technique. For instance, blood tests and response graphs of immunisation carried out on small animals—and, indeed, upon patients—can be constructed only by those who are competent to work with very small quantities of blood, and the same applies to all serial blood-testing.

Further (I think there can be no doubt about this), a man who proposes to carry out a crucial experiment should possess some native experimental ingenuity; whereas a man requires absolutely none for carrying out an ordinary statistical experiment.

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PRINTED IN GREAT BRITAIN BY ROBERT MACLEHOSE AND CO. LTD.
THE UNIVERSITY PRESS, GLASGOW