The Croonian lectures on the behaviour of the leucocytes in infection and immunity : delivered before the Royal College of Physicians of London on June 14th, 16th, 21st, and 23rd, 1910 / by F. W. Andrewes.

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BY

F. W. ANDREWES, M.A., M.D. Oxon., F.R.C.P. Lond.

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LECTURE I.

Delivered on June 14th.

MR. PRESIDENT AND GENTLEMEN,—Before I commence these lectures I have two duties to fulfil, the one sad, the other pleasant. I must, in the first place, deplore the circumstance which has led to my standing before you to-day. The death of Dr. Arthur Gamgee, who was to have been your Croonian lecturer this year, has left a void which it is hard to fill. I cannot hope to carry out the task as he would have done. The loss which we suffer is not ours alone but that of the whole world of science and of medicine. I have next to offer my thanks to the College for the honour conferred by asking me to deliver the lectures in his place. In any case an unworthy substitute, I must crave your special indulgence in view of the fact that I have had only a year for the preparation of my subject, instead of the two which are by custom allowed to the Croonian lecturer.

In the year 1906 it was my privilege to deliver the Horace Dobell lecture before this College. I then endeavoured to picture the stages through which I conceived bacteria to have passed in the course of their evolution into pathogenic agents and the gradual development of those powers of aggressive parasitism which, at the present day, render them such dangerous foes to the animal body. I could have wished to use the larger opportunity afforded by Dr. Croone's foundation, by taking as my subject the converse theme—the evolution of the means of defence possessed by the body against invading microbes—and to trace it from its earliest beginnings to the complex mechanisms which we see brought into play in the human body. Such a task, however, I found beyond my powers, for our knowledge of the means of defence in the lower animals is very scanty, and time failed me for any serious attempt at its investigation. I

have nevertheless ventured to choose for my subject one part of the field of immunity—as I think a very important part. It would be presumptuous for me to attempt to deal with immunity as a whole: I have not the learning or the critical power for such an enterprise. But in one part of the field I have worked, and in view of these lectures worked very specially—that, namely, which concerns the behaviour of the leucocytes in connexion with infection and immunity. During the last 20 years so much of the investigation of immunity has been devoted to the purely "humoral" side of the subject, that I feel it worth while to recall your attention to the cellular elements concerned in defence. While most of the facts which I shall have to put before you are the result of my own observations, I cannot avoid reference to current doctrines of immunity, and I must here own my indebtedness to the critical writings which have appeared during the last few years—those of Metchnikoff and Levaditi, of Ehrlich, Bordet, Pfeiffer, Sauerbeck, Wright, and many others. My own observations have been in the main experimental, derived from a study of the behaviour of the leucocytes in the rabbit under various conditions of infection and immunity, but I shall endeavour to harmonise them with known clinical facts in human disease.

I propose, in the first place, to put before you a short historical sketch of the development of our knowledge as to the part played by the leucocytes in repelling microbic aggression, after which I must perforce attempt to assess the rôle of the leucocyte in immunity. Having thus stated what I conceive to be the present state of our knowledge on the subject, I shall deal at greater length with my own observations on the leucocytes and the conclusions which I am disposed to draw from them.

The Croonian lecturer enjoys a wide range of choice in the selection of his subject, but I can imagine none which would more thoroughly fulfil the intentions of the founder, could he revisit the College after more than 200 years, than one which so directly concerns the prevention, control, and cure of disease.

Historical Sketch.

We owe to Metchnikoff the first serious attempt to explain the resistance of the body against bacterial invasion. The crude and speculative views which had previously been put forward by Pasteur and Chauveau were based upon the supposition that the animal body was a mere culture medium for the growth of micro-organisms which might become exhausted of the necessary pabulum or choked up by bacterial products. Metchnikoff first formulated the conception of an active bodily defence, finding its mechanism in the functions of certain cells of the body.

Theory of Phagocytosis.

It was already known that the amœboid movements of the leucocytes enabled them to take up foreign particles into their interior. Virchow was aware of the increase of circulating leucocytes in certain septic conditions. It had even been suggested by Panum in 1874, and by Roser in 1881, that bacteria might be ingested by leucocytes. But it remained for Metchnikoff to develop the idea that certain cells possessed as a normal function the power not only of ingesting but of digesting and destroying foreign particles and fragments of dead tissue. Amongst the foreign particles thus destroyed he found invading parasitic organisms, and he came to regard the process, which he named "*phagocytosis*," as the main, and indeed the only, defensive agency of the body against bacterial invasion. He first propounded this doctrine in 1883.

Metchnikoff was a biologist and his views were founded upon the long series of patient studies upon the lower forms of life recorded in his well-known work on inflammation. He was able to bring forward evidence of a uniform phagocytic process occurring from the lowliest up to the most specialised members of the animal kingdom, and this breadth of view helped to gain acceptance for his theory. Nor can it be doubted that the fact of his having commenced his studies in lowly invertebrates, where the means of defence are in their simplest and most primitive condition, played no small part in the reasoning by which he built up his hypothesis.

The fundamental starting points of the theory were—(1) the observed phenomena of intracellular digestion, and (2) the behaviour of the mesoblastic tissues in presence of foreign particles. Practically all phagocytic cells were found to be of mesoblastic origin, and Metchnikoff divided them into "macrophages"—fixed tissue cells which more particularly took up cell débris, and "microphages"—the leucocytes, and especially the polynuclears, whose more particular function it was to deal with micro-organisms.

At this early stage of the theory of phagocytosis the matter appeared simple enough. Successful phagocytic defence implied immunity, failure meant susceptibility. The phagocyte was the only means by which the body repelled microbic attack; the properties of the microbe were not yet discovered, and the influence of the fluids of the body had not entered into anyone's mind. From the first the simplicity of the theory and the readiness with which its central fact could be verified gained it wide acceptance.

It may have been an accident that the date at which Metchnikoff drew attention to the leucocytes as defensive agents coincided with a period at which increasing attention began to be paid to the morphological study of the blood. This was for the first time rendered accurate by the staining methods devised by Ehrlich's chemical ingenuity and summed

up in his monograph of 1891. Instruments of precision had further been introduced, such as the hæmocytometer, by which the content of the peripheral blood in red and white cells could be exactly ascertained.

The impulse thus given to the clinical study of the blood bore rich fruit and a new branch of pathology arose—"hæmatology." During the last decade of the nineteenth century important monographs appeared in quick succession, amongst which I may mention those of Rieder, Türck, von Limbeck, Cabot, and Grawitz. So valuable has the study proved that it is now of daily application in clinical pathology. As regards infective disease its importance has proved twofold. It has become a well-established fact that in some infections there is a marked increase in the number of circulating polynuclear leucocytes, in others none, in a few an actual decrease. A diagnostic value may thus attach to the leucocyte count. It is further established that in those infections habitually attended by an increase in the polynuclear count, the presence and extent of this increase serve as some sort of index of the power of the body to react against the infection. In other words, the leucocyte count has at times a prognostic import.

This has come about quite independently of Metchnikoff's theory as a direct result of clinical observation on human disease. But it indirectly supports his views, for it points to one special cell—the microphage of his terminology—as of high moment in the bodily reaction against certain infections. It would seem that the capacity of the body to produce and put into circulation an adequate number of polynuclear leucocytes in response to infection has in some cases a determining influence in recovery.

Humoral Theories of Immunity.

While the doctrine of phagocytosis was at first received with much favour subsequent developments in knowledge tended to throw it, for a time at least, into the shade. It was shown that the fluid elements of the blood, judged from the properties of the serum in experiments outside the body, were able to destroy bacteria apart from the direct coöperation of cells. This power, first noted by Fodor in 1887, was further studied by Nuttall, Buchner, Hankin, and others, and in 1889 Buchner propounded his well-known "*Alexin*" *theory*, in fundamental antagonism to the views of Metchnikoff. Buchner's original conception of alexin was that of a non-specific protective substance existing in normal blood serum, acting upon bacteria after the fashion of a ferment, and easily destroyed by heat, or, indeed, on mere standing outside the body. He suggested its possible origin from leucocytes. To this somewhat primitive conception

may be traced the elaborate humoral theories of immunity which have since so largely held the field.

It would take me too far from the subject of these lectures to enter in detail into the history of all these humoral views. I will only mention the more important landmarks. In 1892 Behring was able to announce the discovery of antitoxins in tetanus and diphtheria, and thus first arose the idea of *chemical immunity*. As a sequel to this came the discovery of *passive* immunity, produced by the transference of the specific antibody in the serum of an immune animal to a normal one.

Although Buchner and others had proved the destruction of bacteria by a suitable serum, Pfeiffer, in 1894, first placed the doctrine of *bacteriolysis* upon a firmer basis and indicated the dual nature of the active substance. In the following year Bordet elaborated and amplified this idea, formulating a doctrine of lytic action which has since gained universal acceptance, and which was later extended by Bordet himself to cover the phenomena of hæmolysis.

At this period Ehrlich was developing his theory of antitoxin formation, and he went on to study lytic action and other humoral phenomena. The bent of his mind was essentially chemical, and he succeeded in framing a purely chemical theory to account for the nature, properties, and formation of all humoral antibodies. This—the celebrated "*side-chain theory*"—was enunciated by Ehrlich in the closing years of the last century. It is impossible to withhold our admiration from a generalisation based on such a wide groundwork of facts, and serving to explain, in terms of exact chemical science, the processes of cell chemistry in general, and immunity as a special instance of these. So immediate was the recognition of the intellectual value of the side-chain theory, so lucidly did it seem to explain and reconcile the facts of immunity, and so fruitful did it prove in the stimulation of research, that it speedily became the dominating influence in our views as to the defence of the body against bacterial invasion. The disciples of Metchnikoff, at least in Germany, and indeed in this country, hid their diminished heads, and phagocytosis was relegated to the position of an interesting but relatively unimportant adjunct to humoral means of defence.

In France this was less the case, but Metchnikoff was compelled to meet the new facts: the primitively simple conception of phagocytosis had to be modified and amplified in such fashion as to include the action of humoral elements. The "alexin" of Buchner and Bordet, which is the "complement" of Ehrlich, was for Metchnikoff a product of the phagocytes, essentially intracellular in the living cell, but liberated on its disintegration. The doctrine of "*phagolysis*" was added to that of phagocytosis to explain the extracellular destruction of microbes. As we now know, there

are many facts in support of the conception that alexin, or complement, is derived from the leucocytes. Later it became necessary to include the "substance sensibilisatrice" of Bordet, which is the "immune body" or "amboceptor" of Ehrlich in the scheme, and this Metchnikoff somewhat reluctantly did; he held it to circulate in normal blood, and as "fixator" to have an adjuvant action in phagocytosis. But these efforts at reconciling the facts of humoral pathology with the main doctrine of phagocytosis, though they were supported by numerous ingenious experiments, failed for the time to stem the current of humoral views, and for many years the views of Metchnikoff remained in comparative neglect.

Opsonin Theory.

Such was the position of affairs during the first year or two of the present century. The next landmark in the development of theories of immunity lies in the "opsonin theory," under which I include all those views which recognise phagocytosis as the essential means of defence, but consider the presence of a humoral element necessary to prepare bacteria for ingestion. The idea of such humoral coöperation was quite foreign to Metchnikoff's original theory, but in 1895 Denys and Leclef, working at immunity against streptococci, showed that the difference between the immune and the normal animal lay, not in the leucocytes but in the presence of a specific humoral element in the serum which determined the occurrence of phagocytosis. The experiments by which they proved this were ingenious and conclusive; it is a matter for some surprise that they did not arouse more widespread attention, for in this and in the later work of the Louvain school certain of the fundamental positions of the opsonin theory were clearly anticipated, including even the anchoring of the effective substance by the specific bacterium.

Nevertheless the credit of the opsonic theory belongs essentially to Wright. In 1903 Wright and Douglas proved the presence in *normal* serum of a humoral element which determines the occurrence of phagocytosis. They coined for it the name "opsonin," and showed that it was thermolabile, putting it forward as a substance hitherto overlooked. The acceptance of the opsonic theory in this country was in the main due to the immediate practical application to which Wright turned it. He devised a method of measuring the opsonic value of a given serum and employed the "*opsonic index*" as a guide in the application of the "vaccine" treatment which he was then beginning to develop. The brilliant results which this method of treatment has yielded in suitable cases drew immediate attention to the methods by which Wright had been guided in the dosage and administration of

his vaccines. The opsonic index became almost a household word, and in this country and in America a very large amount of work has been carried out on the subject. On the continent the opsonic theory made slower headway, though it has now attained due recognition there also. It is a little singular that although the opsonic doctrine presupposes Metchnikoff's theory of phagocytosis as its basis, Wright himself, if we may judge by his writings, is practically a humoralist in the sense that his attention is concentrated on the humoral adjuncts to phagocytosis: to this point I shall return later.

Wright's opsonin, as found in normal serum, is a thermolabile substance. In the year following his original publication Neufeld and Rimpau drew attention to the presence in immune sera of a similar humoral substance, conditioning phagocytosis, but differing from Wright's opsonin in that it was thermostable. This they termed "*bacteriotropin.*" We now know that there are several substances adjuvant to phagocytosis differing in their thermostability. The term "opsonin" may conveniently be used as a generic one, and there is no reason for separating the bacteriotropin theory of Neufeld and Rimpau from the opsonic theory of Wright. The question which now occupies the minds of students of immunity is the relation between opsonins and previously known antibodies. Are they truly substances *sui generis*, or are they our old friends amboceptor and complement under another name? Time will show, but there is a growing body of opinion that opsonic action is only a newly recognised manifestation of substances already known from other aspects. In any case the discovery has helped to bridge the gulf between phagocytosis and the purely humoral view of immunity, and to reinstate Metchnikoff's doctrine as to the essential nature of bodily defence against bacterial invasion.

Aggressin Theory.

More recently a somewhat new turn has been given to the point of view from which we may look on immunity by the "aggressin theory" of Bail. All the preceding theories have as their starting point the reactions of the body against invading bacteria. The aggressin theory more logically begins with the properties of the parasitic organism. It denies that the various lytic antibodies, interesting as they may be in test-tube experiments, have much to do with immunity in the animal body; in the tissues, where infection runs its natural course, bacteriolysis does not occur. Bail focusses his attention in the first place upon the means by which the bacterium is enabled to multiply in the body. Assuming phagocytosis as the essential means of bodily defence, it is plain that any property which enables the

bacteria to paralyse the phagocytes must facilitate their invasion. This property is found by Bail in substances formed by pathogenic bacteria, chiefly within the animal body, which he terms "aggressins," and which are supposed to act by negative chemiotaxis upon the leucocytes; otherwise he imagines them to be non-toxic. Immunity, on the aggressin theory, depends upon the formation by the body of "anti-aggressins," which once more permit the phagocytes to exercise their normal functions of defence. An anti-aggressin is obviously but an opsonin under another name. Only the point of view has been changed. Logically the change is, perhaps, for the better, for the bacterium is always the attacking element, and the names which we choose to apply to the weapons in the hands of the attacking and defending forces are a matter of detail.

It cannot be said that Bail's theory has gained wide acceptance, and it may well be that his facts as to the specific nature of aggressins are capable of interpretation in another way. Nevertheless, the aggressin theory has drawn attention to means by which bacteria may defend themselves against the mechanisms brought against them by the body. Amongst these must be reckoned the remarkable structural change which, in the case of some species of bacteria, has been shown to occur in those individuals which have successfully invaded the animal body—in other words, which are in a highly virulent condition. The change consists in the development of a capsule, often of great thickness. So long ago as 1897 Bordet showed that streptococci might under these circumstance develop a capsule which apparently hindered their ingestion by phagocytes. There seems to be a relation between the virulence of the pneumococcus and the degree of development of its capsule. The anthrax bacillus affords another striking example of the same thing, well shown by the work of Gruber and his pupils. It would seem that when bacteria are experimentally introduced into the animal body (and there is no reason for doubting that the same process occurs in natural infection) the majority perish. These are the more weakly ones, least able to withstand the defensive mechanisms of the body. If the invasion is successful, the victorious host consists of the lineal descendants of the more capable survivors, inheriting their parents' capacities. The rapidity with which generation succeeds generation (which may be a matter of only half an hour) enables natural selection to act with unparalleled intensity, and thus a resistant—i.e., a virulent—strain of the microbe is speedily produced. The properties essential to virulence are seen to become kinetic in the very course of the infection; in the test-tube they were merely potential. Thus is simply explained the exaltation of pathogenetic power long known to result from passage through the body of a susceptible animal. In some cases at least the phenomenon is associated

with the development of a well-marked capsule ; in others, merely with an increase in the apparent size of the bacterium. The term "animal bacilli" has been employed for such strains, as opposed to the "culture bacilli" of the test-tube. The evidence that such strains owe their virulence to the power of resisting the natural defensive mechanisms of the body seems clear as regards phagocytosis. I have already mentioned Bordet's observations on streptococci. Gruber and his co-workers have shown that capsuled anthrax bacilli resist phagocytosis, where the uncapsuled ones succumb. Rosenow has demonstrated the inverse relation between the liability of pneumococci to phagocytosis and their virulence. It has even been shown that, in a given case of pneumonia, the pneumococci cultivated from the lung were susceptible to phagocytosis, while those cultivated from the blood resisted it.

All this so far supports Bail's conception in that it begins the study of immunity from the point of view of the bacterium. Whether or not the microbe forms chemical aggressins it at least exhibits structural adaptations which enable it to defy the defence of the body. It may be that the converse proposition is justified and that the body defends itself against the bacterial poison by some analogous structural adaptation ; hypotheses of immunity on such lines have been suggested by Sauerbeck, Wassermann and Citron, and others.

Such are the chief views as to the immunity of the body against bacterial invasion which have been held during the past quarter of a century. There are indeed others, but they are too novel or too little supported by facts to be included in this short historical survey. I may add that, during the last few years, an altogether new fact has also been brought to light, evidently closely connected with immunity, and so far only explained by chemical hypotheses. I refer to the phenomenon of supersensitisation to alien proteins, known as "*anaphylaxis.*" No intelligible theory of immunity has yet been founded upon the phenomenon, and I mention it here only because I shall have later to describe my own observations on it in relation to the behaviour of the leucocytes. I shall enter fully into the subject in another lecture.

Summary and Criticism.

I must now ask your permission to look back over the history which I have just sketched, and consider how much solid nucleus of established fact may, at the present day, be held to have condensed from the nebula of conflicting opinion about immunity.

We have seen that the first theory of active bodily defence was that of phagocytosis pure and simple, unconditioned by

any humoral adjunct. The discovery of the power possessed by the higher animals of forming antibodies reacting, not only against bacteria, but against foreign cells of all descriptions, and even against foreign proteins and bacterial toxins, threw the simpler explanation of Metchnikoff into the shade, and humoral theories of defence reigned almost supreme for 10 or 15 years.

It is fatally easy to explain a newly observed property by assuming a hitherto overlooked substance in the serum. I do not for a moment desire to cast any doubt on the truth of the fundamental propositions of lytic action: they are most firmly established. But it is plain that a school of thought which translates the observed properties of a fluid into terms of personified entities for which there is no actual chemical evidence is not without its scientific dangers. Not till antibodies and their antigens have been brought under the dominion of weights, measures, and chemical formulæ, not until their chemical and physical properties have been ascertained by methods lying outside biology, shall we be able to feel sure that we are dealing with things and not phantoms. In other words, the solid and final advance in our knowledge of humoral immunity must come from the development of physical chemistry. To explain biological facts by a transcendental pseudo-chemistry may be useful enough as a working hypothesis so long as we are content to recognise it as such.

Metchnikoff's view was essentially based upon the facts of natural immunity, while humoral theories have been chiefly derived from the study of the acquired property. Metchnikoff, again, relies mainly upon what is observed to take place within the living body; the facts of the humoral school are chiefly based upon the behaviour of the serum in experiments carried on outside the body. There is much to be said against the assumption that the serum of shed blood necessarily corresponds in its properties with the fluids of the living body. The gulf is not one to be lightly bridged. Could it be shown that the observed facts of immunity corresponded in all cases with the properties of the serum outside the body no serious difficulties would arise. But this is not the case. The instance of anthrax is one often quoted: rabbit's serum is bacteriolytic for the bacillus, yet the animal is susceptible; the hen is immune, yet its serum has no bacteriolytic power against the bacillus. The doctrine of humoral antibodies, as Sauerbeck has urged, is not quite the same thing as the doctrine of immunity; the phenomena of the test-tube will not explain all that we see in the living body.

The established fact which emerges from all the humoral observations of the last decade and a half is that there exists a restricted group of foreign chemical substances to the presence of which the animal body responds in a special manner. This group—the so-called "antigen" group—is

by no means synonymous with the group of poisons: the simpler chemical poisons are not antigens, and many substances, in themselves harmless, may nevertheless act as such. The group includes proteins of all kinds foreign to the affected organism, including bacterial proteins; it further includes bodies of unknown but certainly complex composition, such as enzymes and bacterial toxins, together with certain other animal and vegetable toxic agents. The response of the body when such substances are introduced into it in suitably small doses is delayed for a period of some days or weeks. After this needful incubation period the serum is found to possess a new property—that of reacting with the antigen in such fashion as to neutralise it. The action may be simple and direct, as in the case of toxin and antitoxin, or it may be more complex, involving two distinct properties, one of which is abolished by heating for half an hour to 55° C., while the other resists higher temperatures.

These are demonstrable biological facts which admit of no doubt or cavil. They are naturally, and probably rightly, interpreted as indicating the formation of chemical substances having the specific power of neutralising the antigen, chemically or physically. How and where they are formed, and how they interact with the antigen, is still a matter of speculation. By far the most ingenious explanation is that offered by Ehrlich's side-chain theory. There is, indeed, no other theory worth mentioning. We may well believe that the processes of cell chemistry are of excessive complexity, but it must be confessed that when, to the side-chain theory, there are added all the accessory hypotheses and corollaries which seem requisite to explain all the observed facts, we are rather staggered in our allegiance.

And when we further consider the relation of all this to the actual facts of immunity, we must feel somewhat sceptical as to the adequacy of these purely humoral explanations. Except in a few isolated cases there is little evidence as to the extracellular destruction of bacteria within the body by humoral agencies. There are certain bacteria which seem susceptible to such agencies; such are the cholera vibrio and perhaps the typhoid bacillus, but these are exceptions to the rule.

Modern opinion hence has during the last few years tended to swing back towards Metchnikoff's doctrine; not, indeed, in its original simplicity, but in the sense that the ordinary means by which invading bacteria are destroyed is phagocytic in essence, conditioned by the presence of a humoral element. The opsonin theory and the aggressin theory accept phagocytosis as their basis, and we may regard an opsonin and an anti-aggressin as much the same things under different names. Numerous and somewhat conflicting attempts have been made to identify opsonins with lytic antibodies. The thermolabile opsonin of normal

serum is identified with complement by Muir and Martin, Gruber, and others. The thermostable opsonin of immune serum may be identical with an ordinary lytic amboceptor, as Dean has argued. Sauerbeck suggests that opsonic action and lytic action may be different degrees of the same process. To enter into the arguments would lead me too far from my subject. It is certain that the means of defence against bacterial invasion is not in all cases identical. The body employs different means according to the nature and properties of the attacking organism.

Immunity and Evolution.

If I may venture to express the view of immunity which appeals to me I shall do so from the standpoint of evolution. An infective disease represents a struggle between the invading microbe and the body of its host. Each party in the conflict possesses its own weapons of offence or defence, specially adapted to counteract those of the other party. In my former lecture before this College I endeavoured to indicate the probable course of evolution in a conflict of this sort. I compared the gradually increasing complexity of the situation to a game of chess, in which the bacteria had always been the attacking side. It is reasonable to assume that at the earliest dawn of infective disease upon this earth the invaders, probably accidental ones, were unprovided with any special weapons of offence, and were no more to the host than any other inert food particles. To repel such casual intruders the body required no special means of defence. The process of phagocytic ingestion followed by intracellular digestion probably sufficed. Intracellular digestion is the primordial form of that function; seen to perfection in the amœba, it is still carried out by the mesoblastic cells of animals higher in the scale. Such, I take it, must have been the germ from which the battle of infective disease originally sprang. The primitive means of bodily defence must almost inevitably have been phagocytic. It must have included the two factors of *ingestion* and of *digestion* by whatever enzyme the cell possessed for its normal nutritional processes. It is possible that in certain cases of infection, as we see it to-day in the higher animals, these factors have become in a measure dissociated. The serum may possess the power of extracellular disintegration of certain bacteria, but it may quite well be that this is due to an active agent which is primarily intracellular. On the other hand, bacteria may be ingested by phagocytic cells without any sign of intracellular digestion; in gonorrhœa the cocci seem to thrive in the phagocytes.

In any case it seems to me impossible to doubt the truth of Metchnikoff's doctrine as the starting point of bodily

defence against bacteria in bygone ages. His studies in comparative pathology show that phagocytosis remains the most essential method of repelling microbic invasion, up to the higher vertebrates. In man himself we see a process fundamentally identical, and still essentially a property of the mesoblast, but long before we reach this point we find various complicating factors introduced into the struggle. These fall naturally into two groups: (1) the development of aggressive weapons by the attacking force; and (2) improvements in the means of defence on the part of the body attacked. It is logical to consider them in this order.

Evolution of the Pathogenic Bacteria.

In my Horace Dobell lecture I endeavoured to trace the evolution of the pathogenic bacteria. Starting from the obligate saprophyte, I suggested the next stage as one of permanent attachment to the surfaces of the animal body, external and internal, still as a saprophyte, but with occasional chances of access to the blood and tissues. Following this must have come a stage at which the bacterium developed the power of taking advantage of these chances and of living for awhile as a facultative parasite. The parasitic power, once acquired, seems in certain species to have become highly developed, so highly in some cases that other modes of life were no longer necessary. Thus must have arisen the obligate parasites, spreading as pathogenic agents from case to case.

What, now, are the means by which the microbe has succeeded in acquiring the power of growth within the living tissues? From the very outset it must have been confronted with the phagocytic powers of certain cells of the body. It is reasonable to assume that the first thing a bacterium had to do in order to invade the living body with any prospect of success was in some way to antagonise the action of the phagocytes. If one wishes to gain access to a pleasant garden guarded by fierce dogs there is more than one way of attaining the end in view. One might put on armour, or by chemical means one might render oneself so physically unpleasant to the animals that they would shrink away in disgust; or again one might poison the dogs. All these things, in varying degree, bacteria seem to have done; we can trace to-day, amongst virulent bacteria, indications of all three methods of antagonising the defensive mechanisms of the body, but we cannot say which method arose first. I have mentioned the formation of capsules by certain bacterial species—structures which, according to many reliable observers, in some way hinder phagocytosis. There is again evidence that certain bacteria or their products exercise a repellent influence upon the leucocytes, a

negative chemiotaxis. This conception lies at the root of Bail's aggressin theory. Again, we know that many pathogenic bacteria produce toxins acting on various cells of the body, sometimes with great severity. Although Bail regarded his aggressins as non-toxic some of his critics do not share this opinion (e.g., Sauerbeck), and it is possible that negative chemiotaxis may represent a minor manifestation of toxicity.

Such seem to be the weapons of offence and defence in the hands of the bacterial army. By their aid the microbe succeeds in eluding the defensive agencies of the body, for it must be remembered that infective disease is a reciprocal affair, and that there is an immunity of the bacteria against the body no less than an immunity of the body against the bacteria.

Improvements in Bodily Defence.

Let me now turn to the opposite side of the conflict and consider the improvements in bodily defence which have been evolved in the higher animals in response to bacterial assaults. I start with the supposition that phagocytosis is a fundamental power of certain cells of the body, adequate for defence against microbes which have no special powers of aggression. It probably still suffices for such bacteria. I have conducted a few experiments in which I have injected into the circulation of animals harmless air and water bacteria, which, being incapable of multiplication at the temperature of the body, may certainly be regarded as devoid of any active powers of aggression. I will give the details of these experiments in due course. Here I will only say that the immunity reactions which I have observed in such cases are of trivial extent compared with those excited by more highly specialised pathogenic bacteria. These harmless organisms are so readily destroyed within the body, even when injected in immense numbers, that it would seem hardly worth the body's while to have developed any special means of defence against them.

But as soon as we turn from these necessarily impotent species to bacteria which have acclimatised themselves, as saprophytes, to the physical and chemical conditions obtaining in the animal body, and which have had chances in the past of occasional access to the tissues, we find coming into play factors other than spontaneous phagocytosis. These factors become more and more conspicuous and important in defence as we pass on to those microbes which have developed the power of aggressive parasitism. It may be noted, however, that even in the latter case some traces of spontaneous phagocytosis still survive. In any bacterial culture which is used for an opsonic experiment there are a few weakly and degenerate individuals which are ingested, even in the absence of opsonin. If, for example, we look at

the protocols of the experiments in the original paper of Wright and Douglas the controls with washed leucocytes are not absolute blanks, though the ingested bacteria are exceedingly few in comparison with those taken up in the presence of serum; I take them to represent decadent bacteria devoid of any powers of resistance. I may further refer to the experiments of Rosenow, who found that pneumococci lost their power of resisting phagocytosis on prolonged culture. In one strain, long cultivated and wholly non-virulent, he found that phagocytosis took place freely in absence of opsonin. Spontaneous phagocytosis—i.e., phagocytosis occurring in absence of any humoral adjunct—seems still to occur, according to the testimony of many observers, where a pathogenic organism has reverted to complete impotence; in any culture there are likely to be some individuals in this condition.

But although this is so, it is certain that, in the struggle against those pathogenic organisms whose invasion of the body causes its commoner and more serious infective diseases, spontaneous phagocytosis plays little or no part. It is amply proved by the work of those who have founded and developed the opsonic doctrine of immunity that in experiments outside the body a humoral element is needful to act upon the bacteria before phagocytosis can take place. It is impossible to prove this point in the same way under the conditions occurring in the body itself, but there is every reason for the belief that experiments *in vitro* here, at least, afford safe ground for arguing as to what obtains *in vivo*. The property which is personified under the term opsonin would appear to have become an integral part of the means of defence against pathogenic bacteria.

I conceive this factor to be the chief improvement which evolution has effected in the primitive means of phagocytic defence. At first a function of the mesoblast, as a whole, it would seem that phagocytosis, so far as the destruction of bacteria is concerned, has chiefly been relegated to the polynuclear leucocytes. This is the explicit opinion of Metchnikoff, and it accords well with observed facts both within and without the body. I know of no evidence that the actual processes of ingestion and digestion have undergone any striking change in the course of evolution. The modern polynuclear leucocyte may be more active and efficient than its remote mesoblastic ancestor, but I think that is the most that can be said.

There is, however, a further respect in which it is reasonable to assume that improvement has been effected. We are entitled to believe that the polynuclear phagocytes have developed increased powers of scenting their prey from afar. The evolution of a closed blood vascular system has vastly increased the readiness with which the wandering cells can be concentrated on any given spot. The mechanism by which this is accom-

plished seems to be a physico-chemical one. In presence of a local tissue attack by certain micro-organisms, and notably by the pyogenic cocci, we find that the polynuclear leucocytes tend to converge upon this spot from all over the body, and this with truly remarkable rapidity. So prompt is the reaction, so obviously purposeful its aim, that it is at first sight difficult to believe it anything but an intelligent response on the part of the active agents. When the police assemble to suppress a street disturbance, we naturally attribute the fact to intelligent action on their part, though even here we are apt to overlook the discipline and routine which really render the phenomenon almost mechanical. We cannot, however, seriously attribute conscious and intelligent action to the leucocytes. I do not quite know why, because I do not know what consciousness and intelligence really are, but the tendency of modern science is to seek for the most prosaic explanation of all observed facts.

In this case a sort of physico-chemical explanation is forthcoming. It is believed, on good experimental grounds, that cells or syncytia capable of amœboid movement are governed, as to the direction in which they move, by chemical stimuli. This is the doctrine of *chemiotaxis*, established by Pfeffer, Stahl, and others. One chemical substance may attract the mobile cell, another may repel it. Chemiotaxis may be positive or negative. Precisely how the chemical substance acts is a matter of speculation, but there are experimental grounds for supposing its action due to changes in surface-tension. Certain bacteria are observed to exert a positive chemiotactic action upon the polynuclear leucocytes; in the case of other bacteria this action is lacking or is replaced by a negative chemiotaxis. It is assumed that those infections which are specially associated with a dense congregation of polynuclear leucocytes—e.g., suppurative processes—owe this peculiarity to a specially intense positively chemiotactic influence on the part of the infecting organism or its products. In presence of a local pyogenic infection the leucocytes are attracted from the blood passing through the neighbouring vessels and are to be seen in active emigration into the affected focus. More than this, the assumed chemical influence, wafted by the blood-stream, appears to attract out into the current the reserves of leucocytes present in the bone marrow, explaining the circulatory leucocytosis observed under such conditions.

All this is commonly admitted and taught; my only reason for referring to the matter lies in its relation to the process of evolution. It is inconceivable that the bacteria should have gone out of their way to develop chemical substances attractive to their hereditary foes. The man who hates cats does not spread valerian root in his back garden. But it is readily conceivable that the more mobile phagocytes should have become educated to respond to the chemical aroma of

the natural foes of the body. On such lines it is intelligible that the phagocytic reaction against intruding microbes may have become specialised and improved, and such considerations may perhaps explain why this reaction has been relegated to the mesoblastic cells specially able to respond to chemiotactic influence.

Development of Humoral Adjuncts to Phagocytosis.

These speculations concern only spontaneous phagocytosis. I must next consider how that important adjunct to the process—opsonic action—can have arisen. Here I can be very brief, for the opsonic school has not yet furnished an evolutionist to trace back the process to its earliest beginnings, as Metchnikoff has done for the more central factor of phagocytosis itself. Such a research would be full of interest, and had the time at my disposal been longer I should have attempted it. But as matters stand we are wholly ignorant of how far down the animal scale opsonic action can be traced, and it would be futile to speculate on its origin. There are, nevertheless, certain fundamental facts which cannot be overlooked.

If we believe spontaneous phagocytosis to have been the primitive means of defence, and if it be conceded that in the evolution of the processes of infective disease the bacteria have always been the attacking side, it is difficult to avoid the conclusion that the more highly developed pathogenic bacteria, against which alone opsonic action has hitherto been adequately studied, have acquired the power of neutralising spontaneous phagocytosis, presumably by developing some chemical substance repelling the phagocytes. At first sight this may appear to contradict what I said just now in considering the education of the leucocytes to respond by a positive chemiotaxis to the presence of bacteria. But we are considering two different things—spontaneous phagocytosis and opsonic action. I do not find it illogical to conceive that certain inherent products of the natural bacterial body may have become positively chemiotactic to the leucocytes—thus explaining the facts of spontaneous phagocytosis—while believing that the more highly specialised parasites have secondarily acquired the power of producing another chemical substance which may keep phagocytosis in abeyance. It would be this substance which confers "virulence," and it would be an "aggressin" in Bail's sense. The inverse relation between virulence and opsonic action compels us to admit something of this kind, and I submit that the first move in this game must have come from the bacteria. So far, I consider the aggressin theory justified, and it may be conjectured that an opsonin

is a substance which antagonises the aggressin and permits the normal positive chemiotactic influence of the bacterial body upon the leucocyte to resume its sway.

We have next to ask how and in what connexion did these humoral adjuncts to phagocytosis come to exist. If we believe an opsonin to be a substance apart, having nothing to do with the lytic antibodies, we cannot guess its primitive origin till we have the data as to its presence or absence in the various lower animals. But if it be conceded that opsonic action is probably only a newly recognised manifestation of antibodies already known (and there is increasing reason for this belief) we may with some probability trace it back to the phenomena of primordial cell nutrition. This conception of humoral antibodies has been developed by Metchnikoff to some extent, but in far greater detail by Ehrlich, in whose hands it became the very basis of the side-chain theory. The substances capable of acting as antigens are foreign proteins or allied substances, of a kind which must have served as food from the beginnings of animal life. It is conceivable that the simple processes of intracellular digestion by enzymes may have become elaborated in the course of time and in the higher animals into a response to the presence of all food-like substances, even when these are harmful, by which the primitively digestive secretions act as neutralising or destructive antibodies. These may be liberated from the attacked cell by disintegration, as Metchnikoff's idea of phagolysis suggests, or may be cast off from it by over-production, due to over-stimulation, as Ehrlich would have it. In any case, the result would be a humoral antibody with the property of disintegrating and destroying, or at least injuring or neutralising, the antigen in question.

Ehrlich and Metchnikoff are at one in this, at least, that they trace back their conceptions of immunity to the phenomena of primitive cell nutrition. Ehrlich lays stress on the chemical and digestive aspects of the process, Metchnikoff on the mechanical fact of ingestion. We may candidly recognise the value of both factors in bodily defence against bacterial invasion and well believe that the body has, in the course of evolution, exploited every means in its power to antagonise the dangers which threaten it. In certain cases the effective means may have been purely chemical, and an antitoxic immunity in Ehrlich's sense may be the essential defence. In other cases the defence may be a purely phagocytic one, but this is nowadays rarely an unconditional and spontaneous phagocytosis, because the attacking agents are as a rule provided with aggressive powers. These require to be antagonised before phagocytosis can be accomplished, and to this end the body employs one or another of its lytic antibodies in what we term opsonic action. In exceptional cases the lytic antibodies can defend the body apart from direct cellular coöperation, but such

immediate bacteriolytic action is seldom seen in the living tissues, though it can be produced in certain cases *in vitro*.

On some such lines as these I can dimly foresee a simplification, though not a unification of the present conflicting doctrines of immunity. I can believe them all traceable in their origin to the ingestion and digestion of a casual bacterium by an amœba. But I can believe this elementary process to have been elaborated in various ways into several different mechanisms of defence, some suited to antagonise one, some another kind of microbic assault. And it would appear that the body now employs whichever weapon is best suited to the immediate end in view, or which it happens to have at its command. Looking at the facts at present established, it appears that the essentially antitoxic means of defence is limited to a very small number of pathogenic organisms, and that the essentially bacteriolytic means of defence is equally limited in its application. In the great majority of cases the defence is in its essence phagocytic, but dependent upon opsonic action, an opsonin being a substance which neutralises the agency by which the bacterium attempts to ward off phagocytic attack.

Adequacy of Defensive Agencies in Normal Conditions.

Whether or not this imperfect sketch of the means by which the warfare of the body with the bacteria which invade it is carried on, and of the stages by which it has been evolved in the past, is a true one, it is clear that the defensive agencies of the body are on the whole very adequate under normal conditions. We see the cutaneous and mucous surfaces of the body swarming with bacteria, many of which are potential parasites, yet health is, as a rule, undisturbed. And this is not because the bacteria do not gain access to the tissues. They do so to a degree which we are only beginning to realise. It is commonly taught that the normal organs and tissues are sterile, although the lungs and lymphatic glands are admitted to contain bacteria. Conradi has, however, recently shown that this is by no means the case; bacteria are not uncommonly present, though in such small numbers that special methods are required for their detection. He examined 162 portions of tissue taken fresh from the slaughter-house in oxen and pigs. The portions of tissue were rigidly sterilised externally by means of oil at 200° C., and then in 2 per cent. mercuric chloride. After this they were incubated at 37° C. for 20 hours in a sealed moist chamber, when cultures were taken from the interior of the mass. Of the 162 pieces of tissue examined no less than 72 yielded bacteria. In the case of the lungs 80 per cent. yielded growth; in the liver, 66 per cent.; while the kidneys and muscles showed growth in 31 and 30 per cent. of the

portions examined. The bacteria found were in order of frequency: B. coli communis, B. lactis aerogenes, Streptococcus acidi lactici, B. mesentericus, B. fluorescens non-liquefaciens, Diplococcus pneumoniæ, &c., pointing plainly to the intestine as their main source. We must give up the idea that the saprophytes of our mucous surfaces only occasionally gain access to the tissues; they evidently do so frequently, but they are present in the tissues in extremely sparse numbers, because the defence of the body is so good that they are done to death in a very short time.

More than this, bacteria, if not of marked virulence, can be artificially injected into the peritoneum or into the blood stream in immense numbers, with no disturbance of health and no inflammatory reaction. As regards the peritoneum, we know from the observations of Grawitz that even pyogenic organisms, unless of a virulent strain, can be injected without setting up peritonitis, provided that the volume of fluid in which they are suspended does not exceed the absorbent powers of that cavity. I shall have later to describe certain experiments in which I injected living bacteria into the circulation in rabbits, in doses of several hundred millions, and watched their disappearance from the blood stream by taking measured samples of the blood for culture every two minutes. Even the pneumococcus in full virulence fell very rapidly in numbers till in a quarter of an hour they had fallen to about one-tenth of the number first observed: harmless organisms disappeared even more rapidly. The disappearance of bacteria from the blood stream does not of course necessarily mean their destruction, but in the case of harmless bacteria the animals remain in perfect health, and there can be no doubt that disappearance from the blood is followed by destruction.

Lines of Defence against Bacterial Invasion.

It seems therefore clear that the defensive mechanisms of the body in health are not only fully adequate to deal with the bacteria which gain access to the tissues from the intestine and elsewhere, but that much larger numbers of harmless bacteria can be disposed of with equal readiness. Only when the resistance of the body is lowered by what we term the "predisposing causes" of disease, or when the strain of microbe gaining access is one of special virulence, is this normal equilibrium upset. Under ordinary conditions the work of defence ceaselessly goes on without any obvious fuss or disturbance. I have elsewhere spoken of this as the *first line of defence* against bacterial invasion.

But when this first line breaks down, and bacteria begin actually to multiply and hold their own in the tissues, leading to infective disease, a new phenomenon appears. A *second line of defence* is brought into play in which the resources of

the body are concentrated upon the area attacked, should this be localised, as is usually at first the case. The work of defence is now by no means inconspicuous and tranquil; the chief clinical symptoms of the disease depend upon it, for it is the process which we term *inflammation*. The presence of a closed vascular system enables the body to concentrate upon any desired point both the leucocytes and the humoral elements which may be contained in the blood. I need not here insist that the phenomena of acute inflammation are essentially vascular, and that the outpouring of fluid and the emigration of leucocytes are its central features. It is true that acute inflammation is not necessarily of bacterial origin; it is a response to injury. But the commonest bodily injuries are microbic in origin, and there are few bacterial invasions which are not associated with inflammation. We now justly look upon inflammation as a defensive and reparative process—a means of defence brought into play, and usually with success, where a bacterial invasion threatens the body. Its actual weapons are not, perhaps, different from those employed in the daily extermination of casual invaders, but its clinical features depend upon the acute local vascular phenomena which enable these weapons to be concentrated upon the threatened area. Where the invasion is from the first general and the microbes multiply in the blood stream itself, we have a septicæmia in which the phenomena of inflammation are absent or scattered over various areas. This is commonly a fatal condition, but there are few septicæmias which are not secondary to a primitively local infection. Should the second line of defence be absent or fail, the condition of the patient is a grave one indeed.

There is another factor in the second line of defence which I will only mention here, because it lies outside my immediate subject—namely, the rise in bodily temperature which habitually attends acute infections. It is still *sub judice* how far fever is to be regarded as a defensive mechanism of the body, but there is much to be said in favour of such a view. It is clear that true fever, as distinguished from mere hyperthermia, is in the main associated with bacterial invasion, and there is evidence that a raised bodily temperature is inimical to pathogenic bacteria, attenuating their harmful properties.

I fear that in this lecture I have gone somewhat beyond the limits of my central theme. But it is plain that one cannot attempt to criticise the part played by leucocytes in immunity without reference to doctrines of immunity in general. I have tried to look at these from an independent and unbiassed point of view, though I am only too conscious of the imperfections of my sketch. In the following lectures I shall concentrate my attention upon the leucocyte and its behaviour in infection and immunity.

LECTURE II.

Delivered on June 16th.

MR. PRESIDENT AND GENTLEMEN,—In the preceding lecture I attempted a short sketch of the chief doctrines of immunity, and I indicated my own conviction that the primitive means of defence lay in phagocytosis, and that this process still remains the main element in such defence, even in the higher vertebrates. So far as bacteria are concerned the chief phagocytes are admitted to be the polynuclear leucocytes, and it is to these and to their behaviour in the body under conditions of infection and immunity that I have now to draw your attention.

I wish that I could deal equally fully with the other kinds of leucocyte. That the lymphocytes, the eosinophils, and the basophils have some duties to perform in connexion with bacterial infections is not improbable, for they may exhibit remarkable fluctuations in infective conditions. But we know almost nothing of the meaning of these fluctuations. I shall have in some cases to record them, but I can do little more.

The leucocytes of the blood are, by general consent, specialised representatives of the primitive wandering cells seen in the body cavity of the lower invertebrates. The idea that the different types of leucocyte in normal mammalian blood are merely stages in the development of a single kind of cell is a defunct hypothesis. It is now clearly recognised that the lymphatic system provides one kind of cell—the *lymphocyte*—ungranulated to ordinary staining methods, though not to all, which seems specially associated with chronic inflammatory processes, and according to many observers is related to the plasma cells, and through them to certain reactions on the part of the fixed tissues. The bone-marrow, on the other hand, apart from such tiny foci of lymphoid tissue as it may contain, supplies the blood with at least three kinds of leucocyte, distinguished by the possession of specific granulations in their protoplasm—oxyphil, basophil, and neutrophil. The parent cells of these, as seen in the bone-marrow, are mononuclear, and are known as *myelocytes*. The adult cells, as seen in the blood, have a lobed or twisted nucleus, often apparently separated into distinct masses. The more powerful nuclear stains commonly reveal threads of chromatin connecting the separate masses, and hence the term "*polymorphonuclear*"

is more correct than "*polynuclear*." The polymorphous character of the nucleus is chiefly conspicuous in the neutrophils of human blood, and when one speaks of polymorphonuclear cells it is these which are meant. In other mammals the granulations in the protoplasm of these essentially polymorphonuclear cells are not necessarily neutrophil. In the rabbit, with which animal all my experimental work has been performed, they are "amphophil," staining both with acid and basic dyes. But the amphophil leucocytes of the rabbit are the strict homologues of the neutrophil polymorphonuclears of man, and for convenience I shall refer to them simply as "polynuclears."

Functions of the Polynuclear Leucocytes.

Let me turn now to the "polynuclear" leucocytes, neutrophil in man, amphophil in the rabbit, and consider what we know of their functions. They are the most actively mobile of the cells in the body and seem to possess in special degree the property of being attracted or repelled by chemical stimuli. They are, further, the most actively phagocytic cells, and this function is pre-eminently displayed in relation to bacteria. They are the "microphages" of Metchnikoff. There is ample evidence that in most cases they are able to digest and destroy the microbes which they take up, and they must do so by the aid of a digestive secretion provided by their own protoplasm. This is not the only enzyme with which these leucocytes appear concerned; there are those who maintain that the "thrombo-kinase" or activating element in the formation of the fibrin ferment is derived from them. Ainley Walker has, indeed, suggested a general analogy between enzymes and bacterial antibodies. He brings forward evidence with regard to several enzymes that when inactivated by heat they can be reactivated by a "kinase" which may be complement under another name, and traceable to the leucocytes. As regards the humoral elements in defence against bacteria there is ground for the belief that an element, at least, of these is of leucocytic origin. Buchner, in the earliest of the humoral theories, was inclined on experimental evidence to trace his "alexin" to the leucocytes. The modern alexin—i.e., the "complement" of Ehrlich, which seems to be the "microcytase" of Metchnikoff, and, according to some, the opsonin of Wright (at least the thermolabile opsonin of normal serum), is with considerable probability to be traced to these cells. The evidence is, of course, circumstantial, but as a working hypothesis we may believe that the leucocytes furnish complement either as a secretion or by their disintegration. If so, we must look upon them as incomparably the most important cells in the body as regards defence against bacteria,

for not only are they the chief mechanical agents in phagocytosis, but they furnish also a fundamental element in humoral defence. Whether we are disciples of Metchnikoff or Ehrlich, the polynuclear leucocyte stands out as the cell pre-eminent in defence.

The Distribution of Polynuclear Leucocytes in the Normal Body.

I do not know that any physiologist has attempted to determine the distribution of this cell in the normal body. In the course of the experiments I am about to describe I desired information on this point; the only paper I have found which mentions the subject is one by Bruce.[2] This observer, studying the leucopenia which follows the injection of peptone into the circulation in the rabbit, examined the distribution of the polynuclear cells in the organs of two normal rabbits, finding them chiefly abundant in the spleen and in the lung. It is now generally admitted that the red marrow of the bones is the sole normal birthplace of the polynuclear leucocyte. It is well known that in its mature form it is the most abundant circulating leucocyte in the blood. It is conjectured that its grave may be the spleen. At the time when I began to investigate the subject I was unaware of Bruce's paper, but the methods which I devised are substantially identical with those he used, and my results are in harmony with his. I have, however, endeavoured to obtain data expressing the number of polynuclear leucocytes per cubic millimetre of tissue.

The rabbit lends itself with particular ease to the investigation. The amphophil granulations in its polynuclear leucocytes are large and conspicuous, and in sections stained with Ehrlich's triacid stain these cells stand out so plainly that they cannot be overlooked or confused with other cells. My method has been as follows. A healthy normal rabbit is killed either by a blow on the head or better by the intravenous injection of a small dose (½ cubic centimetre of a 5 per cent. solution) of potassium cyanide. The moment it is dead the various tissues and organs are removed and placed in 10 per cent. formalin. After one or two days appropriate blocks are cut and soaked for 24 hours in normal (0·9 per cent.) saline solution. Each block is next measured in millimetres as regards its three diameters, and then passed, through weak and strong alcohol, into xylol and lastly into paraffin. Before the final embedding in paraffin measurements of the three diameters are again made and recorded. The object of these measurements is to determine the degree of shrinkage of the blocks in the process of

[2] Proceedings of the Royal Society, vol. lv., 1894

embedding. The measurements of the three diameters of the fresh hardened block are multiplied together and divided by the product of the three similar measurements of the shrunken block ready for embedding in paraffin. The result is a figure by which, when the figures obtained from the stained section are divided they are reduced to terms of fresh tissue. In the case of most organs, lung, liver, spleen, kidney, &c., I find this factor approximately 2. If one is careful to leave the block for not more than two or three hours in melted paraffin it shrinks to about one-half of its cubic content when fresh.[3] In the case of most organs I am content to accept 2 as an average shrinkage factor without the trouble of actual measurement in each case. With the bone marrow this is not so, as the varying fat content makes the shrinkage much more variable; I have obtained shrinkage factors from 1·23 to 11·65, and I always take measurements in each case.

Sections are cut in paraffin in the usual manner and are then stained for from one to three hours in Ehrlich's triacid stain, washed carefully in water and in alcohol, cleared in xylol, and mounted in balsam. If, now, one knows the superficial area of a given microscopic field, and the thickness of the section—data which are easy to obtain—it is clear that one can count the numbers of any sufficiently conspicuous cell in the known volume of tissue seen in the field, and go on to find the average number of many fields. As a rule, the whole field offered by a 1-12th inch oil immersion lens is too large for counting, besides being out of focus at the edge. Messrs. Leitz have made for me a metal disc with a square hole in it. This, when placed in the eyepiece of the microscope, restricts the field to a convenient area for counting. Having obtained the average of a sufficient number of fields (60 to 100), the numbers of the cell in question can be calculated per cubic millimetre of tissue, and this figure divided by the shrinkage-factor gives the number per cubic millimetre of fresh tissue. The method, which I first used in the case of bone marrow,[4] cannot pretend to absolute accuracy. One is apt to count fragments cut off tangentially from cells belonging to adjacent sections, so that no cell should be counted which does not present a nucleus. Nevertheless, I find it a method capable of sufficient accuracy to enable a reasonable comparison to be made between different organs or between the same organs in different animals.

Applying it to the tissues and organs of two normal rabbits, I obtained results which were concordant in the two animals, and which showed very remarkable differences in the number of polynuclear leucocytes in different tissues.

[3] Actual figures are:—For lung: 2·01, 1·72, 2·13, 2·27, 2·07, 2·12; for liver: 2·18, 1·93, 2·11; for kidney: 2·33, 2·14, 2·96; for spleen: 2·26, 2·15, 2·15; for brain: 1·61.

[4] See Local Government Board Reports for 1907–08

In two tissues—viz., the spleen and bone marrow—it is impossible to state the number of *polynuclear* neutrophiles with accuracy, for a different reason in each case. In the spleen I find them absent from the lymphoid tissue of the Malpighian bodies, but extremely abundant in the spleen pulp. Here, however, they are in various degrees of disintegration. The opinion that the spleen pulp is the grave of the leucocytes is fully confirmed from my observations; in no other tissue have I found these disintegrating leucocytes. I find in the spleen every gradation between the perfect cell and amorphous masses in which the neutrophil granulations have fused together and are evidently breaking down. The eosinophil cells seem to break down into similar masses. In the counts which I give below I have tried to include only such polynuclear neutrophils as were still clearly recognisable. In the bone marrow, on the other hand, the difficulty is to distinguish them from the neutrophil myelocytes. In other tissues one recognises them by their specific granulations, for the nuclear staining with Ehrlich's triacid stain is by no means deep. I can only give the total neutrophil count for the marrow, including both myelocytes and adult polynuclears. From a series of eight or ten normal rabbits I find this count at the level of the middle of the femur, to lie anywhere between 100,000 and 200,000 neutrophil cells per cubic millimetre of fresh marrow tissue, and of these the majority are certainly myelocytes.

For tissues other than the bone marrow I have obtained the following results and they are borne out by those which I shall presently relate, derived from abnormal animals.

Tissue.	Polynuclear leucocytes per cubic millimetre of fresh tissue.	
	Rabbit 1.	Rabbit 2.
Spleen pulp	68,000	38,400
Lung...	20,000	12,000
Liver	2,900	1,700
Kidney	250	—
Voluntary muscle	—	20
Intestine...	—	0
Medulla oblongata	—	0

It is apparent that even if the method I have used were less accurate than I believe it to be, such variations as these

must be of deep physiological significance. We might be prepared for a great excess of polynuclear cells in the bone marrow and in the spleen; this, indeed, I find. But the differences in the other organs are enormously greater than those which the mere vascularity of the tissue would explain. The lung, the liver, and the kidney are all very vascular organs, and I know of no differences in the diameter of their capillaries such as could explain the variations seen in the above table. A leucocyte does not easily get through an ultimate capillary; one often finds the polynuclears squeezed into an elongated sausage form in the act of passage, but this is seen equally in lung, liver, or kidney.

Failing any mechanical explanation for the discrepancies in the distribution of the polynuclear leucocytes in the various tissues, it must be assumed that there are physiological reasons underlying them. It would seem that when once these cells are discharged into the blood they tend to be arrested in certain situations, either because they have some function to perform there, or because a sojourn there is requisite for their own needs. In such tissues as brain, muscle, and intestine they would appear to have no function to perform and no benefit to gain. They are hence relatively sparse passengers through the capillaries, and are seen hardly or not at all in sections of such tissues. In the more vascular kidney and liver they are seen in larger numbers, but I am unable to offer any conjecture as to the reasons for this fact. When, however, we turn to the lung we find the polynuclear leucocytes present in altogether disproportionate numbers. After the bone marrow and spleen, the lung tissue, though so largely occupied by air, comes far ahead of any other tissue I have examined in respect of the polynuclear leucocytes present in it. Failing any evidence that the narrowness of the lung capillaries mechanically arrests them, one is tempted to assume that they voluntarily tarry in this tissue. I cannot suggest any special function which they have to perform here; if I may speculate I should suggest that they sojourn here for the sake of oxygenation. I shall presently have to show that this accumulation of polynuclear leucocytes in the lungs is much exaggerated in certain pathological conditions. It may be that if the bone marrow is the birthplace of these cells and the spleen their ultimate tomb, while the blood is their means of transit, the lung may serve as a week-end at the seaside, where they may recuperate their energies. I cannot otherwise explain the accumulation of polynuclears in the pulmonary tissue.

The Circulating Leucocytes.

My experimental work has all been carried out on the rabbit—an animal which is convenient for the purpose on account of the readiness with which blood can be obtained

from the ear.[5] In studying the effects of infections and of vaccines, I have in most cases used the intravenous method of inoculation on account of the rapidity and intensity of the changes induced in the blood when the living or dead bacteria are introduced directly into the circulation. I have as a rule used fairly large doses of bacteria, desiring to produce marked and unequivocal results. By a fairly large dose I mean one or two hundred million bacteria; by a very large dose I mean a thousand million or more. The drawback to this method is that it has little parallel with naturally occurring disease in man or animals; it is only rarely that the body has to respond to the sudden irruption into the blood stream of large numbers of bacteria. In estimating the significance of the results I have obtained this discrepancy must be borne in mind. It is, I think, far outweighed by the simplicity and rapidity with which the effects of the dose are determined. My main object was to study the varying leucocytic response to the presence of bacteria of different kinds, and for this the method sufficed very well.

I employed ordinary hæmatological methods in the work and I have striven to make it as complete and accurate as possible. The complete study of the changes in the circulating leucocytes after a given intravenous dose of bacteria is very laborious on account of the frequent counts which it entails. The immediate effect of the injection is often precipitate and the blood must be examined every few minutes. The routine which I ultimately came to adopt was as follows. A drop of blood is withdrawn from the rabbit's ear to obtain the initial data. The dose of bacteria is then injected, and blood counts made after 1, 2, 5, 10, 15, and 30 minutes; again at the hour, and thenceforward hourly for 5 to 7 hours or more. On the following day two or three counts are made, and thenceforward once or twice a day till the animal is well again or dies. Each time the blood is counted films are

[5] This is a suitable place to state the characters of the leucocytes in the rabbit. The average number of these cells per cubic millimetre of blood is not very different from that seen in man, being a little less than 7000. The range of variation in different normal rabbits appears wider than in man, so that in some counts of 15,000 and even 20,000 may be met with, while in others the count may be 2000 or 3000. In my experience these differences depend more on fluctuations in the lymphocytes than in the polynuclears. The lymphocytes are more numerous than in man; on an average they form 50 per cent. of the total, but they may reach 70 per cent. or more. The average number of lymphocytes per cubic millimetre is rather more than 4000. The cell which corresponds to the human polynuclear neutrophil bears so-called "pseudo-eosinophil" or amphophil granulations. These cells form on an average 40 per cent. of the total leucocytes—less than 4000 per cubic millimetre. The eosinophil cells are fewer in the rabbit than in man, forming less than 1 per cent. of the total. The basophil cells are, on the contrary, much more numerous than in man, though very variable in numbers. I have known them to form over 20 per cent. of the total leucocytes in an apparently normal rabbit; the average is 6 or 7 per cent.

made and stained with Leishman's dye; a differential count of 200 or more leucocytes is then carried out at leisure. It thus becomes possible to state the actual numbers of the different kinds of leucocyte present per unit of peripheral blood throughout the course of the experiment, and to represent them in curves on graphic charts. I cannot too strongly insist on the advantages of this method of procedure. Unless differential leucocyte counts are made, important events may be overlooked; a slight but definite polynuclear leucocytosis may be completely masked by a lymphocytic leucopenia. Further, the differential counts afford a valuable check upon the accuracy of the total counts: the lymphocytes and the polynuclears do not vary together as a rule, and when one finds their curves parallel suspicion may justly be aroused as to the correctness of the total counts upon which these are based.

It is not so easy as it might appear to make accurate total counts of leucocytes from a rabbit's ear. I have been compelled to make more than one special set of observations to determine the existence of fallacies; the chief fallacy is that of local leucocytosis. I commonly shave the animal's ear before the experiment begins, as it is thus easier to collect the drop of blood, but I find that the act of shaving induces a local leucocytosis which may easily vitiate the results obtained. Half an hour after simple shaving of the ear the leucocyte count from it may be double that obtained from the unshaven ear, owing, I presume, to slight inflammatory changes leading to an accumulation of polynuclear leucocytes in the small veins and capillaries. If the blood flows freely from the needle prick the fallacy is negligible, but if it flows poorly and any squeezing is employed it is a serious one. I now shave the ear a day or so beforehand.

If one has injected into the circulation living bacteria, and especially pyogenic cocci, fallacies may arise from local inflammatory changes in the animal's ears. Some of the injection may escape into the subcutaneous tissues, and even if counts are taken from the opposite ear the earlier needle pricks may permit of the escape of bacteria, which may then induce inflammatory changes. Counts taken from any inflamed area are open to grave suspicion when they show an apparent leucocytosis. After three years' work at this subject I am, I trust, sufficiently alive to the danger of these fallacies to have allowed for them in all doubtful cases.

Some of the investigations which I am about to record have been carried out in the course of researches for the Local Government Board during the past few years, partly in conjunction with Dr. M. H. Gordon and Dr. T. J. Horder. I am glad to have the opportunity for summarising this work, and adding to it the special researches which I have carried out in view of these lectures. It is plain that the labour of the hæmatological investigations I have described is too great for a single observer, and I have much pleasure

in acknowledging the able assistance of Mr. A. E. Gow in my more recent work. I can only regret that I have not had a longer time at my disposal to pursue the interesting lines of research which the work has opened up.

Leucopenia.

I propose first to give an account of the observations which I have made on diminution in the numbers of the circulating leucocytes. Less attention has perhaps been paid to this than to the increase known as leucocytosis. The subject of leucopenia nevertheless offers more than one problem of importance in connexion with immunity.

The number of circulating leucocytes may be reduced in several ways. They might conceivably be destroyed to an excessive degree, but I know of no good evidence of this. They may be withdrawn from the peripheral circulation and accumulate elsewhere; this is well known to occur. Or the supply of leucocytes may become exhausted and there may be a failure to replenish the circulation. There are thus different kinds of leucopenia, but my experiments throw light on only two varieties—the leucopenia of marrow exhaustion, and the leucopenia which appears as an initial phenomenon when a dose of bacteria is introduced into the circulation. I will deal with the latter first, because it has more particularly engaged my attention.

The Phenomenon of Initial Leucopenia.

The importance of this remarkable form of leucopenia is in the main theoretical, for we have no opportunity of observing it in human infections; indeed, the conditions which evoke it can rarely occur in man. It has long been known that an experimental leucocytosis can be induced in animals by the injection of certain chemical substances, notably of certain organ-extracts, of albumose, and of bacterial proteins. It was found, on closer investigation, that this leucocytosis was preceded by a marked diminution in the circulating leucocytes; both the diminution and the subsequent increase were found mainly to concern the polynuclear leucocytes. Löwit, who was one of the first to describe this initial leucopenia, considered that the foreign substance introduced actually destroyed the leucocytes, and that it was the products of their dissolution which evoked the subsequent leucocytosis. Goldscheider and Jacob, and also Bruce, later showed that the decrease in the circulating leucocytes was not due to their destruction but to their accumulation in the capillaries of the internal organs, and especially of the lungs. I know of no important light which

has been shed on the subject since the appearance of Bruce's and of Goldscheider and Jacob's papers in 1894.

I have closely examined the initial blood changes, after the intravenous injection of bacteria, on 30 or 40 occasions mostly by means of differential leucocyte counts. Frequently the counts have been made at intervals of only two or three minutes. The bacteria employed for the experiments have included staphylococcus aureus, streptococcus pyogenes, streptococcus fæcalis, micrococcus citreus agilis, the pneumococcus, bacillus coli communis, the typhoid bacillus, Gärtner's bacillus, the diphtheria bacillus, the tubercle bacillus, the anthrax bacillus, and bacillus fluorescens aureus. These various organisms have been injected both living and dead; the effect as regards the initial leucopenia seems the same in either case.

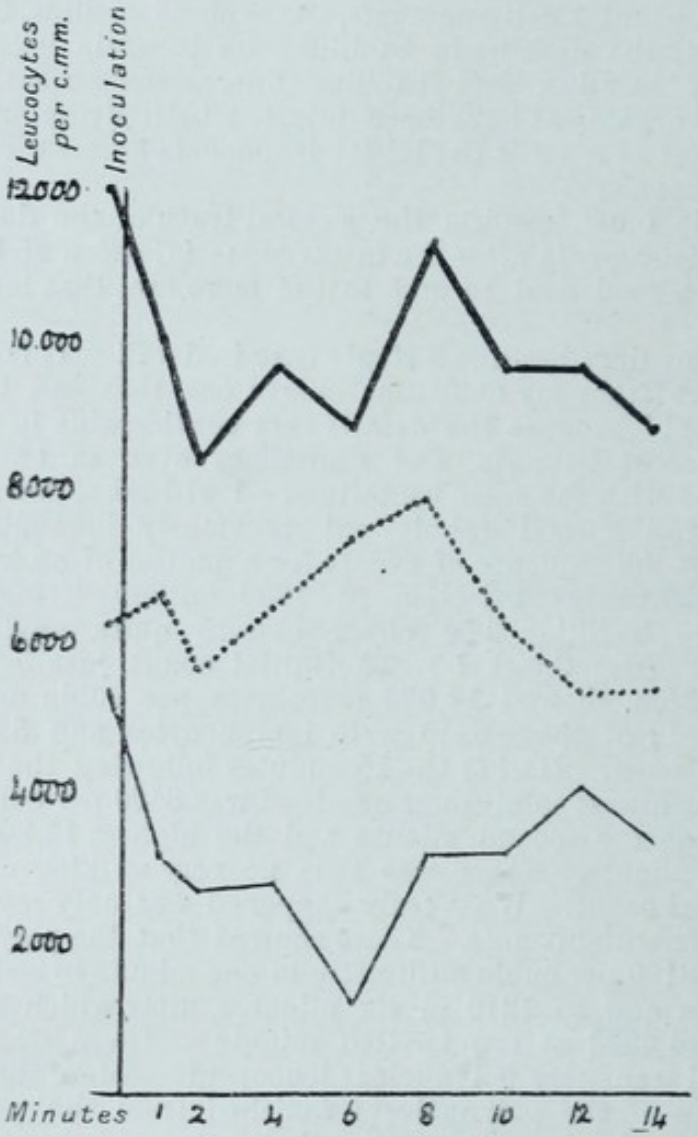
While I can confirm the general truth of the doctrine of initial leucopenia after the intravenous injection of bacteria, I have a good deal to add to the mere fact that leucopenia occurs.

Let me first describe a single case in detail. It is not easy to select from my numerous charts one that can be called "typical" because the details vary considerably in the case of different bacteria, and sometimes even in two rabbits injected with the same bacterium. I will take, however, the case of a normal rabbit not previously immunised and describe the sequence of events for a quarter of an hour after the intravenous injection of 1000 million living bovine tubercle bacilli. The changes were quite moderate in degree. (See Chart 1.) The initial count, just before the inoculation, showed 12,000 leucocytes per cubic millimetre of blood; of these 6240 were lymphocytes and 5340 were polynuclears. During the 15 minutes following the inoculation the lowest total count obtained was 8400 per cubic millimetre at the second minute and the highest 11,200 at the eighth minute. There was thus no very striking change in the total count. What truly happened was only revealed by the differential counts. These showed that the polynuclears fell to 3150 per cubic millimetre in one minute to 2646 in two minutes and to 1276 in six minutes, after which they rose again to 4032 at the twelfth minute. There was thus a marked transitory polynuclear leucopenia. More than three-quarters of the polynuclears vanished from the peripheral circulation in six minutes, though they soon reappeared. In the total counts the fact was masked by an irregular rise in the lymphocytes, which reached 7896 per cubic millimetre at the eighth minute.

I have chosen this case to start with because it illustrates the fundamental fact of initial polynuclear leucopenia, though in a moderate degree, and because it shows the necessity for differential leucocyte counts if that fact is to be appreciated in its minor manifestations. Chart 2 is a much more extreme example of initial leucopenia. It shows

the changes occurring after the intravenous injection—not of bacteria, but of sheep's corpuscles—in a rabbit which had already been immunised against these cells. In this instance the polynuclears actually vanished from the circulation at the tenth minute; in a count of 200 leucocytes lymphocytes were alone found. I now pass on to discuss the matter more fully.

CHART 1.

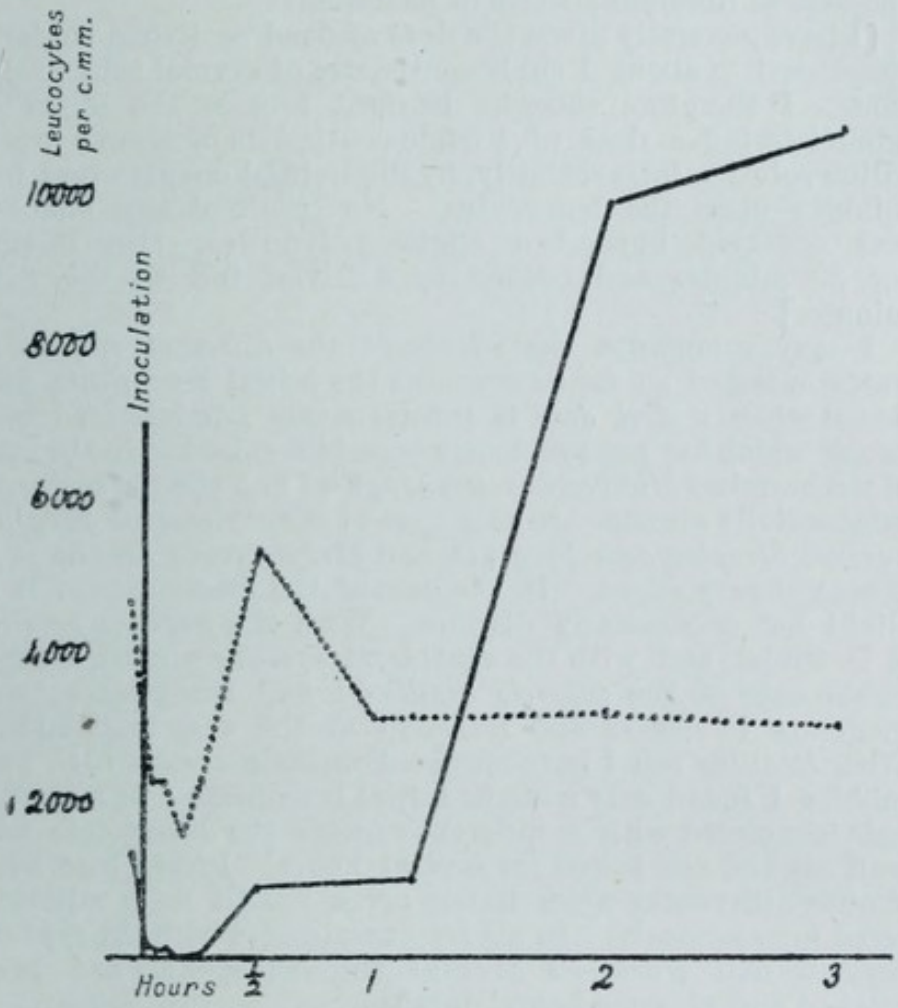


Showing the changes in the circulating leucocytes for 14 minutes after the intravenous inoculation of a normal rabbit with 1,000,000,000 living tubercle bacilli. Thick line= Total leucocytes. Dotted line=Lymphocytes. Thin line= Polynuclears.

In the first place, I find that the phenomenon of initial leucopenia is by no means equally marked after injections of differing bacteria. Some species excite it to a high degree and for several hours; others to a trivial extent and for a few minutes only. The group of bacteria in which I have

found the property of exciting an initial leucopenia most highly developed is that including the typhoid bacillus and B. coli. The staphylococci and the diphtheria bacillus have the power only feebly developed, nor under ordinary circumstances is it well marked in the streptococci, the pneumococcus, the tubercle bacillus, and the anthrax bacillus. I have never found initial leucopenia quite absent when the

CHART 2.



Showing an extreme initial polynuclear leucopenia following the intravenous inoculation of 3 cubic centimetres of a 5 per cent. suspension of sheep's corpuscles into a partially immunised rabbit. Dotted line=Lymphocytes. Continuous line=Polynuclears.

blood has been investigated every few minutes during the first quarter of an hour after an intravenous injection, but it may be trivial and transitory or intense and lasting.

Thus in two animals inoculated for the first time with a dose of (100 million) B. coli communis vaccine, the polynuclear leucopenia lasted for four hours; in one the fall was from 3960 polynuclears per cubic millimetre before inoculation, to 700 at the third hour; in the other it was from 6400 before

inoculation, to 1344 at the second hour. In both cases a polynuclear leucocytosis then supervened. In animals, on the contrary, inoculated for the first time with living and dead diphtheria bacilli, the leucopenia was trivial and soon over; less than one-fifth of the polynuclear leucocytes disappeared from the peripheral circulation, and in an hour the normal numbers were exceeded. It is clear, therefore, that the power of exciting a polynuclear leucopenia when injected into the circulation is one present in very varying degree in the case of different species of bacteria.

[I have generally given the dose of dead or living bacteria suspended in about 1 cubic centimetre of normal saline solution. I therefore thought it right to test the effect of administering a dose of 1 cubic centimetre of simple warm saline solution intravenously, by differential counts every few minutes upon the leucocytes. No result of any moment was observed beyond a slight polynuclear rise lasting for 30 minutes and broken by a trivial fall at the fifth minute.]

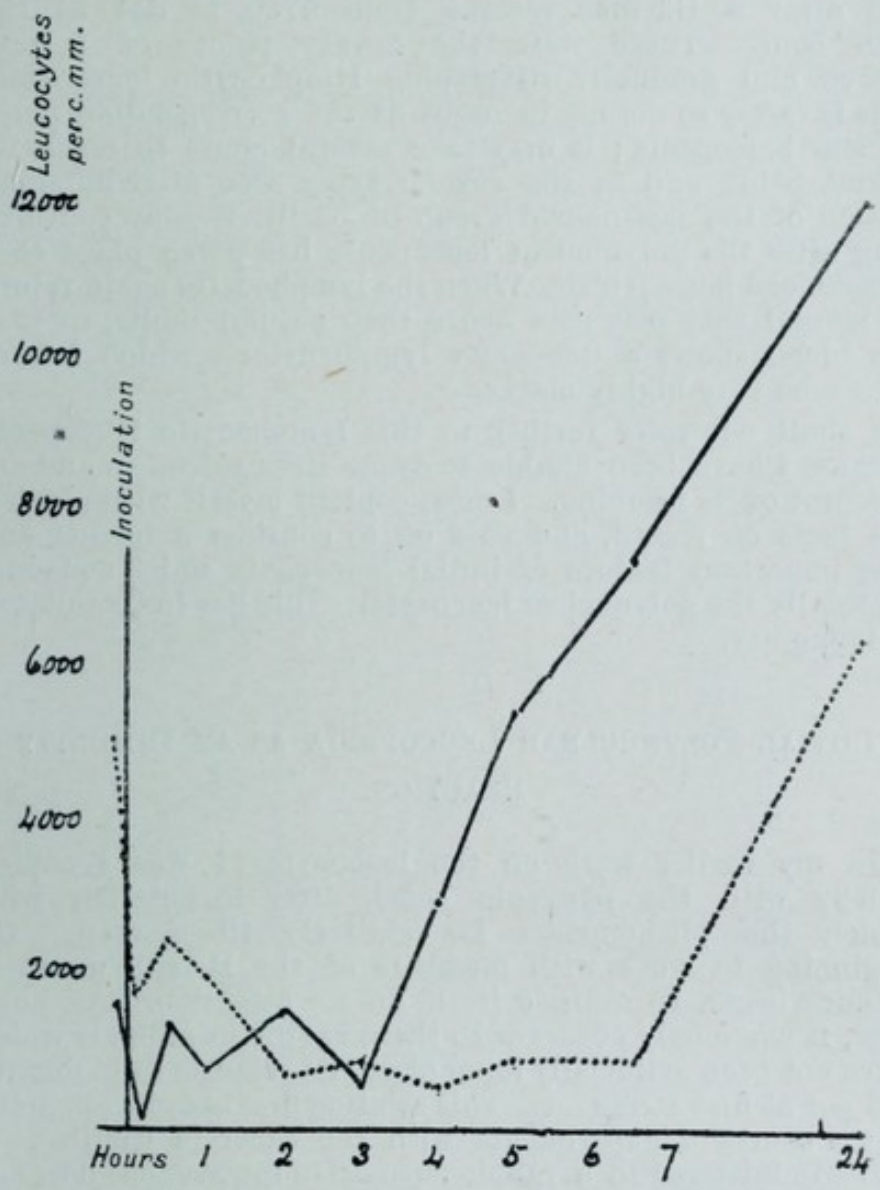
I may summarise the effects of the different species of bacteria tested so far as concerns the initial leucopenia produced when a *first dose* is intravenously administered to a rabbit which has not previously been immunised. In the case of the harmless *Micrococcus citreus agilis* I find the phenomenon substantially absent. In the case of *Staphylococcus pyogenes aureus*, *Streptococcus pyogenes*, and *Streptococcus fæcalis* it is absent or very slight. In the case of the *Pneumococcus* it is slight but occasionally distinct. With the *anthrax bacillus* it is trivial, and with the *diphtheria bacillus* almost absent. In the case of the *tubercle bacillus* I find the phenomenon moderate in degree and transient at the first inoculation. With *Bacillus coli* I have obtained variable results: in two rabbits I found only a slight initial leucopenia; in a second pair inoculated with a different vaccine the leucopenia was well marked and lasted for several hours. I have met with similar differences when living colon bacilli were administered intravenously. In all my experiments with the *typhoid bacillus* and *Gärtner's bacillus* the leucopenia has been marked and of some hours' duration.

The phenomenon is seen, even in the case of unimmunised animals, to vary in its extent and in its duration. The intensity and the duration commonly, but not invariably, go hand in hand. It is not always an absolutely initial phenomenon: I have more than once found it preceded by a very transitory rise in the polynuclear leucocytes—e.g., after the injection of staphylococcus aureus. With B. coli and B. fluorescens aureus I have seen it delayed till the second or third hour, but this is exceptional.

It is commonly held that initial leucopenia is exclusively an affair of the polynuclear leucocytes, and it is true that the fluctuations in these cells are usually more abrupt and noticeable than in the other types. I find, however, that the

circulating lymphocytes undergo similar, though less abrupt, changes, and that the lymphocytic leucopenia may be more lasting than that affecting the polynuclears. A survey of nearly 40 graphic charts of differential leucocytic changes

CHART 3.



Showing the behaviour of the circulating lymphocytes and polynuclears in a rabbit after the intravenous inoculation of a dose of typhoid vaccine (200,000,000). Dotted line=Lymphocytes. Continuous line=Polynuclears. There is seen a lymphocytic leucopenia, slower in onset, but more lasting than that affecting the polynuclears.

after the intravenous injection of living and dead bacteria enables me to state that a lymphocytic leucopenia is hardly ever absent. I find it least marked after first injections with the pyogenic cocci: here it is usually slight and of transient duration. In the case of diphtheria, tubercle, and

anthrax it is more pronounced and may last some hours. It is most pronounced in the case of injections with members of the coli group. After injection of B. coli vaccine I have seen the lymphocytes fall from 3880 per cubic millimetre to 1220 at the third hour; after typhoid vaccine I have seen a fall from 5040 to 504 at the fourth hour; and after a Gärtner vaccine from 4760 to 324 at the fifth hour. These were the lowest points of a prolonged and gradually developing lymphocytic leucopenia This is rarely so abrupt in onset as the corresponding polynuclear leucopenia; it may take several hours to reach its lowest point, and in the severer types seen after administration of the last-named group of bacilli it may continue long after the polynuclear leucopenia has given place to a pronounced leucocytosis. When the lymphocytes again return to normal they may pass above their natural limits, so that the blood shows a temporary lymphocytosis which I have once seen very highly marked.

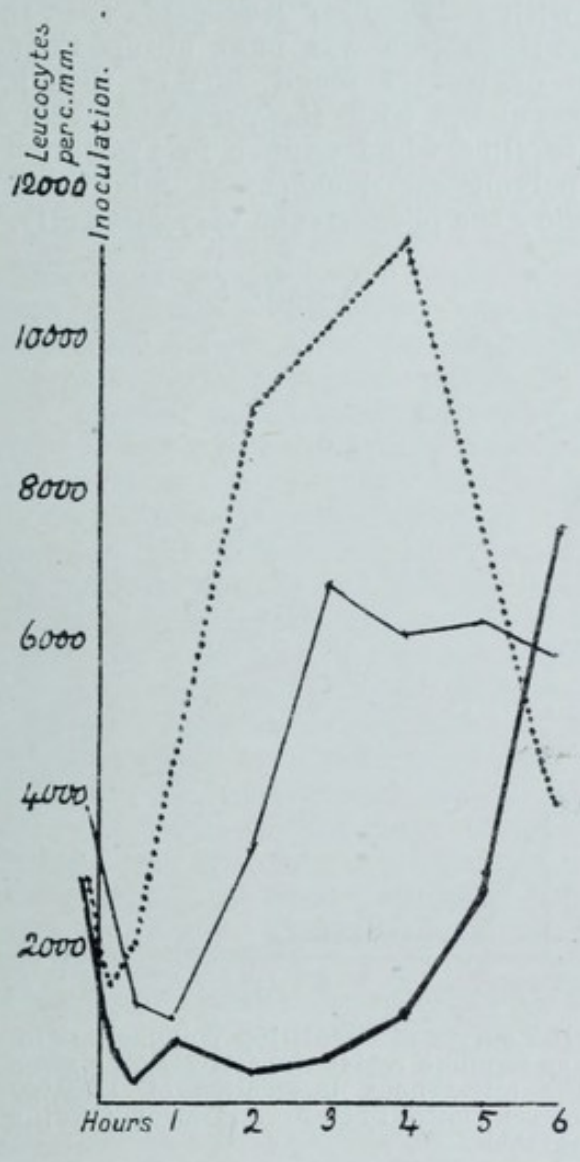
I shall not refer further to this lymphocytic leucopenia because I have been unable to trace its mechanism and am ignorant of its meaning. I must content myself with placing the facts on record, and pass on to consider a further and very important feature of initial leucopenia which concerns especially the polynuclear leucocytes. This lies in its relation to immunity.

Initial Polynuclear Leucopenia as an Immunity Reaction.

In my earlier work on the leucocytes I was occupied chiefly with the pyogenic cocci, after inoculation with which the phenomenon is relatively ill marked. On beginning to work with members of the B. coli group it at once began to obtrude itself on my attention. As, however, it commonly occurs with these bacilli in animals which have not been artificially immunised, its relation to immunity did not at first strike me. This relation first became apparent in the course of experiments with the tubercle bacillus. I had administered to a rabbit successive intravenous doses of killed bovine tubercle bacilli (varying from 100 to 200 million), with intervals of about a month between the doses. I had prepared complete differential leucocyte charts on the occasions of the first, second, and fourth inoculations, and on comparing these I noticed a very remarkable progressive increase in the initial polynuclear leucopenia. On the first occasion this had lasted less than an hour and the lowest polynuclear count observed had been 1544 per cubic millimetre; on the second inoculation the polynuclears fell to 1118 at the end of one hour, but in two hours had nearly regained their initial figure. On the fourth occasion they

fell to 224 per cubic millimetre in half an hour and were still very low (532 per cubic millimetre) at the third hour; they did not regain their initial number till between the fifth and sixth hours.[6]

CHART 4.

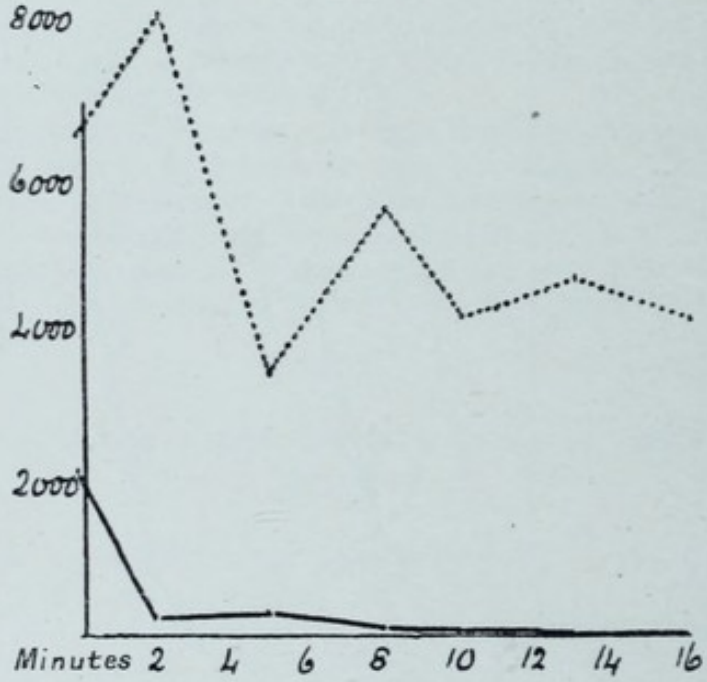


Showing the superposed curves of the circulating polynuclear leucocytes in the rabbit for six hours after three intravenous inoculations of killed tubercle bacilli in doses of from 100,000,000 to 200,000,000. The effect of the first inoculation is shown by the dotted line, that of the second by the thin line, and that of the fourth by the thick line. The duration of the polynuclear leucopenia is seen to increase with the progressing immunity. (Data for the third inoculation are lacking.)

[6] A fifth inoculation yielded a somewhat discordant result; there was a more abrupt polynuclear fall than on any previous occasion, for they fell in five minutes to 264 per cubic millimetre, but the leucopenia was not maintained so long as on the fourth occasion—indeed, in two hours the number had surpassed the initial figure.

I was convinced that there must be some relation between the increasing immunity of the animal and the more marked polynuclear leucopenia seen, and I began to consider other charts which I possessed from this point of view. In the case of animals vaccinated against members of the B. coli group I found that succeeding doses were more constantly associated with polynuclear leucopenia than the first dose, and that the leucopenia was more abrupt in onset and more extreme in degree. I found further that animals intravenously inoculated with the pyogenic cocci and with the diphtheria bacillus, which on their first injection showed only a trivial polynuclear leucopenia, might on subsequent injections show the phenomenon very distinctly.

CHART 5.



Showing the curves of circulating polynuclears in a normal and in an immune rabbit respectively for sixteen minutes after the intravenous inoculation of 1,000,000 virulent pneumococci. Dotted line=Normal rabbit. Continuous line=Immune rabbit.

I now instituted experiments to test the point and commenced to examine the blood at short intervals after inoculation, sometimes killing the animals, for a purpose I shall shortly describe, a quarter of an hour from the beginning of the experiment. I began with the tubercle bacillus. I took the animal mentioned above, which had been vaccinated five times, three weeks having elapsed since its fifth vaccination, together with a normal control animal which had not been treated in any way. Each animal received an intravenous dose of about 1000 million living bovine tubercle bacilli, and was

killed after a quarter of an hour. The leucocytes were counted every two minutes, with the following result. In the case of the immune rabbit the polynuclears numbered 1562 per cubic millimetre before inoculation ; in one minute they fell to 136 and in two minutes to 34 per cubic millimetre; after this they rose a little and numbered 432 per cubic millimetre before the animal was killed. In the case of the non-immune rabbit the polynuclears numbered 5340 per cubic millimetre : they fell to 1276 at the sixth minute, but this was the lowest figure seen ; in 12 minutes they had risen again to over 4000. There was thus a most striking difference between the immune and the non-immune animal.

In an experiment with the pneumococcus the difference was even more remarkable. I possessed a rabbit which had survived a dangerous pneumococcal septicæmia, and which had later been inoculated with living attenuated pneumococci ; 18 days had elapsed since the last infection. I took this rabbit, together with a normal untreated animal as a control, and I injected intravenously into each 1000 million living, virulent, capsuled pneumococci from cultures made the day before from a case of human pneumococcal septicæmia. Both animals were killed after 16 minutes. The effects upon the circulating polynuclears were as follows. The immune animal had 2160 per cubic millimetre before the inoculation ; in two minutes they had fallen to 264 ; they had risen slightly in five minutes, but at the eighth minute they were only 56 per cubic millimetre, and this number fell to 10 per cubic millimetre at the sixteenth minute ; the polynuclear cells were practically wiped out of the circulation. In the non-immune animal the result was very different ; its initial polynuclear count was 6596 per cubic millimetre ; this had risen in two minutes to 8136, but in five minutes was only 3480 per cubic millimetre, the lowest number observed. Thenceforward the counts were between 4000 and 6000.

With streptococcus fæcalis the results were less extreme. In this experiment I used a rabbit which had received three intravenous doses of vaccine, the last 23 days previously. No normal control animal was tested on this occasion. A does of 1000 million living streptococci was given intravenously and the animal was killed after 15 minutes. The polynuclear leucocytes numbered 2990 per cubic millimetre before the injection ; in two minutes they had fallen to 504, but they began to recover at the eighth minute, and in 14 minutes they exceeded the initial number.

I carried out a similar experiment with micrococcus citreus agilis upon an animal twice immunised with living cultures of this organism, but no noteworthy leucopenia was induced. The polynuclear count was 2920 per cubic millimetre before inoculation, and the lowest subsequent count was 1888 at the eighth minute ; after this the counts were above normal.

I shall have later to mention experiments with typhoid

immune rabbits in which the polynuclear leucopenia was rapid in onset, extreme in degree, and of long duration, but I need hardly labour the point further. It is clear that there is a relation between the degree of immunity and the intensity and abruptness of the polynuclear leucopenia induced by an intravenous injection of the homologous bacterium. In other words, we may describe the initial polynuclear leucopenia as an immunity reaction. I cannot claim to be the first to recognise this. While I was engaged in these experiments and had already arrived at the above conclusion, I came across a reference to observations by Sacerdotti, published in 1907. He had immunised rabbits against the blood-platelets of the dog and found that these platelets produced an extreme but transient leucopenia when injected into the immune animal, whereas in the normal rabbit they caused so such effect. He therefore put forward the phenomenon as an immunity reaction, and his observations encouraged me to pursue the matter further, as I shall presently describe.

It may be objected that the leucopenia is seen in animals which are not immune—or rather which have not been artificially immunised. I have already stated that this may be so, in marked degree, with the B. coli group of bacteria. But it does not follow that because one has not intentionally immunised an animal it is destitute of immunity: some degree of natural immunity is often seen and it may well be better marked against some bacterial species than against others. The question is a much larger one than this, as I hope soon to show.

The Mechanism of the Leucopenia.

In a marked case nine-tenths or more of the polynuclear leucocytes may disappear from the peripheral circulation within a minute or two of the bacterial injection. Nor is this all, for a considerable number of the lymphocytes usually vanish also at the same time. Where the latter go to I do not know, but with regard to the polynuclears I can most fully endorse the results of Goldscheider and Jacob and of Bruce. They are held up in the lung, screened off, as it were, by the pulmonary capillaries.

I have already described the method by which I have endeavoured to measure the number of polynuclear cells in unit volume of given tissue; in two normal rabbits, suddenly killed, I found them to number respectively 12,000 and 20,000 per cubic millimetre of lung. In no other organ (except the bone marrow and spleen pulp) are these numbers approached. The accumulation of the polynuclears in the lung after a bacterial injection appears to be an exaggeration of a natural physiological process.

I have examined the lungs and other organs of seven rabbits killed while in the leucopenic condition—mostly

about a quarter of an hour after the bacterial injection. Where the leucopenia has been pronounced the number of polynuclears in the lung is very much increased. Thus, in a rabbit immunised against streptococcus fæcalis and killed a quarter of an hour after a large dose of the living organism I found 51,000 polynuclears per cubic millimetre of lung tissue. In a coli-immune animal dying two hours after a moderate injection of vaccine, with a circulating polynuclear count of only 205 per cubic millimetre, there were 60,000 polynuclears per cubic millimetre of lung. But in an animal killed 15 minutes after the injection of micrococcus citreus agilis, and which showed hardly any circulating leucopenia, the lung figures were normal—viz., 11,000 per cubic millimetre.

The relation of the phenomenon to immunity is shown by two pairs of animals. I have already described the early circulatory changes in the immune and non-immune rabbit, after the intravenous injection of very large doses of the tubercle bacillus and of the pneumococcus, and have shown the much more intense leucopenia in the immunised animal. In all cases the animals were killed in about a quarter of an hour. In the case of the tubercle bacillus, the immune animal showed 57,000 polynuclears per cubic millimetre of lung, the non-immune only 35,000. With the pneumococcus the corresponding figures were 72,000 and 41,000.

No such increase was found in any organ except the lung. The figures for the spleen, liver, and kidney did not exceed those found in the normal animals (except in the case of the animal dying after the B. coli vaccine, in which the liver showed 13,000 polynuclears per cubic millimetre). I feel justified in asserting that the polynuclears are held up, to all intents and purposes, solely in the lung. And calculation shows that the observed increase in the lung tissue is sufficient to account for the observed decrease in the peripheral circulation.

It may naturally be asked by what mechanism the polynuclear cells are thus screened off, as it were, in the lung. I do not know, just as I do not know why the process occurs to no inconsiderable extent in the normal animal. It looks as if the sojourn in the lung were a voluntary one on the part of the leucocytes, fulfilling some need which they feel, possibly for oxygenation. Levaditi, in his experiments with cholera immune guinea-pigs,[7] lays stress on the phagocytosis seen in the leucocytic accumulations in the lung. At first I was inclined to think that where phagocytosis had occurred in the circulation the leucocytes retired to the lung to digest their prey, just as a dog may retire under a sofa with a bone. It is true that in certain cases the leucocytes which have accumulated in the lung may be seen full of ingested bacteria; in my experiment with streptococcus

[7] Annales de l'Institut Pasteur, vol. xv., p. 894.

fæcalis this was noticeable, but in a similar experiment with virulent pneumococci I could find no phagocytosis in the lung or elsewhere. In the case of an immune rabbit inoculated with living tubercle bacilli I made smears from the lung and stained them for the tubercle bacillus. Polynuclear leucocytes were abundant in the films, but out of 400 which I enumerated only 5 contained bacilli (1·25 per cent.). Clearly, then, the phagocytic theory will not explain matters, and I shall shortly show that the phenomenon occurs in immunity against unorganised antigens, putting phagocytosis completely out of court. A possible explanation, though a somewhat speculative one, lies in the suggestion that in the circulatory reaction between antigen and antibody, complement is used up and that, assuming an origin of complement from the polynuclear leucocytes, these cells need a free supply of oxygen to recuperate their powers.

In some cases the initial leucopenia may be transient; in other cases it may endure for four or five hours or more. There then ensues, in most cases, a polynuclear leucocytosis. Watching the blood from hour to hour one finds the polynuclears slowly regaining the normal and then more quickly rising high above it. The question of leucocytosis I must defer till another lecture, but it is relevant here to ask whether, as the leucopenia is passing off, the returning polynuclears are those which had for the time being retired into the lung, or whether they are a new supply derived from the bone marrow. I have no sufficient data for answering the question, but in a case of severe and protracted leucopenia in a typhoid-immune rabbit which had received a large intravenous dose of living typhoid bacilli I noted in the blood about the fourth and fifth hours, as the leucopenia began to pass off, considerable numbers of nucleated red corpuscles (normoblasts) and a few myelocytes. This suggests an over-activity of the marrow.

The Constitutional Symptoms associated with Initial Leucopenia.

So long as I was working with the pyogenic cocci, which do not cause much leucopenia except in the highly immune animal, I did not notice any marked constitutional symptoms after the intravenous injection of bacterial vaccines. The rabbits were at times quiet for an hour or two after an injection, but they were not manifestly ill. When, however, I began to experiment with vaccines prepared from the B. coli group, I speedily met with symptoms of marked illness after the injection. The animals were not merely quiet; there was sometimes much respiratory distress, the symptoms coming on in from 30 minutes to an hour or so after the inocula-

tion. In a marked case the animal lay down with its limbs extended, and the respirations rose in frequency to 60 or 80 per minute. (The respiration frequency in a normal rabbit at rest is about 30 per minute.) I soon noticed that these symptoms bore some relation to the leucopenia; they were marked in proportion to its intensity, and as the leucopenia passed off and leucocytosis set in the animal would recover its liveliness and begin to eat again. During the height of the leucopenia it was often difficult to obtain a flow of blood from a punctured vein; it is easy in the natural animal to induce a vascular dilatation, during which blood flows readily from the smallest prick, but in a leucopenic rabbit it may be difficult to induce this vaso-dilatation.

My attention was at last very decisively aroused by the occurrence of actual death in an immune animal, following a not immoderate dose of B. coli vaccine. I had two rabbits which had been three times immunised with doses of 100 million killed B. coli communis at intervals of about a fortnight. They had then been left for three months without treatment. Fearing lest their immunity should fade, I gave each a double dose—200 million of the same vaccine, which had been standing in the laboratory for some months. All the injections were intravenous. An hour after the injection I noticed that one of the rabbits was lying down and breathing rapidly, while the other showed similar symptoms in lesser degree. In an hour and three-quarters the first animal had a respiration frequency of 130, and in two hours of 150 per minute. Suddenly it became convulsed and died in less than a minute. Just before death its total leucocyte count was 1000 per cubic millimetre, of which 205 were polynuclears. The fellow animal, though ill, recovered. I may add that I have repeatedly administered doses of 200 million killed B. coli intravenously without serious symptoms, but never before in an immune animal after so long an interval as three months.

Now the symptoms I have described in initial leucopenia in rabbits and the mode of death in the single animal which died are the symptoms associated with the condition known as "*anaphylactic shock.*" Increased frequency of respiration is the most obvious symptom of the anaphylactic animal after the injection of a second dose of antigen. It is known that anaphylaxis can be induced by bacterial proteins, and notably with those of the B. coli group. I do not doubt that the phenomena seen in the leucopenic rabbit after the injection of living or dead bacteria are the phenomena of anaphylactic shock. I have come to the conclusion that the initial leucopenia seen after intravenous injection of bacteria and bacterial vaccines, especially in the immunised animal, is intimately bound up with anaphylactic shock, and is an integral part of this condition. To substantiate this opinion I must offer a few remarks on anaphylaxis.

The Relation of Leucopenia to Anaphylaxis.

By the term "anaphylaxis" is meant a condition of induced super-sensitisation to an alien protein. The protein may be harmless to a normal animal, but in the sensitised animal even a moderate dose causes severe symptoms and sometimes death. The term anaphylaxis was coined by Richet in connexion with his observations on a protein derived from the sea anemone, and against which he sensitised dogs. For awhile the phenomenon remained a mere curiosity, but renewed attention was drawn to it by the observation of Arthus in 1903, that although normal horse serum was harmless to the rabbit, highly toxic symptoms occurred if the animal had previously been several times injected with this substance, provided that a sufficient interval had elapsed since the last injection. It was later shown that a single previous injection sufficed to sensitise the animal. The practice of employing guinea-pigs for standardising diphtheria antitoxin led to a rediscovery of the phenomenon here also, and Theobald Smith in America drew attention to the frequently fatal results which followed second injections of serum. Inasmuch as horse serum is now extensively used in the treatment of human disease, the matter was soon seen to be of considerable importance, for it became apparent that there was some connexion between anaphylaxis and the so-called "serum disease" in man. The subject has now been attentively studied by numerous observers, and though no complete explanation is yet forthcoming, it is possible to make certain general statements about the condition.

It would seem that an animal can be supersensitised against any alien protein or allied substance capable of acting as an antigen—that is, of evoking the formation of an antibody. The anaphylactic condition is closely associated with the development of the antibody; after the primary sensitising injection an incubation period of two or three weeks is required before the supersensitisation is fully established, and this period coincides fairly with that known to be requisite for the formation of an antibody, but is rather longer. The reason seems to be that something more than the formation of antibody is required; before the animal is fully sensitised the antigen must also have disappeared from the body. When this condition is reached and antibody alone is present (a state of affairs which may last for many months) the introduction of a new dose of antigen, especially if it be injected directly into the circulation, leads to a violent disturbance of the system, the so-called "anaphylactic shock," in which the animal may die. Should it survive it is found to be for the time desensitised and is said to be in a condition of "anti-anaphylaxis"; a further injection of antigen causes no such systemic storm. Anaphylaxis may in

time return or may be reinduced by suitable injections of antigen.

It must be noted that in order to induce the condition of supersensitisation the antigen must be administered by way of the circulation, the peritoneum, or the subcutaneous tissue, not by the alimentary canal. Although some have claimed to obtain anaphylaxis by feeding, the great majority of observers have failed to get any results in this way.

The symptoms which follow the introduction of a second dose of antigen into the anaphylactic animal vary somewhat with the species. The most obvious clinical symptoms are usually respiratory; the animal is restless and begins to breathe quickly; the respirations may be enormously accelerated. The heart's action is feeble and it has been shown that there is an extreme fall in blood pressure. The temperature falls and urine and fæces may be passed involuntarily. In a fatal case convulsions end the scene. The clinical picture is that of "shock" in an extreme degree. Post-mortem examination reveals nothing to account for death. Local hæmorrhages have sometimes been found, but there is no visible organic lesion sufficient to explain the symptoms. If the animal does not die it recovers from its apparently dangerous condition with the most remarkable rapidity.

Besredka, who has largely employed the intracerebral method of injection for administering the second dose of antigen (in which an extremely small dose suffices to produce a fatal result), has found that if an anaphylactic guinea-pig be deeply narcotised with ether the desensitisation can be carried out without harm to the animal.

I do not propose to enter here into the highly speculative theories which have been put forward to explain the remarkable facts that I have mentioned. The phenomena bear a striking resemblance to those which have long been known to ensue when peptone is intravenously administered, and it may be that the matter is bound up with the functions of the intestinal wall or portal area in defending the body against the toxic effects of the initial products of proteid digestion. It is stated that the phenomena seen when a dose of antigen is administered to a supersensitised animal are associated with a disappearance of complement from the serum. If this be so, and if complement is derived from the leucocytes, we may obtain some inkling of the reason for the participation of the polynuclear cells in the events of the anaphylactic shock. In any case it may confidently be asserted that the phenomenon of anaphylaxis is closely bound up with that of specific immunity.

It has been known for some years that bacterial proteins are capable of setting up anaphylaxis (Wolff-Eisner, 1904). The subject has been carefully studied by Rosenau and Anderson in America and by Kraus and Doerr in Austria.

The most striking results have been obtained with bacteria of the B. coli group (including the bacilli of typhoid and dysentery), and with the cholera vibrio and allied organisms; the bacterial proteins have been prepared by autolysis with weak caustic soda, or by alternate freezing and thawing. Definite results have also been obtained with tubercle and anthrax bacilli, and even with species which are not pathogenic—such as the hay bacillus. It seems to be the alien protein, rather than the toxin present, which evokes the supersensitisation—indeed, Kraus and Doerr found that in guinea-pigs sensitised against dysentery bacilli only the bacillary bodies were able to induce anaphylactic shock; the germ-free toxin produced no effect. Nevertheless, hypersusceptibility to true bacterial toxins, such as that of the diphtheria bacillus, is known to exist—witness the observations of von Behring and Kitashima on horses in course of immunisation. Whether this latter phenomenon is identical with that of anaphylaxis against alien protein is not yet settled.

It must be noted that there is such a thing as *natural anaphylaxis*, as opposed to the artificially induced condition. It is held by many that the idiosyncrasies of some persons against certain food-stuffs—crab, mussels, &c.—are an example of this. And there is no difficulty in believing that anaphylaxis may naturally exist against certain bacteria, inasmuch as the serum of some individuals or species is known to contain antibodies capable of destroying some sorts of bacteria. Such natural anaphylaxis may well be in truth a post-natal acquirement: if the intestinal bacteria tend to invade healthy tissues so frequently as we must now believe, a sort of continuous auto-vaccination on a small scale will serve to explain the presence of antibodies and inferentially of anaphylaxis.

LECTURE III.

Delivered on June 21st.

MR. PRESIDENT AND GENTLEMEN,—I devoted the last part of my second lecture to a short recital of the more important facts known concerning anaphylaxis in order to justify the statements made, that the constitutional symptoms observed during initial leucopenia are actually those of anaphylactic shock, and that leucopenia is an integral part of the phenomena of anaphylaxis.

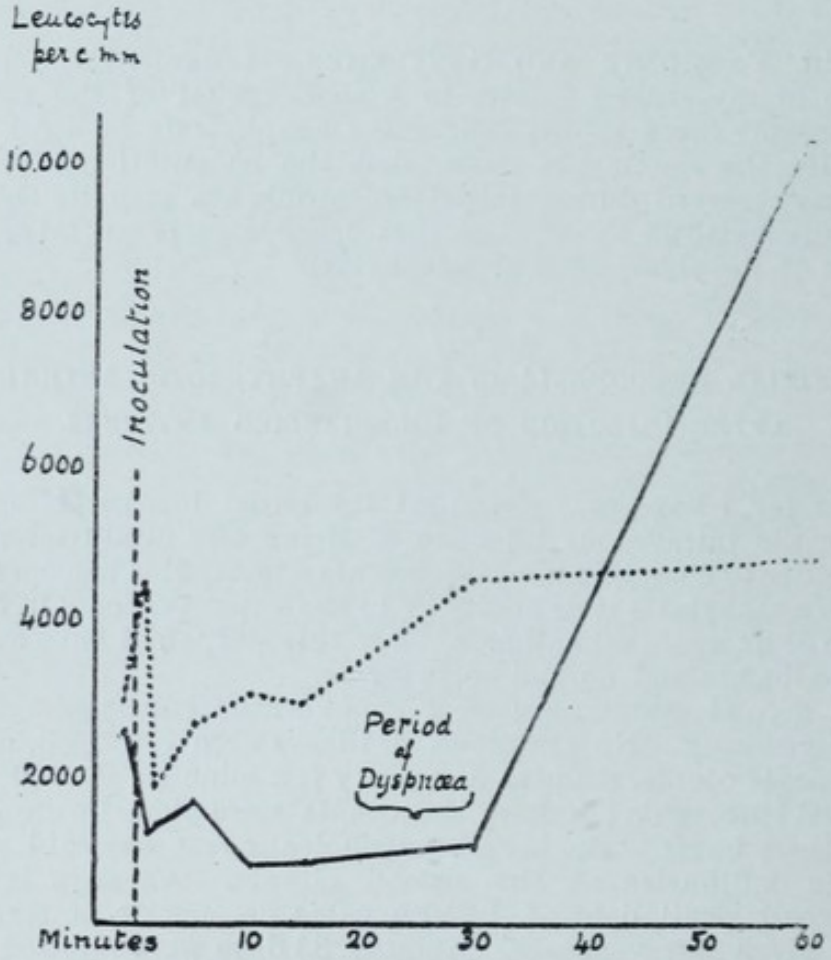
INITIAL LEUCOPENIA IN THE ANAPHYLACTIC ANIMAL AFTER INJECTION OF UNORGANISED ANTIGENS.

So far I have only described the initial leucopenia seen after the intravenous injection of living and dead bacteria. In order to complete the evidence associating this leucopenia with anaphylaxis it is necessary to show that it occurs in the case of unorganised antigens. For this purpose I have used egg-albumin and normal horse serum.

A normal rabbit received ¼ cubic centimetre horse serum intravenously. No symptoms of illness were produced, and leucocyte counts, made at first every few minutes, showed no initial leucopenia; indeed, the counts were almost constant for three hours. The lowest polynuclear count was 7614 per cubic millimetre at the second minute. 42 days later a second small dose of 1-250th cubic centimetre of serum was given intravenously. Another 33 days were allowed to elapse, and then the animal was tested by the intravenous injection of 3 cubic centimetres of serum. The total leucocyte count fell from 5800 per cubic millimetre before inoculation to 3600 in two minutes; the polynuclears fell from 2552 to 880 per cubic millimetre in 10 minutes and remained low till the thirtieth minute, when a pronounced leucocytosis set in (14,406 polynuclears per cubic millimetre in 1¼ hours.) The animal showed no marked symptoms till 20 minutes after the injection had been given; it then suddenly lay down at full length with intense dyspnœa (respirations 216 per minute). This lasted about eight minutes when, with the onset of leucocytosis, the symptoms passed off as rapidly as they had set in.

A normal rabbit was given ¼ cubic centimetre of egg-albumin (fresh white of egg diluted to 1 cubic centimetre with normal saline) intravenously. The animal remained perfectly well. No leucopenia occurred; indeed, the polynuclear count was slightly raised during the ensuing 15 minutes at each of the five observations made. 23 days afterwards the same dose was repeated: in 20 minutes the animal was lying on its side with respirations of 140 per minute, in 50

CHART 6.



Showing the curves of the circulating lymphocytes and polynuclear leucocytes after the intravenous injection of 3 cubic centimetres normal horse serum into a rabbit previously sensitised against that substance. Dotted line = lymphocytes. Continuous line = polynuclears

minutes it was recovering, and in 2½ hours it was apparently normal again and taking food. On this occasion the total leucocytes fell from 6600 per cubic millimetre before inoculation to 1400 at the tenth minute, and the polynuclears from 1716 per cubic millimetre before inoculation to 88 one minute after. They then rose, and in 2½ hours there was a polynuclear leucocytosis (12,402 polynuclears per cubic millimetre).

It is thus plain that an initial leucopenia similar to that seen after bacterial injections occurs also in the anaphylactic animal when a second dose of antigen is administered, even though this be an unorganised protein.

The Effect of Deep Ether Narcosis on Anaphylactic Shock and Leucopenia.

I have endeavoured still further to complete the evidence associating leucopenia with anaphylaxis. Besredka has shown that deep ether narcosis so far modifies anaphylactic shock that guinea-pigs can safely be desensitised under it, whereas the unanæsthetised control animal perishes. I was therefore wishful to see how far such anæsthesia modified the phenomenon of initial leucopenia in the immunised animal.

I possessed two rabbits of about the same size and weight which had been immunised against the typhoid bacillus by repeated doses of vaccine—the last doses having been given two months previously; both animals were in good health. I administered to each animal intravenously a dose of 1000 million living typhoid bacilli of an old laboratory strain; one rabbit was not anæsthetised, the other was plunged into deep ether narcosis before the injection was given and kept fully under for 15 minutes afterwards. I am indebted to Mr. H. E. G. Boyle for the skill and care with which the anæsthetic was given. He secured absolute muscular relaxation, with complete abolition of the corneal reflex during the whole experiment.

The result of the experiment was not quite what I expected. The initial leucopenia was during the first quarter of an hour extreme, and practically identical in both, though delayed by a few minutes in the anæsthetised animal. But whereas in the unanæsthetised animal it persisted for five or six hours, the anæsthetised animal showed its lowest polynuclear count at 15 minutes; at two hours it had regained the normal, and in three hours a marked polynuclear leucocytosis had commenced, which by the fourth hour had reached nearly 13,000 polynuclears per cubic millimetre. There was a corresponding difference in the symptoms exhibited by the two animals. The one to which no anæsthetic was administered was rendered markedly ill for many hours; indeed, it was two days before it completely recovered. The symptoms came on towards the end of the first hour after the injection, and were at their worst during the first half of the second hour. In the anæsthetised rabbit the symptoms were at first masked by the after-effects of the ether, but they seemed much less severe than in the control animal, and next day the animal appeared in its usual health. I could detect no noteworthy difference between the two

animals as regards the rate at which the bacilli disappeared from the blood. In both they had become very scanty in a few hours.

The actual polynuclear counts were as follows. In the unanæsthetised animal these cells were 6102 per cubic millimetre before inoculation. In one minute they fell to 1312, in two minutes they were 288, in five minutes 44, in 15 minutes 14; they then rose a little and for the next hour and a half varied between 170 and 306. By the fifth hour they had risen to 2145 and in seven hours to 6786. 29 hours after the injection they numbered over 55,000; this leucocytosis was practically over on the second day after the injection.

The corresponding facts in the anæsthetised animal were these. Before the anæsthetic the polynuclear count was 11,440 per cubic millimetre; when fully under ether, but before the injection was given this had dropped to 5632. In one minute after the injection it was 3950, in two minutes 266, in five minutes 204, and in 15 minutes 42. This was the lowest point reached. In half an hour the polynuclears were 3380 per cubic millimetre, in two hours 5676, in three hours 8208, and in four hours 12,994. The highest polynuclear count next day was 22,080. It may here be noted that the more severe and prolonged leucopenia in the unanæsthetised animal was succeeded by the higher leucocytosis.

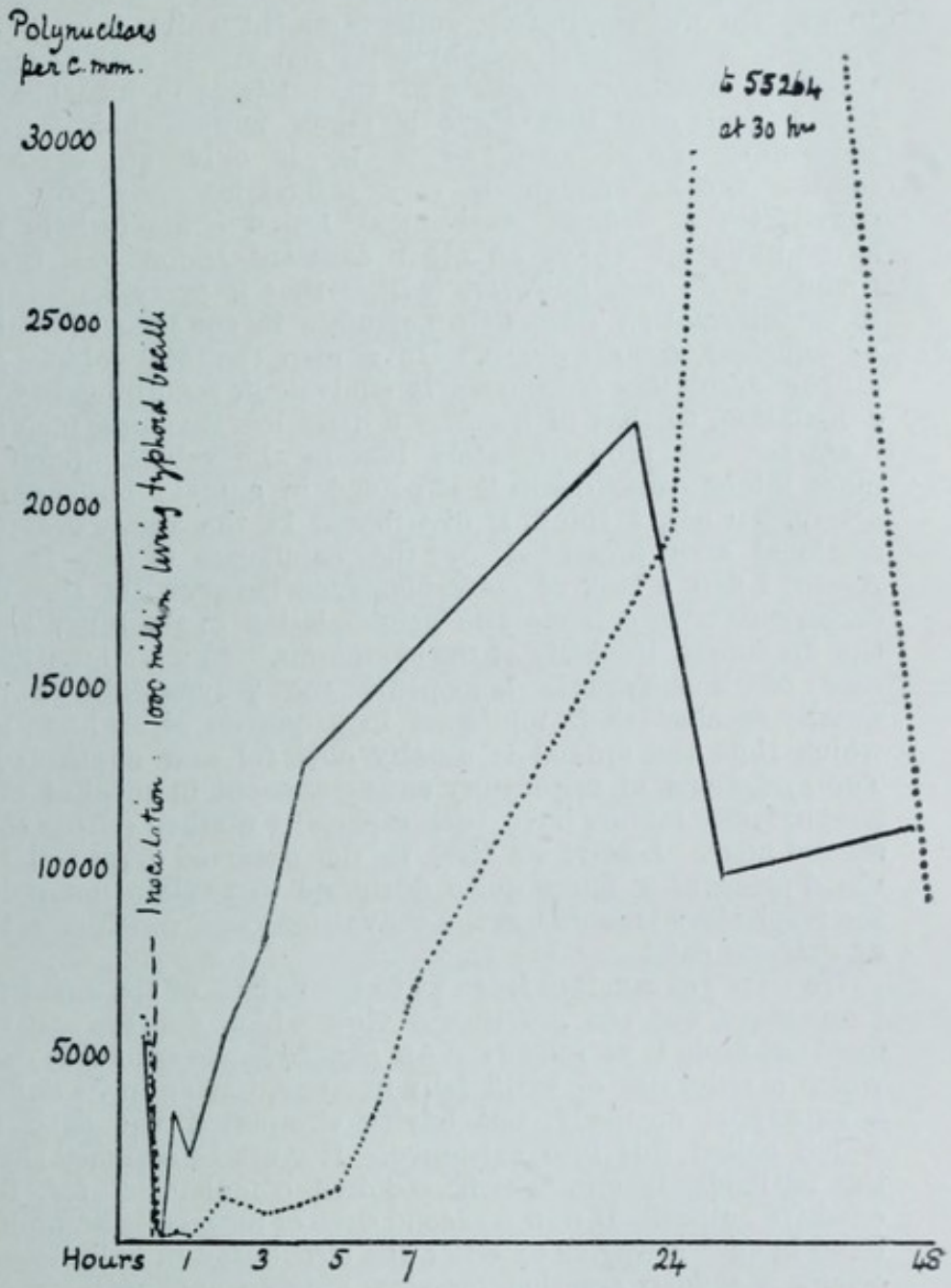
So far as this one experiment goes it would seem that deep ether narcosis diminishes anaphylactic shock in the rabbit, as Besredka had found was the case in the guinea-pig. It scarcely affected the abruptness or intensity of the initial polynuclear leucopenia, but it greatly reduced its duration.

I now determined to test the rabbit which had not been anæsthetised in the foregoing experiment by repeating the injection as soon as the animal had recovered from the effects of the preceding one—i.e., when it might be supposed to be in a condition of "anti-anaphylaxis." On the third day, therefore, I gave it another 1000 million living typhoid bacilli. The animal, however, reacted much as it had done the first time. It was not made so ill and the respiration frequency was not quite so great; the lowest total leucocyte count noted was 2400 per cubic millimetre during the second hour (as against 1400 on the first occasion). The polynuclears were reduced to 90 per cubic millimetre at the tenth minute, their lowest point, and there was no polynuclear leucocytosis on the following day. The animal was less ill than on the previous occasion. There was thus some evidence of lessened anaphylactic shock on the second occasion, but there was not that absence of symptoms which I had anticipated. The animal was very highly immune, and it may be that it was not wholly desensitised by the dose of bacilli three days previously or that antibody was beginning to return again.

I have thus brought forward evidence showing that the phenomenon of initial leucopenia, after intravenous injection

of living or dead bacteria, is an immunity reaction, that it is commonly associated with constitutional symptoms, which may be severe, and that it is part and parcel of the condition

CHART 7.



Showing the effect of ether narcosis in reducing the duration of the polynuclear leucopenia in typhoid anaphylaxis. Dotted line = polynuclear curve of unanæsthetised animal. Continuous line = polynuclear curve of anæsthetised animal.

known as anaphylactic shock. It seems reasonable to speak of this form of diminution in the number of circulating leucocytes as "*anaphylactic leucopenia.*"

Nature of the Anaphylactic State.

At first sight it might seem that the great accumulation of leucocytes in the lung—amounting to leucocytic thrombi in large numbers of the pulmonary capillaries—would serve to explain the respiratory embarrassment which is so conspicuous a feature of anaphylactic shock. It may be that this is a contributory cause of the distress in breathing; but I feel sure that there is much more behind. The pulmonary block, such as it is, is only an episode in the shock, though a very interesting one, for it constitutes a definite anatomical lesion characteristic of a condition in which no other constant lesion has been found. I do not, however, believe that if every leucocyte in the circulation were to accumulate in the lung it would be sufficient to cause death. In a man the total volume of all the circulating leucocytes is equivalent, according to my calculation, to that of a sphere a little less than one inch in diameter and proportionately less in the rabbit. Such a mass might cause death if impacted in a main pulmonary artery, but not, I think, if distributed in the vastly greater sectional area presented by the capillaries of the lung. Again, I find a lack of correspondence between the time of occurrence of the leucocytic accumulation in the lungs and the maximum intensity of the symptoms. In a well-marked case of anaphylactic leucopenia the polynuclears have usually reached their minimum in a quarter of an hour, at which time the animal is usually cheerful and unaffected. The symptoms of respiratory embarrassment in most of my anaphylactic rabbits have been especially marked during the second hour. Lastly, we have in the observed great fall in blood pressure a factor quite adequate to explain not only the respiratory trouble but the convulsions and death seen in an extreme case.

We have yet much to learn as to the nature of the anaphylactic state, but the provisional view which appears best to meet the facts is as follows. An anaphylactic animal is one which is naturally or artificially immune, having in its fluids a supply of antibody, but having eliminated the antigen which called this into existence. It must be assumed that the antibody is one which requires complement for its effective action. If now a second dose of antigen is administered in such a way as to enter the circulation in bulk, there occurs a sudden reaction between antigen and antibody in which the store of available complement is quickly exhausted. Why and how this sudden reaction should evoke such violent constitutional symptoms we do not yet know; we must be content to admit the fact. It may be that, as Besredka holds, the action is chiefly upon certain cell groups in the central nervous system. If further it be assumed that one of the functions of the polynuclear leucocytes is to furnish the

supply of complement we may conceive these cells, exhausted of their supply, to repair to the pulmonary capillaries for oxygenation in order to recuperate.

I am inclined, indeed, to think that initial leucopenia is a more delicate test of the occurrence of the reaction between antigen and antibody in the circulation than the clinical symptoms of anaphylactic shock. I have never seen shock without some degree of leucopenia, but I have seen a marked leucopenia without any symptoms of constitutional disturbance. Of this I will quote a marked example. A rabbit had been twice immunised against washed sheep's corpuscles by the intravenous injection of 1 cubic centimetre of a 5 per cent. suspension, with a six weeks' interval between the doses. Thirty-four days after the second injection I gave the animal a third dose—3 cubic centimetres of a similar suspension. The polynuclear leucocytes fell in one minute from 1312 to 80 per cubic millimetre, and in 10 minutes there were none. In this differential count I found 200 lymphocytes in succession (see Chart 2). Yet the animal was not made ill; shock and dyspnœa were absent throughout. There was nevertheless evidence of disappearance of antibody from the circulation; before the third dose of corpuscles the hæmolytic *titre* of the serum (not inactivated) was 1 in 10; half an hour after the injection it was less than 1 in 5. The immunity was thus feeble at the commencement of the experiment, but practically absent at its close.

Observations on Immunity in the Anaphylactic Animal.

It is at first sight somewhat of a contradiction in terms to speak of an animal as immune, when a dose of the antigen in question produces far more serious symptoms than it does in a normal animal and may even cause a fatal result. The immune condition, so far as it concerns the presence of a humoral antibody, seems to be one of greater sensitiveness and quicker response to the presence of the antigen. When we use the term immunity we understand in an ordinary way an increased power of resisting a naturally occurring infection. The method adopted in producing anaphylactic shock, and that which I have used in my work on rabbits, is a wholly unnatural one. Never under natural conditions can a large dose of alien protein gain sudden access to the blood; all the digestive and portal arrangements of the body are designed to shield the system from such an event. Hardly ever can a sudden large dose of bacteria gain access to the blood; never, unless there is a previous focus of infection; and here one of the conditions necessary for anaphylaxis (previous elimination of antigen) is absent. We must not, then, deny the right of the anaphylactic animal to be termed

immune merely because we can make it ill, or kill it, by an artificial procedure which has no parallel in ordinary life.

As a matter of fact, I do not find that the constitutional illness associated with anaphylactic shock interferes in the least with the rapidity of disappearance from the circulation of the bacteria which have been injected. I have made a few observations bearing on this point, though they mostly cover only the first quarter of an hour after inoculation.

In a rabbit immunised against streptococcus fæcalis and into the ear-vein of which I had injected 1000 million of the living organism, 1-50th cubic centimetre of blood withdrawn every two minutes from the opposite ear yielded colonies too numerous to count at two and four minutes; at six minutes the cocci per cubic centimetre of blood were 33,000; at eight minutes, 13,000; and at 14 minutes only 5900. The animal was leucopenic all the time.

In the case of a rabbit similarly immunised against micrococcus citreus agilis, which showed no appreciable leucopenia when injected with 1000 million of the living coccus, the following figures were obtained: at two minutes 120,000 cocci per cubic centimetre of blood; at four minutes 26,000, dropping quite regularly to 1200 at the fourteenth minute.

The figures are very striking in the case of the two rabbits I have already mentioned as treated in this way with virulent pneumococci. One animal was immune, the other normal; both received the same dose (approximately 1000 million) of the same emulsion of cocci. For the first few minutes a confluent growth of pneumococci was obtained from 1-100th cubic centimetre of blood. The numbers progressively fell, but more rapidly in the immune than in the normal animal, till in 16 minutes the immune rabbit had only 15,000 cocci per cubic centimetre of blood, while the normal animal still had 125,000. The highly leucopenic rabbit was able to rid its blood of the cocci more than eight times faster than the normal one, in which leucopenia was almost absent.

In the case of a rabbit repeatedly immunised with diphtheria bacilli I gave 780 million living bacilli intravenously and found them almost gone from the circulation in 2 hours (one or two bacilli in 1-5th cubic centimetre of blood). In the case of typhoid immune rabbits intravenously injected with 1000 million living bacilli I found the bacilli absent from the circulation after 4 to 6 hours, though there had been pronounced leucopenia.

There is thus nothing to suggest that anaphylactic shock and its attendant leucopenia interfere in any way with such immunity as is evidenced by the rate of disappearance of the injected bacteria from the peripheral blood. The facts I have just mentioned do not necessarily mean a complete and final disappearance, for in one case at least, that of the non-immune rabbit injected with virulent pneumococci, it is highly probable that the cocci would not have disappeared beyond a certain point and that the animal would have died from septic-

æmia. But in the case of the animals inoculated with diphtheria and typhoid bacilli—the only ones which were not killed after 15 minutes—recovery was complete.

I have only met with one case in which an immunised animal died from septicæmia while the normal control animal survived, and I do not know why this was the case. I had immunised a rabbit against B. coli communis by six large intravenous doses of killed bacilli, spread over five months, rising at the last dose to 400 million. The animal had, as the result, a fairly high opsonic index against B. coli (about 3), and its serum showed a bactericidal power against this organism which was ten-fold the normal. Four or five weeks after the last vaccination I subjected it, together with a normal untreated animal, to an intravenous dose of 750 million living B. coli communis recently isolated from a case of ulcerative colitis. The normal animal showed a moderate leucopenia (lowest total count 2000 leucocytes per cubic millimetre at 3 hours), but in 5 hours had a leucocytosis of 12,400 and next day of 52,000 and 60,000, the increase being entirely due to the polynuclears. This animal had eliminated all bacilli from its peripheral blood in 24 hours and it recovered. The immune animal, on the contrary, exhibited a progressive leucopenia from which it never emerged. At the end of 5 hours the total leucocytes were only 400 per cubic millimetre and it died, still leucopenic, in 23 hours, with about 1200 B. coli per cubic centimetre of heart's blood ; the leucopenia at the end was lymphocytic no less than polynuclear. The count from the heart's blood immediately after death was, per cubic millimetre, lymphocytes 108, polynuclears 918, large hyaline 72, and basophils 102.

I may in this connexion call attention to some remarkable results obtained by Horder and Gordon with the meningococcus.[2] Searching for a criterion by which they could judge of the efficacy of vaccines and antisera against this organism, they found that whereas a normal rabbit commonly withstands an intravenous dose of no less than six sloped cultures, when given as a single dose, it readily succumbs to a series of doses, no larger in the aggregate, when these are intravenously administered at hourly intervals. Single cultures, given thus every hour, were found to kill after four to six doses, and they adopted this method of "serial dosage" in order to obtain a standard lethal effect. The serial doses produced a marked leucopenia, and it is not a little strange that no meningococci could be recovered from the blood in animals thus treated, even when the heart's blood was examined immediately after death, though in animals receiving a single massed dose of six cultures living cocci were recovered from the blood in diminishing numbers up to 24 hours after the injection.

The effect of previous vaccine treatment was to diminish

[2] Local Government Board Reports, 1907-08.

the percentage of rabbits dying as the result of the serial inoculations. Out of 22 normal animals 72 per cent. died; out of 21 vaccinated animals 28 per cent. died. Testing the effect of a number of the anti-meningococcal sera on the market it was found that when the more potent sera were administered previously to, and concurrently with, the serial doses of the meningococcus, not only was no protection conferred, but in certain cases death ensued earlier than in the control animals which received no serum. Horder and Gordon suggest that this may be due to an abnormally rapid dissolution of the cocci in presence of the antiserum, with consequent sudden liberation of an excessive amount of endotoxin. They do not, however, bring forward any proof of such lytic action. I mention these interesting observations here because they offer some parallel to the case I have quoted in which an immune animal succumbed to a dose of B. coli from which a normal rabbit recovered.

I may add that on the three occasions on which I have had the opportunity of observing the effects of Sclavo's anti-anthrax serum in human cases of cutaneous anthrax, without excision of the pustule, a local exacerbation of the inflammatory symptoms has in each case followed the administration of the serum, associated with a disappearance of the bacilli from the local lesion. In one case cultures were made every hour or two from the lesion, and the bacilli, at first abundant, were extremely scanty in 12 hours and absent in 24 hours after the serum was given. All three cases made a perfect recovery. It is difficult in such cases to avoid the belief that the exacerbation of local symptoms is in some way associated with a liberation of toxin from the bacilli which are being destroyed.

Before quitting the subject of anaphylactic leucopenia I may remark that it is followed by a polynuclear leucocytosis. The law of negative and positive phases is here also apparent, and the height of the leucocytosis seems to bear some relation to the intensity and duration of the preceding leucopenia. It is difficult to draw the line between this leucocytosis and that which is commonly associated with the presence of active tissue infections. The one may often pass into the other, just as the leucopenia of the shock may pass into that of marrow exhaustion in a rapidly fatal blood infection.

The Leucopenia of Marrow Exhaustion.

If a normal or immune rabbit is killed during the height of initial leucopenia following an intravenous injection of living or killed bacteria no noteworthy changes are found in the bone marrow. I have already stated that the number of cells bearing neutrophil granulations present per cubic millimetre of the normal rabbit's fresh bone marrow at the mid-femoral level may be anywhere between 100,000 and 200,000.

I have examined the marrows of six rabbits killed a quarter of an hour after the injection of large doses of living micro-organisms. Four of these were immune animals and three showed a marked leucopenia at the time they were killed, but neither these nor the ones which had exhibited no leucopenia showed any sign of marrow depletion. The neutrophil counts per cubic millimetre of marrow were in every case within the limits of normality, as the following table shows :—

Infecting organism.	Immunity.	Preceding leucopenia.	No. of cells bearing neutrophil granulations per c.mm. femur marrow.
Tubercle bacillus.	Immune.	Very marked.	159,000
,, ,,	Not immune.	Slight.	185,000
Virulent pneumococcus.	Immune.	Extreme.	193,000
,, ,,	Not immune.	Slight.	208,000
Streptococcus fæcalis.	Immune.	Moderate.	138,000
Micrococcus citreus agilis.	,,	None.	134,000

The bone marrow in these cases was in other respects also quite normal to microscopic examination; the cells and their granulations were natural and well stained; there was no sign of any gelatinous or mucoid degeneration. It would seem, then, that whatever the mechanism of initial leucopenia exhaustion of the bone marrow has nothing to do with it.

There is, however, a form of leucopenia which we may reasonably attribute to marrow exhaustion—that, namely, which is associated with severe and rapidly fatal septicæmias. It is well known that in certain fulminant human infections, of which malignant small-pox is a good example, a circulatory leucocytosis is absent, or if at first present is soon replaced by a pronounced leucopenia, in which myelocytes and nucleated red corpuscles may be found in considerable number in the circulation. Muir has studied the bone marrow in such cases and found it on the whole a "depleted" marrow, with widened blood spaces and a diminished number of cells.[3] The marrow appears to have been robbed of its cells by the drain upon its resources, while no compensating hyperplasia has had time to occur. I find phenomena of this kind in rabbits dying from acute septicæmia. I have never seen them so marked in infection with staphylococcus aureus as with the pneumococcus and

[3] Transactions of the Pathological Society, vol. liii.

with B. coli communis, but I should be prepared to meet with them in any acute and rapidly fatal blood infection.

If one injects into the circulation of a normal rabbit a dose of 200 to 400 million living virulent staphylococcus aureus the animal commonly dies. It may die in the course of the second day acutely septicæmic and with little in the way of local signs of disease; or it may live for 4 to 10 days and die with pyæmic abscesses in the kidney and sometimes in the heart wall. There is a difference in the changes seen in the blood and in the bone marrow in these two cases.

I have already said that the phenomenon of anaphylactic leucopenia is practically absent when staphylococci are intravenously administered to a normal rabbit. I have seen four animals die under such circumstances in 40 hours or less from the time of inoculation; no one of them had a severe leucopenia at any time, though in three of them counts lower than those found before inoculation were obtained at times, especially towards the end. As a rule I have found intermittently high leucocyte counts, not maintained and falling again to normal or below it as death approached. Myelocytes and nucleated red corpuscles may be found in the last stages. I have examined the bone marrow in three out of these four staphylococcal cases and found counts below the average but still within the limits of normality. The neutrophil counts at mid-femoral level were 112,000, 110,000, and 127,000 per cubic millimetre of fresh marrow. It would appear that the efforts at leucocytosis were beginning to exhaust the marrow but that the supply of neutrophil cells had not yet seriously failed, though insufficient time had elapsed for compensating structural changes in the marrow.

If, however, the animal survives as long as four days after the dose of staphylococci a neutrophil leucoblastic reaction begins to take place in the marrow and progressively increases. I have notes of four rabbits showing this, but I will defer mention of the facts till I come to speak of polynuclear leucocytosis. Rabbits dying four days or more after intravenous staphylococcal infection commonly show no terminal leucopenia but maintain intermittently high leucocyte counts to the end.

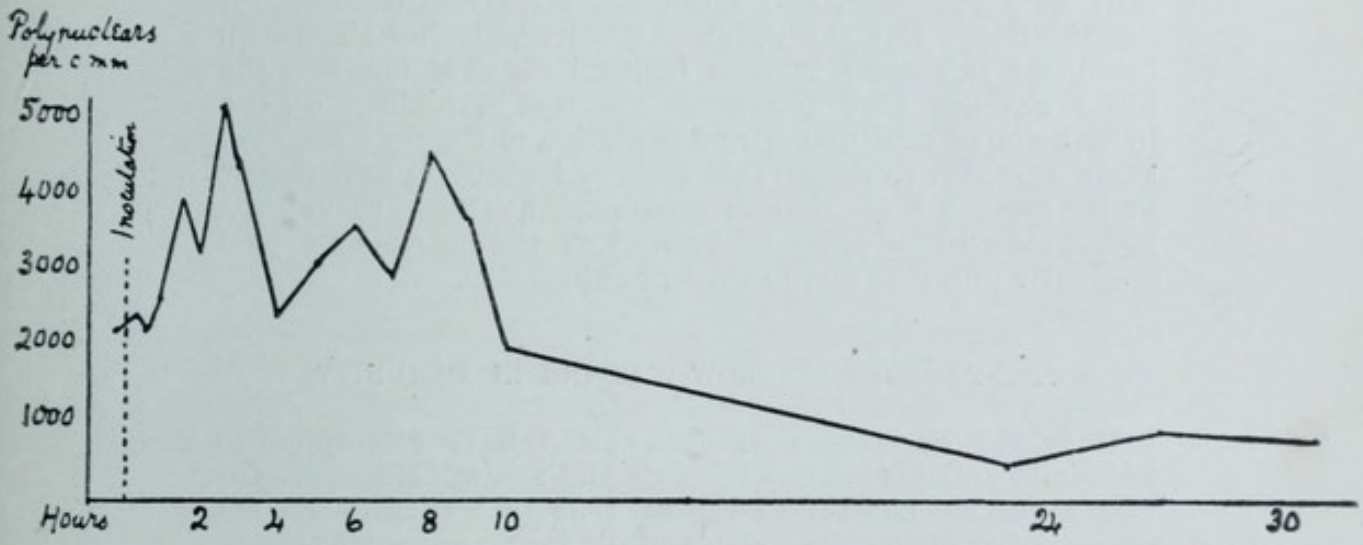
I have only once seen a really depleted marrow after death from acute staphylococcus aureus infection. This was in an animal greatly over-immunised against the coccus by five successive weekly doses of vaccine (200 million cocci). The animal was wasted and had a low opsonic index (0·5); it succumbed to a dose of living cocci in 11 hours without exhibiting any noteworthy leucocytosis, although a normal animal survived the dose for eight days. The bone marrow of the over-immunised animal yielded a neutrophil count of only 41,000 per cubic millimetre. I shall have directly to mention other instances in which a depleted marrow is found in the condition of wasting which not rarely attends unsuccessful efforts at immunisation. The low count of

neutrophils in this rabbit's marrow was probably associated with the wasting.

Much better examples of leucopenia due to marrow exhaustion are furnished by two rabbits dying from acute septicæmia after intravenous injection of the pneumococcus and of B. coli communis respectively.

A normal rabbit, weighing 2900 grammes, received an intravenous dose of about 200 million virulent pneumococci, isolated the day before from the pericardial fluid of a fatal human case of pneumonia with pericarditis. Before the inoculation the total leucocyte count was 8800 per cubic millimetre, and of these 2156 were polynuclears. Counts were made three times during the first hour, but no initial polynuclear leucopenia was detected, though there was a heavy fall in the lymphocytes 20 minutes after inoculation. The polynuclears

CHART 8.



Showing the polynuclear curve in a rabbit intravenously inoculated with 200,000,000 virulent pneumococci. There is no sustained attempt at leucocytosis and leucopenia soon ensues. Death occurred in about 40 hours, with no local lesions.

rose somewhat at first and at two and a half hours reached 5148 per cubic millimetre, the highest point observed. By the tenth hour they had fallen below their initial number and on the following morning they were only 480 per cubic millimetre. Septicæmia was now pronounced (250 colonies of the pneumococcus from 1-40th cubic centimetre of blood, although two hours after the inoculation this amount of blood had proved sterile). General œdema supervened and the animal died during the second night, probably about 40 hours after inoculation. The leucopenia persisted and involved lymphocytes as well as polynuclears. The latest count (31 hours) showed 3 per cent. of myelocytes, and there were a few nucleated red corpuscles. The bone marrow of this animal showed an extreme degree of depletion; the neutrophil count was difficult to carry out, as the cells were

not only reduced in numbers, but seemed in large part to have lost their granulations. I could only count 24,000 cells per cubic millimetre with recognisable neutrophil granulations, and in many of these the granulations were poorly stained and scanty; there were others which showed a pinkish sub-granular protoplasm. This was so however carefully I stained the sections.

I have already mentioned a rabbit immunised against B. coli communis which succumbed to an intravenous dose of 750 million B. coli in 23 hours, dying with some 1200 bacilli per cubic centimetre of blood. This animal was leucopenic almost from the first. . The neutrophil count from its bone marrow was 59,000 per cubic millimetre.

These two animals did not present the clinical phenomena of anaphylactic shock. Their leucopenia was progressive and endured to the end. At the time the experiments were carried out I had not begun to study initial leucopenia so fully as I did later, and I did not preserve the lungs for examination. But the strict parallel which they show with fulminant infections in man inclines me to believe that the blood changes they presented are best explained on the hypothesis of exhaustion and depletion of the bone marrow. I am disposed to regard this form of leucopenia as different in its essence from that of anaphylactic shock, though the latter may conceivably be present at the outset and pass gradually into that due to marrow exhaustion.

Spontaneous Marrow-wasting in Immunity.

It is the experience of many who have attempted to immunise animals against bacteria and against alien proteins that a certain number of the experimental animals do not prosper, but ultimately waste and die for no apparent reason, and without any cause for death being found on post-mortem examination. It has been noted, for example, in experiments on anaphylaxis that a sensitised animal may survive the immediate anaphylactic shock consequent upon a second dose, but may then pine away and die some weeks later. In my experiments on the immunisation of rabbits by the intravenous route, chiefly against bacteria, I have observed this unexplained wasting and death at least nine times. Occasionally I have felt able to attribute it to over-immunisation, but as a rule I could find no reason. I have several times observed that of two rabbits treated in the same way one has prospered and grown fat, while the other has wasted and perished. Sometimes these wasted animals develop sores on the rump or head, and in such cases I have noted polynuclear leucocytosis during life and a leucoblastic reaction of the bone marrow after death. These are, I believe, secondary to the septic sores; in animals simply wasted I have found not only no leucoblastic reaction of the marrow, but a gelatinous

kind of marrow poor in cellular elements. Thus in three rabbits dying wasted while in course of immunisation with vaccines of the diphtheria bacillus, the tubercle bacillus, and Gärtner's B. enteritidis respectively, the neutrophil counts per cubic millimetre of marrow (mid-femur) were 100,000, 87,000, and 78,000.

These observations are in accord with what is known as to mucoid transformation of the bone marrow in man and other animals. It is a condition associated especially with wasting and inanition. Cadbury records it in many cases in his marrow studies in pulmonary tuberculosis.[4]

Another Form of Leucopenia.

There is a form of leucopenia, well known from clinical observation in man, but with which I have not met in my observations on the rabbit. It is that seen in uncomplicated typhoid fever, and in many cases of acute general tuberculosis. It is a persistent condition, and it does not fit in with either of the forms of leucopenia which I have been describing. There is nothing to suggest that it is related either to anaphylactic shock or to marrow exhaustion. In those diseases in which it is seen there is a considerable amount of evidence that the polynuclear leucocytes play little part in the defence of the body against the infecting organism. This fact might well explain the absence of leucocytosis, but not an actual circulatory leucopenia. I do not know that the typhoid bacillus and the tubercle bacillus are distinguished by any negatively chemiotactic powers sufficient to account for the facts. Having no experimental evidence on the matter, I must be content to admit the existence of this type of leucopenia without attempting to explain it.

I now pass on to those conditions in which the circulating leucocytes are increased in number and in particular to the subject of polynuclear leucocytosis.

Leucocytosis.

When we say that a man or an experimental animal has leucocytosis we commonly mean that the number of leucocytes in the peripheral blood is increased. With "local" leucocytosis—i.e., an accumulation of one or another kind of leucocyte in the tissues at any given point—I am not for the moment concerned. But there is another factor in the definition of the term leucocytosis. A distinction must be drawn between leucocytosis and that other, and usually more extreme and persistent, increase in the number of circulating

[4] Fifth Annual Report of the Henry Phipps Institute.

leucocytes, presented by the group of diseases known as the "leukæmias." We know little or nothing of the causes of leukæmia, and it is therefore very difficult to draw any line between leukæmia and leucocytosis. Leukæmia is perhaps only a symptomatic condition, but it is probably fair, in the present state of our knowledge, to put the matter thus. Leucocytosis is a deliberate and purposeful increase in the circulating leucocytes: leukæmia is an apparently purposeless and wanton increase. This difference may vanish when we know more about leukæmia: at the present it holds good.

Corresponding to the purposeful nature of leucocytosis we find it almost invariably due to an increase in one special kind of leucocyte—that of which the function is in request. Here I shall deal chiefly with *polynuclear leucocytosis*—which I define as the putting out into the circulation of an abnormal number of polymorphonuclear neutrophil leucocytes, presumably to fulfil some special end. What this end may be we do not in all cases know. Often, as in invasions by the pyogenic cocci, it is clearly the phagocytic destruction of the bacteria; but we also see it after the injection of peptone and, in the immune animal, after injection of foreign proteins. Various toxic leucocytoses are seen in man, and drug leucocytoses such as that produced by cinnamic acid and its allies. We are driven to infer some function of the polynuclear leucocytes other than phagocytosis in order to explain the increased output in such cases. We may guess that where an antibody is reacting with an antigen and using up complement the functions of the leucocytes in furnishing complement are in request. But in many cases we are quite in the dark as to the meaning of the leucocytosis.

The Various Types of Polynuclear Leucocytosis in Infections.

I have endeavoured to show that there is more than one type of polynuclear leucopenia. My animal experiments lead me to believe that, similarly, an increase in the circulating polynuclears occurs under more than one condition of infection. When a dose of bacteria is intravenously injected into a rabbit there sometimes ensues a practically instantaneous increase in the circulating polynuclears, over in a few minutes, and followed by the ordinary initial leucopenia. In other cases I have seen a high leucocytosis coming on in an hour or two and soon followed by a progressive leucopenia. When initial leucopenia is well marked it is almost always followed by a leucocytosis lasting a few hours, or, it may be, a day, and then subsiding. When the bacteria are not promptly exterminated but gain a foothold somewhere in the body and set up local disease,

there is generally seen a polynuclear leucocytosis of longer duration and often of greater intensity than in the preceding cases, lasting till death or recovery. This is the form of leucocytosis which has its parallel in human infections, and corresponds to that met with clinically. I am not prepared to say that it occurs only in local infections, but it is certainly much more characteristic of these than of septicæmic conditions, which tend to leucopenia in proportion to their gravity.

I am therefore disposed to recognise at least three types of polynuclear leucocytosis in infective conditions experimentally induced by intravenous inoculation, and I will now give my reasons for this opinion in further detail.

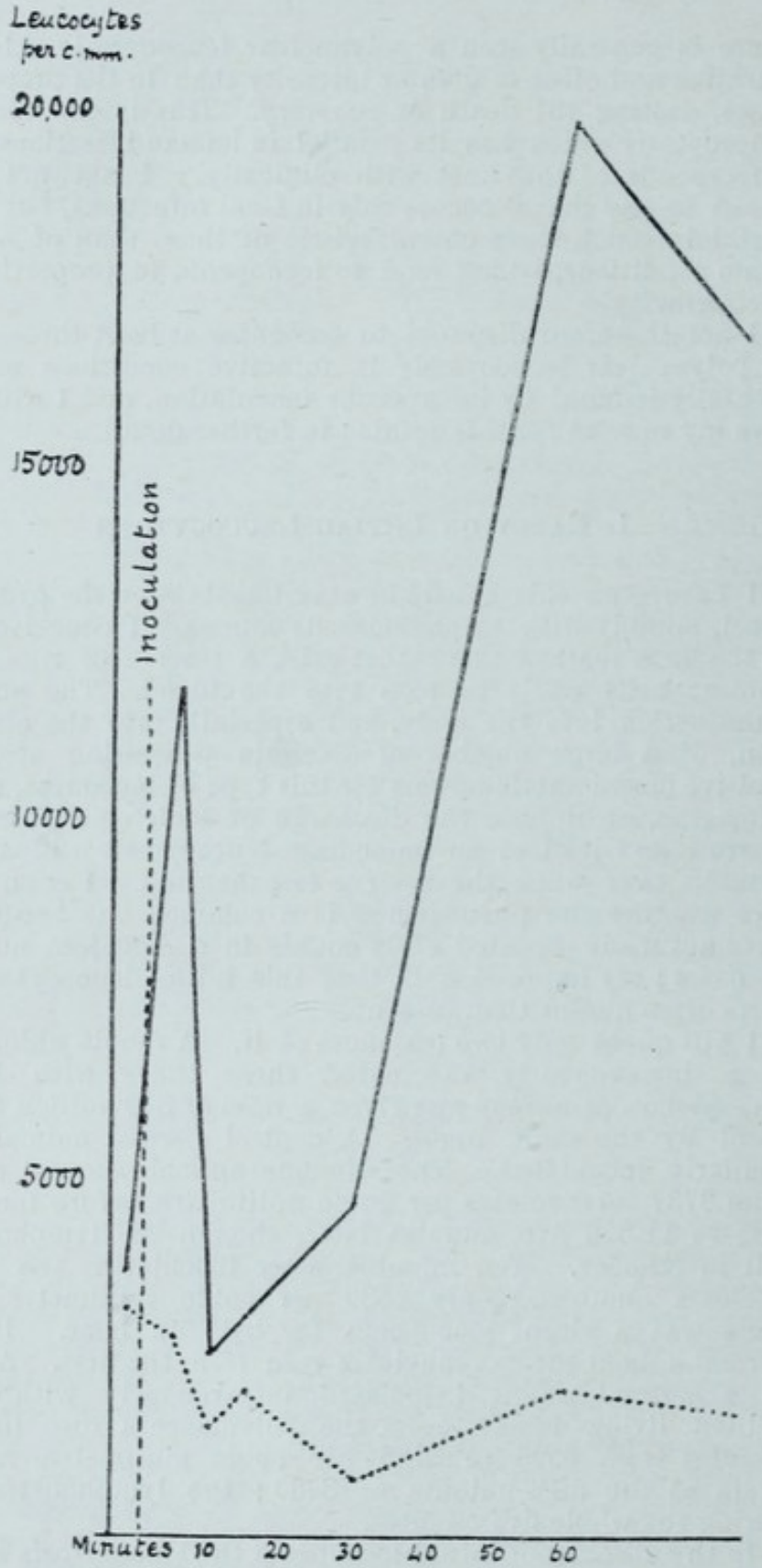
1. Early or Initial Leucocytosis.

I have seen this chiefly in experiments with the pyogenic cocci, notably with staphylococcus aureus. I conceive that in the bone marrow there must exist a reserve of ripe polynuclear cells ready to pass into the blood. The sudden introduction into the body, and especially into the circulation, of a large number of bacteria possessing strongly positive chemiotactic powers for this type of leucocyte, must, I imagine, precipitate the discharge of such cells from the marrow and lead to an immediate leucocytosis which will soon be over when the reserve is exhausted. I cannot be sure whether the phenomenon is a common one because I have not made repeated early counts in a sufficient number of cases; my impression is that this initial leucocytosis is more often absent than present.

I will quote only two instances of it. A rabbit which had been intravenously vaccinated three times with killed staphylococcus aureus was given a dose of 240 million living cocci by the same route. A control normal animal was similarly inoculated. The immune animal showed a rise from 3737 polynuclears per cubic millimetre before inoculation to 11,808 five minutes later, though the lymphocytes fell in number. Ten minutes after inoculation the polynuclears numbered only 2530 per cubic millimetre, and there was a slight leucopenia for half an hour. In the normal animal the polynuclears rose from the first. Again, in a normal rabbit inoculated intravenously with 1000 million living pneumococci the polynuclears rose in two minutes from 6596 to 8136 per cubic millimetre, falling again at the fifth minute to 3480; the lymphocytes fell during the whole five minutes.

In the absence of initial leucopenia the leucocytosis set up by the injection of the pyogenic cocci may come on in half an hour or an hour, and the same is true of harmless cocci. Thus, of two normal rabbits into the veins of which I injected living micrococcus citreus agilis (450 million), one

CHART 9.



Showing the behaviour of the polynuclear leucocytes in an immune rabbit intravenously inoculated with 240,000,000 living staphylococcus aureus of low virulence. There is seen an initial polynuclear rise (continuous line). The lymphocyte curve is shown by the dotted line.

showed 16,000 polynuclears per cubic millimetre in 30 minutes, and the other 17,000 in 1½ hours. In a normal rabbit, injected intravenously with a dose of virulent staphylococcus aureus (220 million), the blood showed an hour later a total leucocyte count of no less than 114,000 per cubic millimetre. This is an exceptionally high count, but the majority of the rabbits, normal and immune, which I have intravenously inoculated with staphylococcus aureus have had a leucocytosis by the end of the first hour. In a rapidly fatal case this may be replaced in from 12 to 24 hours by the leucopenia of marrow exhaustion. Or again, if the infection is easily overcome the leucocytosis may subside in a few hours.

2. Reactive Leucocytosis after Anaphylactic Shock.

I have observed the early leucocytosis, which I have just described, mainly in experiments with the pyogenic cocci—i.e., with bacteria having a marked positively chemiotactic power towards the polynuclear leucocytes. There is evidence that phagocytosis occurs very early in such cases, at least when the cocci are not of extreme virulence. I have mentioned the evidence which I have obtained of this in the lung of an animal inoculated with streptococcus fæcalis and killed after 16 minutes. In the reactive leucocytosis seen after non-fatal anaphylactic shock, phagocytosis cannot be invoked in explanation of the rise. Just as we see a high leucocytosis following the leucopenia consequent on injection of peptone, so, in the rabbit sensitised against a foreign protein, do we see polynuclear leucocytosis following the anaphylactic leucopenia. The phenomenon is seen in marked degree in animals immunised against the typhoid bacillus; yet there is good clinical evidence that in human typhoid fever, although suppurations may occur, the polynuclear leucocyte plays a very subordinate part compared with that which it occupies in infections by the pyogenic cocci.

So invariably have I seen a polynuclear leucocytosis to follow on the leucopenia of anaphylactic shock, whatever the foreign protein concerned—bacterial or non-bacterial—that I am disposed to regard it as an integral part of the phenomena of anaphylaxis when the shock is not attended by a fatal result. Again and again I have seen the minor symptoms of shock pass off concurrently with the onset of leucocytosis after the injection of bacterial vaccines. During the leucopenic period the rabbit refuses food, its respirations are increased in frequency, and it is difficult to obtain blood from the ear owing to lowered blood pressure. At a given moment the animal becomes cheerful, begins to eat and move about its cage; it is now found to bleed readily, and the polynuclear leucocytes are increased above the normal.

For these reasons I incline to recognise a distinct type of experimental polynuclear leucocytosis as a reactive form

having no necessary connexion with the phagocytic needs of the organism, and, indeed, well seen in those cases where the disturbance is due to a foreign protein and not to an organism having any special chemiotactic influence upon the polynuclear leucocytes.

If it be asked why this leucocytosis occurs the answer is probably to be sought in the humoral functions of the polynuclear leucocytes. If it be true that these cells furnish complement, and that complement is used up in the processes associated with anaphylactic shock, the reason for their appearance in excess is sufficiently explained. In the case of the injection of bacterial vaccines phagocytosis cannot be altogether excluded as some part of the aim of reactive leucocytosis, but I doubt whether it has much to do with the matter. If phagocytosis occurs it does so early, during the leucopenic stage; the dead bacteria in the vaccine are probably easily dealt with, and their multiplication locally in the tissues is here out of court. For these reasons I incline to regard the reactive leucocytosis seen to follow the initial leucopenia consequent on intravenous administration of a vaccine as of the same nature as that seen after an alien protein, such as horse serum or egg albumin. And it may well be that, when living bacteria are substituted for the dead vaccine, the same is true when the infection is easily overcome during the first few hours.

I have seen the phenomenon of reactive polynuclear leucocytosis after the intravenous injection of horse serum, egg albumin, foreign blood corpuscles, and bacterial vaccines, especially of the coli-typhoid group, but also with diphtheria and tubercle bacilli, and it is better marked in the immune than in the normal animal. I have seen it exceedingly well marked when living typhoid bacilli are injected into the immune rabbit.

3. The Leucocytosis of Local Tissue Infection.

I am not quite clear whether I am right in calling the leucocytosis seen in established infective disease a phenomenon of *local* tissue infection. I am not prepared to deny that such polynuclear leucocytosis in human septicæmic conditions may depend upon the generalised blood infection and not upon a local lesion, but I am far from prepared to affirm it. In practically all septicæmias there exist primary, or at least secondary, foci of local infection. In those grave conditions where the blood infection is the predominant feature leucopenia is usually seen; leucocytosis seems more pronounced in proportion to the evidence of local foci of inflammation. My experiments upon the rabbit lead me to lay stress upon local infection as the main condition upon which depend the higher and more enduring forms of polynuclear leucocytosis.

It is probably safest to argue from those infections which depend upon the pyogenic cocci, since these have the most intense positively chemiotactic influence upon the leucocytes. I have repeatedly inoculated rabbits, both normal and immune, with moderate to large doses of living virulent staphylococcus aureus. Sometimes the animal dies within two days, with a pronounced septicæmia; such cases may show a high early leucocytosis, but it is one soon over and replaced, as the animal becomes more ill, by a leucopenia which persists till death, and no focal lesions can as a rule be found in these animals. The spleen may be enlarged and a few subserous hæmorrhages may be seen, but that is all. It is well known that such post-mortem appearances may be all that can be found in certain fulminant septicæmias in man. But in a rabbit dying some four to seven days after an intravenous infection with the staphylococcus the course of events is very different. There may be an initial leucocytosis or the polynuclear leucocytes may not at first be much increased in the circulation, but in two or three days a leucocytosis, essentially polynuclear, becomes established and persists, often with striking fluctuations, to the fatal issue. Such cases always exhibit local foci of suppuration, most invariably in the renal medulla, but often in the heart muscle and sometimes elsewhere. And I shall presently show that they also exhibit a very striking neutrophil leucoblastic reaction in the bone marrow, absent in the cases of earlier death.

In two animals which had been highly immunised against staphylococcus aureus, but which nevertheless succumbed to infections with the living coccus, 8 and 17 days respectively after intravenous inoculation, the local focus of suppuration was found in the spinal meninges. It is noteworthy that in several of these cases of delayed death after intravenous inoculation with staphylococcus aureus, and in which high leucocytosis had been observed to the end, the cocci could not be recovered from the blood during life or even from the heart's blood after death. The condition was not essentially a septicæmia, but a local visceral infection.

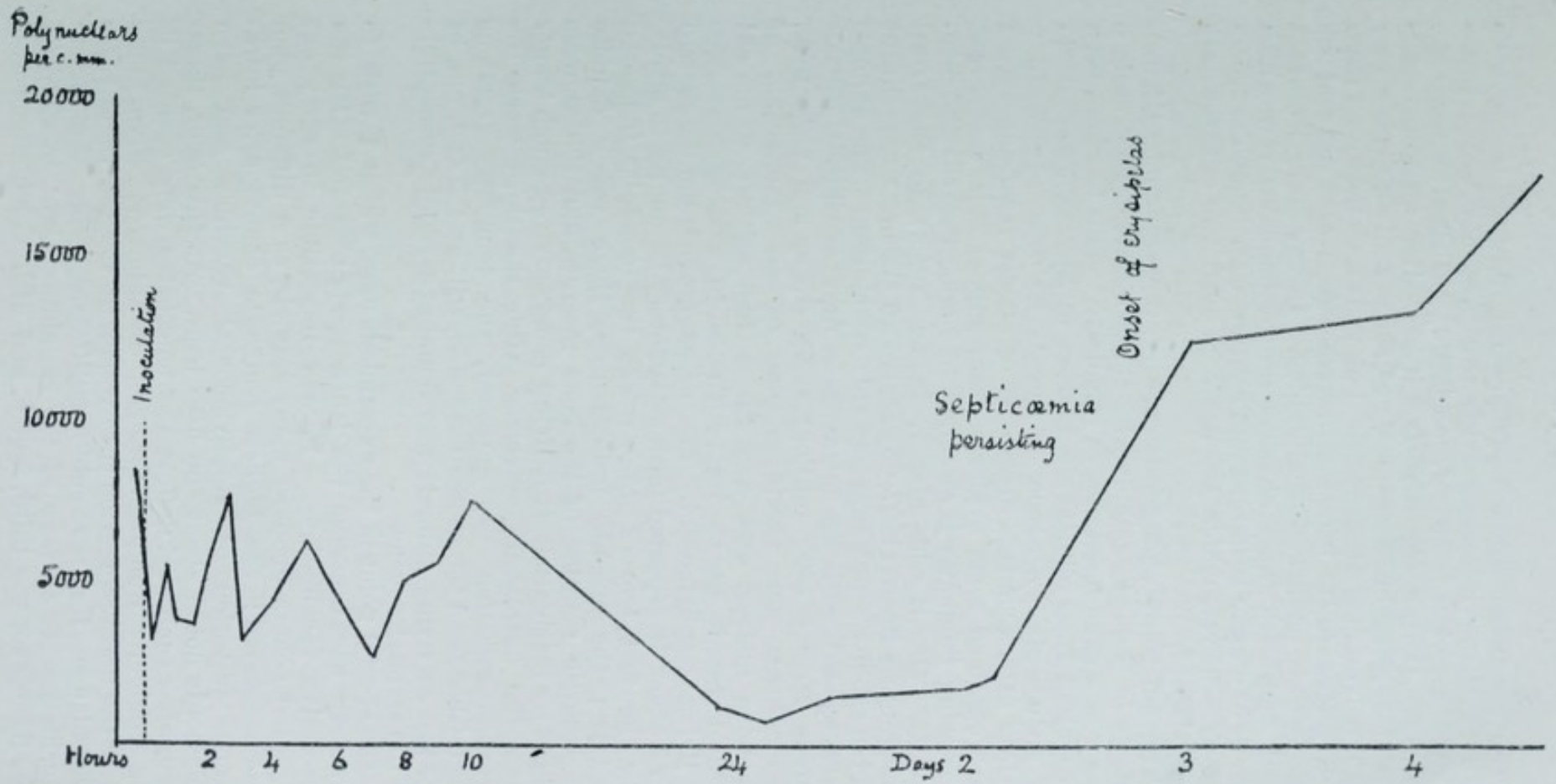
I may further mention an experiment with the pneumococcus which adds force to the preceding facts. It is well known that the rabbit is highly susceptible to this coccus, and when infected with a virulent strain succumbs to a diffuse septicæmia without local lesions. I have already mentioned such an instance in speaking of the leucopenia of marrow exhaustion. I had inoculated another normal rabbit at the same time with the same strain of pneumococcus, using the actual pericardial fluid from a fatal human case, and not a culture. Half a cubic centimetre of the fluid was injected intravenously; I cannot state the number of cocci present in this. Before inoculation the polynuclears of this animal were rather high, numbering 8480 per cubic millimetre. They fell in 20 minutes to 3268, and during the 10 hours on the day of

inoculation during which leucocyte counts were made, they varied from 2656 to 7696 per cubic millimetre, never reaching the initial figure. On the following day the animal was evidently ill, and there was a marked polynuclear leucopenia (700 to 1500 per cubic millimetre). On the third day it was no better, and the polynuclears numbered only 1700 to 2100 per cubic millimetre. Nucleated red corpuscles were present in the blood, and there was still a septicæmia. 1-40th cubic centimetre of blood yielded two colonies of the pneumococcus. I had no doubt that the animal would die, but a new and almost dramatic turn was given to the case by the development of a local lesion. Starting on the third day and rapidly increasing in intensity there arose a typical erysipelas of the inoculated ear, which by and by presented bullæ of clear fluid yielding pure but not abundant colonies of the pneumococcus. (I have once before seen erysipelas occur in the rabbit after inoculation with the pneumococcus, and human cases have been recorded.) With the onset of the local lesion the blood picture entirely changed; on the fourth and fifth days the polynuclears numbered 12,000 and 13,000 per cubic millimetre, and on the sixth day nearly 20,000. They remained intermittently high for many days. The animal recovered and was later used for an experiment I have already mentioned.

The occurrence of a pronounced polynuclear leucocytosis, with the onset of the local lesion, appeared to be the turning point in a case which had threatened to be fatal. The humoral changes in the blood were not followed, though I believe they might have proved instructive. But I think it is legitimate to use the case as an argument in favour of the view that polynuclear leucocytosis is essentially a phenomenon of local tissue infection, rather than of general blood infection. Whether this be so or not I have one or two remarks to offer as to the characters and associations of this form of polynuclear leucocytosis.

The first is that the actual number of circulating leucocytes varies from hour to hour, sometimes in very sudden fashion. It is only when counts are made at frequent intervals that this fact becomes apparent. It would seem as though under the stress of infection the polynuclear leucocytes were discharged into the circulation not continuously and uniformly but in irregular gushes, in the intervals between which fresh crops mature. Making every allowance for possible errors in technique and for the fallacies attending local leucocytosis, I feel convinced that these fluctuations are genuine. In a rabbit intravenously inoculated with the pyogenic cocci, I have seen an increase from 9000 to 72,000 leucocytes per cubic millimetre in less than an hour, and though that is perhaps an extreme case, I have seen the same sort of thing in lesser degree on many occasions. Whether similar abrupt variations in the leucocyte count occur in human beings suffering from similar infections I do not know.

CHART 10.



Showing the curve of the polynuclear leucocytes in a rabbit intravenously inoculated with virulent pneumococci. Septicæmia and leucopenia persisted during the second day. With the onset of a local erysipelas in the ear on the third day leucocytosis set in and the animal recovered.

The persistent, though perhaps intermittent, leucocytosis seen in tissue infections with the pyogenic cocci seems always associated with leucoblastic changes in the bone marrow. During the first few hours after an intravenous infection a rabbit may at times show a high, even a very high, polynuclear leucocytosis, but it is an effort soon over. The reserves in the bone marrow seem soon used up, and the leucocytosis cannot be maintained. In my next lecture I shall deal with those changes in the bone marrow which permit of a more sustained and continuous polynuclear leucocytosis—changes which are not seen until two or three days have elapsed.

The efficiency and persistence of the polynuclear leucocytosis in tissue infections with the pyogenic organisms is a factor of some importance in immunity. This has long been recognised from clinical observation in such human diseases as pneumonia. Absence of circulatory leucocytosis in a serious attack of this disease is of almost fatal augury. I have obtained some evidence from animal experiment that increasing immunity is attended by higher degrees of leucocytosis. Dr. Horder and I, in our report to the Local Government Board (1907–08), record an elaborate experiment on ten rabbits which supports this conclusion. The animals were treated with an intravenous dose of staphylococcus aureus vaccine (200 million) every week, and each week two animals were sacrificed, one for an examination of its bone marrow and one for a test against a normal control rabbit as to its resistance to a dangerous dose of living staphylococci. The doses proved too large and too frequently repeated for a satisfactory immunity to result. For the first two or three weeks the immunity increased rapidly ; the opsonic indices rose, there was a definite leucoblastic reaction of the bone marrow, and the animals tested by inoculation with living cocci survived much longer than the untreated controls. After the third week the over-immunisation was apparent, the immunity reactions diminished, and the protection faded, till, at the end, one of the last surviving animals was found abnormally susceptible to the staphylococcus.

The special point in the experiment upon which I wish to lay stress here concerns the leucocytosis observed after the successive vaccinations. It was impossible to carry out repeated counts upon so many animals, so a different plan was employed: the highest leucocyte counts after intravenous injection of staphylococcal vaccines had been commonly noted about the fifth hour. At this hour, therefore, after each weekly dose the leucocytes were counted in the gradually diminishing band of animals. Five hours after the first vaccination the average of the ten rabbits was 9780 leucocytes per cubic millimetre, after the second vaccination the average of the remaining eight was 24,775. The figures for the third, fourth, and fifth vaccinations were

22,066, 27,700, and 20,600 per cubic millimetre. It would thus appear that with increasing immunity—i.e., in this case up to the second or third week—there was an increased circulatory leucocytic response to the same dose of antigen and that this response lessened at the end as protection faded.

Had I time I could adduce one or two other observations pointing in the same direction. But I must be content to state my belief that in the case of infection with the pyogenic cocci the immune animal responds by a higher and a more ready increase in the circulating polynuclear leucocytes than the normal animal. The facts which I shall have to relate in my next lecture as regards the bone marrow support and explain this.

LECTURE IV.

Delivered on June 23rd.

MR. PRESIDENT AND GENTLEMEN,—The importance of the polynuclear leucocytes in the defence of the body against bacterial invasion has been dwelt on in my previous lectures from more than one point of view. It follows naturally that the mother tissue from which these cells are derived must play no small part in defence, at least when such defence has to be prolonged. This has long been recognised; it has been said that "leucocytosis is a function of the bone marrow," and Ehrlich has aptly termed this tissue a "defensive organ."

THE BONE MARROW IN IMMUNITY.

A distinction must, of course, be drawn between the "red marrow" and the "yellow" or "fatty" marrow which fills the shafts of most long bones. The cavities of the bones are larger than is necessary to lodge the amount of true marrow tissue which suffices for the needs of the healthy body; the extra space is utilised for fat storage. But stained sections of fatty marrow from a long bone show something which is not seen in ordinary adipose tissue elsewhere; here and there on careful search are to be found tiny foci, it may be single cells only, of true marrow tissue with its characteristically granulated cells. When need arises these cells can undergo rapid increase, and the fat can readily be absorbed to make room for them; the yellow marrow is easily converted into red.

The marrow is the mother tissue of red cells as well as of white. Of the leucocytes, it furnishes the granulated oxyphil, basophil, and neutrophil cells, and it further contains islands of lymphatic tissue which may share in the general lymphoid hyperplasia of lymphatic leukæmia. The marrow may therefore undergo a transformation in several different directions in accordance with the special needs of the body at the moment. If the urgent call is for more red corpuscles there is seen an "erythroblastic" reaction in which the predominant marrow cell is the erythroblast; if the call is for leucocytes there is a "leucoblastic" reaction, which is commonly a neutrophil leucoblastic reaction because the need for an increase in the polynuclear leucocytes is a

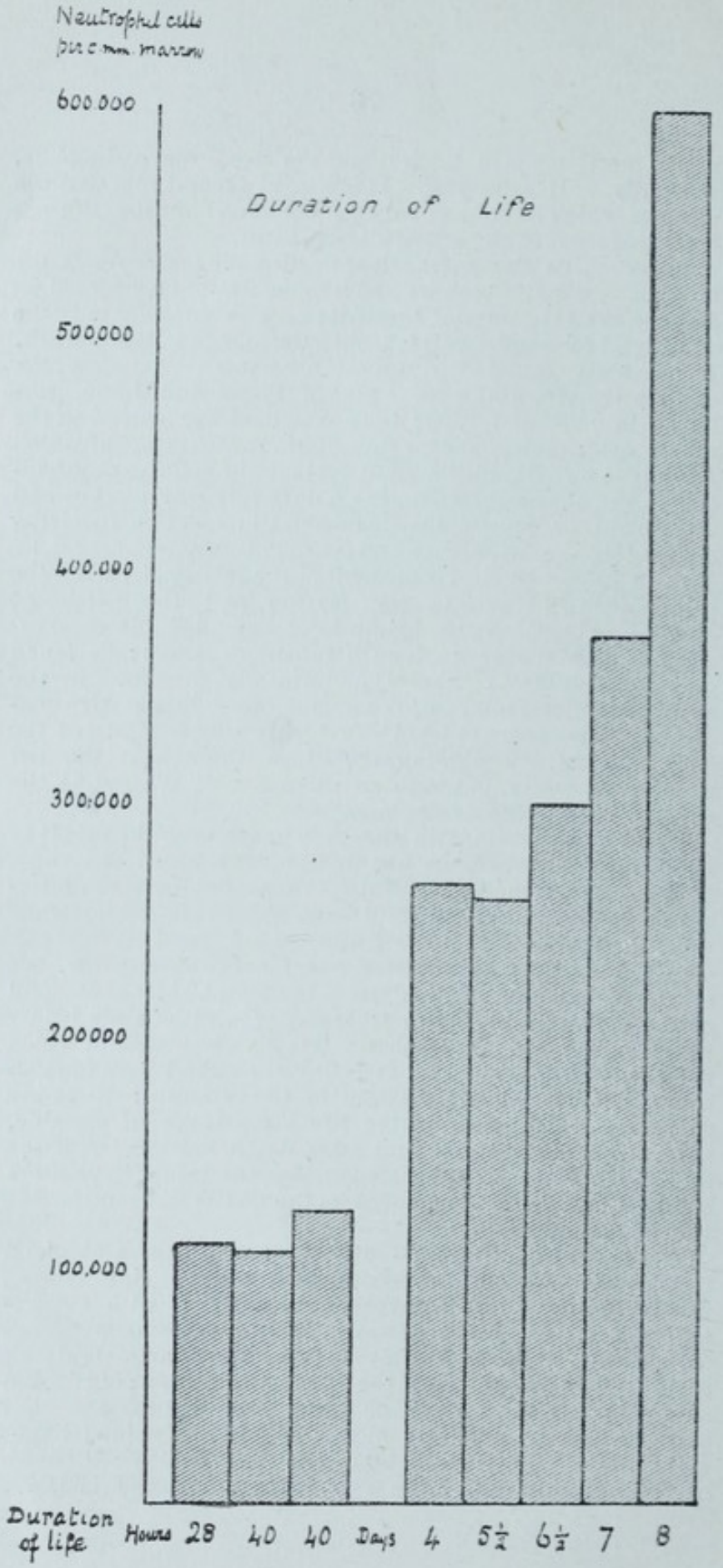
much more common thing than the need for eosinophils, basophils, or lymphocytes. It is a good general rule that the changes which have been seen in the blood during life are found reflected in the marrow after death.

Inasmuch as the polynuclear neutrophil leucocyte is the only marrow cell which we know to be actively concerned in defence against bacteria, I propose here to consider only the neutrophil (or amphophil) leucoblastic change in the marrow. It has been studied by several observers. Amongst the earliest experimental work is that of Roger and Josué, published in 1896 and 1899; they examined the marrow in the rabbit after subcutaneous inoculation with staphylococci. Already in two days they found evidence of cellular proliferation in the bone marrow, and in 5 days this was marked and was found to consist in a leucoblastic reaction involving chiefly the amphophil myelocytes. This was confirmed by Muir in 1901, who clearly recognised the relation between the leucoblastic changes in the marrow and the persistent circulatory leucocytosis. Muir later extended his observations to the marrow in human infections (1902); he found changes, similar to those experimentally produced in the rabbit, in pneumonia, empyema, and other suppurative conditions. The changes involve not only a hyperplasia of the cells bearing neutrophil granulations throughout the red marrow generally, but also an extension of the red at the expense of the yellow fatty marrow.

My own observations on the bone marrow of the rabbit in staphylococcal infections are in complete accord with those of Roger and Josué and of Muir. I have employed the intravenous route of infection and I have already stated that when death occurs rapidly, in 40 hours or less, from acute septicæmia I find no leucoblastic reaction in the marrow, but rather a depletion of this tissue in respect of its neutrophil cells. These are the cases which show a circulatory leucopenia as the end approaches. But in every case in which life has been prolonged for four days or more I have found a neutrophil leucoblastic reaction in the marrow; these are the cases which show during life a persistent, if variable, polynuclear leucocytosis, and after death local foci of tissue infection. I have further found that the longer the animal survives the more pronounced is the neutrophil transformation of the marrow.

The subjoined diagram shows the actual figures obtained from neutrophil marrow counts in a series of eight normal rabbits dying after intravenous inoculation with staphylococcus aureus (seven cases), and streptococcus pyogenes (one case). It is seen that in the three animals dying early (40 hours or less) the figures are low; in those surviving for four days or more the figures are high and become progressively higher till, in the animal living eight days, the neutrophil cells per cubic millimetre of fresh marrow reach the very large number of 596,000.

CHART 11.



Showing the content of the bone marrow in neutrophil cells at mid-femoral level in rabbits dying from acute infection with the pyogenic cocci.

It is thus clear that in presence of an active pyogenic infection the response of the bone marrow is prompt and energetic; the neutrophil cells are trebled or quadrupled in number in the course of a week or so. This is in perfect harmony with the known facts in man: Muir's observations on the marrow in pneumonia and empyema indicate an equally prompt and energetic reaction.

Significance of Leucoblastic Reaction.

From these facts we are entitled to draw two conclusions. I have said that rabbits showing a marked leucoblastic reaction in the marrow exhibit during life a persistent and usually high leucocytosis up to death; in this they contrast strongly with the more rapidly fatal cases. It would seem that the "plant" for the production of polynuclear leucocytes present in normal marrow, though it may suffice for a high transitory leucocytosis, such as that which I have spoken of as reactive leucocytosis after anaphylactic shock, or for that associated with an infection easily overcome, is quite inadequate for the *maintenance* of a high leucocytosis. In presence of a grave pyogenic infection steps are at once taken to increase the plant necessary to keep up a due supply. In spite of this the patient or the animal may die, but it is permissible to assume that in every case showing a *sustained* circulatory leucocytosis this marrow reaction is present and that when, as is commonly the case, recovery occurs, the reaction has been part, and I think a very important part, of the machinery by which the body has overcome the infection. The prognostic value of the leucocyte count in such human diseases as pneumonia rests on a definite anatomical basis.

The second conclusion which may be drawn from the facts is that of the vast importance of the polynuclear leucocyte in the bodily struggle against invasion by the pyogenic cocci. The immediate steps which seem to be taken to provide an increased supply of these cells in presence of such invasion, and the disastrous results which seem to attend failure of the supply, can bear only one interpretation. If, in any given bacterial infection, a sustained polynuclear leucocytosis is found during life, and, after death, a neutrophil leucoblastic reaction in the bone marrow, we are fairly entitled to conclude that the defence of the body against the microbe in question rests largely, perhaps entirely, with the polynuclear leucocytes, and is probably phagocytic in essence. An impartial observer studying an invasion of this country by a foreign foe would note an excess of soldiers on the battlefield and on the railways leading to it; he would see an increased activity in the depôts where soldiers are trained and drilled; and he would justly conclude that we relied on our soldiers for the defence of our native land.

But if he saw few soldiers about, in spite of the imminence of the danger, and if he saw the invasion repelled without the apparent need for any increase in our soldiery, he would with equal justice conclude that in this particular invasion we had some other means of defence. And thus, I believe, and shall shortly endeavour to show, that the habitual absence of a circulatory polynuclear leucocytosis and of a neutrophil leucoblastic reaction in the bone marrow must mean that the polynuclear leucocytes play no part, or only a subordinate part, in that kind of bodily infection.

The Humoral Factor.

In speaking thus of the importance of the polynuclear leucocyte in infections by the pyogenic cocci I do not wish to disparage the humoral accessories which render the action of these cells effective. I am convinced of the importance of opsonic action, but I desire to protest against the exclusive importance which some have in recent years attached to this phenomenon. Salt and mustard are valuable elements in a meal, but the beef-steak and the man who sits down to eat it must not be left wholly out of account.

In the course of my work on the pyogenic cocci I have obtained a certain amount of evidence that the intravenous injection of killed cultures of staphylococcus aureus is efficacious in producing a neutrophil leucoblastic reaction in the marrow of the rabbit. I mentioned in my last lecture an experiment in which ten rabbits received a weekly intravenous dose of 200 million killed staphylococci, two rabbits being sacrificed for observation each week. One of each pair of animals was simply examined as to the condition of its bone marrow. It will be remembered that in this experiment the immunity of the animals showed an increase for the first two or three weeks, but that their protection then faded away, owing probably to over-vaccination. The figures obtained from the bone marrows were as follows. A week after the first dose of vaccine the count showed 249,000 neutrophil cells per cubic millimetre of fresh marrow—a considerable increase above the normal. The animal killed a week after the second dose of vaccine had a marrow count of 315,000. After the third, fourth, and fifth doses the figures were respectively 232,000, 128,000, and 216,000.

There is thus seen a correspondence between the degree of leucoblastic reaction in the marrow and the height of the leucocytosis observed after vaccination as detailed in my previous lecture. Both corresponded with the degree of resistance manifested by the animals which were inoculated with living cocci, and I may add that the opsonic indices of the animals showed the same rise at first, followed by a fall towards the end of the series.

So far as this experiment goes, it shows a fairly close parallel between the degree of immunity on the one hand, and on the other the leucoblastic reaction of the marrow, the capacity for a high circulatory leucocytosis on stimulation, and the opsonic index. These three things seem all to be connected, and, in this experiment at least, to vary together: they are part and parcel of a common mechanism, the object of which is to secure the phagocytic destruction of the cocci. We should, I think, be wrong in assigning exclusive importance to any one factor in the process. The opsonic index probably offers an easy method of determining the general efficiency of the mechanism as a whole, but I should not, on the evidence, be prepared to admit a humoral factor as the sole index of immunity, for I should expect to find it more fluctuating than the anatomical substratum of defence indicated by the changes in the bone marrow, and upon which the use and significance of the opsonic index must depend. I am referring here to the opsonic index against the pyogenic cocci, where phagocytosis plays an undisputed part as the main bodily defence.

Further Observations on Pyogenic Infections.

Before leaving the subject of the changes in the bone marrow in infections by the pyogenic cocci, I may add a few other observations bearing on the subject. In a rabbit which had been three times treated intravenously with a vaccine of streptococcus fæcalis, and which died for no apparent reason, I found 315,000 neutrophil cells per cubic millimetre of bone marrow, but in another animal killed after similar treatment the number was only 138,000, and in a third, which died much wasted, the number was only 128,000. In an animal killed after two intravenous inoculations with micrococcus citreus agilis I found no leucoblastic reaction in the marrow; the neutrophil count was 134,000 per cubic millimetre. Several of my rabbits have contracted a scaly disease of the inner surface of the ear, in which an accumulation of pus and epithelium forms a laminated mass over half an inch in thickness. I do not know the exciting cause of this, but a white staphylococcus of peculiar reactions is the most constant organism in the lesions, and sections of the affected ears show an obvious local accumulation of polynuclear leucocytes. There is also a circulatory polynuclear leucocytosis during life. I have examined the bone marrow in two rabbits affected in this way and in each case there has been an extreme leucoblastic reaction; in one the neutrophil count per cubic millimetre of marrow was 463,000 and in the other 540,000.

The general conclusion to which these observations have led me is that infections by, and vaccinations with, the pyogenic cocci lead in a few days to a neutrophil leucoblastic

reaction in the bone marrow, pointing to the polynuclear leucocyte as the main element in defence.

Non-Pyogenic Infections.

I must now give certain data as to the changes in the bone marrow in infections other than those due to the pyogenic cocci.

My observations here are comparatively few. A considerable number of the experimental rabbits died, wasted, during the course of immunisation, and the low neutrophil counts obtained from such animals cannot be used in argument. The scanty data which follow are from animals which were in reasonably good condition at the time of death.

Tubercle.—An animal which had been five times vaccinated intravenously with doses of dead tubercle bacilli and was fat and well nourished was killed 19 days after the last dose. Its neutrophil marrow count was 159,000 per cubic millimetre.

Bacillus coli.—An animal six times vaccinated intravenously with killed B. coli communis in doses of 200 to 400 million was sacrificed some five weeks after the last dose. It was in good condition and its various humoral reactions (e.g., bacteriolytic power and opsonic index) were raised above the normal. The neutrophil marrow count was only 111,000 per cubic millimetre. Another rabbit, which had passed through three severe infections with living B. coli, intravenously administered, was killed on account of cage-palsy two months after the last infection; it had lost weight, but there was no depletion or mucoid transformation of the marrow. The neutrophil marrow count was here 164,000 per cubic millimetre. A rabbit vaccinated three times with a combination of B. coli communis and streptococcus fæcalis (100 million of each) and had later survived an intravenous infection with living B. coli (1600 million bacilli), died three weeks later for no apparent reason. Its marrow count was 149,000 neutrophils per cubic millimetre. In the coli-immune rabbit which I have previously mentioned as dying from anaphylactic shock after an intravenous injection of vaccine, the neutrophil marrow count was only 86,000 per cubic millimetre.

I have no reliable counts on rabbits immunised against the typhoid bacillus or Gärtner's bacillus; my animals died, wasted or septic, and yielded no data of value. The same is true of a diphtheria immune animal. Dr. J. W. H. Eyre of Guy's Hospital was good enough to furnish me with the femora of a rabbit dead of acute M. melitensis septicæmia three days after intracerebral infection. The marrow count

in this animal was normal, 169,000 neutrophils per cubic millimetre.

These are, unfortunately, all the marrow counts which I have to offer bearing on the effects produced by bacteria other than the pyogenic cocci. Only in one case, that of B. coli communis, are the counts numerous enough to allow of any conclusion being drawn, and though further data are required we may provisionally infer that the colon bacillus has no power of exciting a leucoblastic reaction in the marrow. My single observation suggests that this may also be true of the tubercle bacillus. I may add that in the bone marrows of the cases I have just mentioned, not only was there no neutrophil leucoblastic reaction, but no other change which I could recognise; the marrows appeared in all respects normal.

The Varying Nature of Bodily Defence against Different Bacteria.

It will have been apparent, in the account which I have given of my own observations upon the bone marrow and upon the behaviour of the leucocytes in various conditions of infection and immunity, that the body reacts differently according to the species of bacterium by which it is invaded or against which it is vaccinated. The clinical facts in relation to different human infective diseases have long suggested this, and the more closely the cytological changes in the blood and the humoral properties of the serum are studied, the more apparent is the fact that the body has several means of defence, and that it employs now one and now another in resisting microbic aggression. I propose to devote the remaining time at my disposal to a closer review of these differences.

Observations on Harmless Bacteria.

I will first mention certain experiments which I have made as regards the effect of perfectly harmless bacteria upon the body. While I was working with well-known pathogenetic species it seemed to me that clues might possibly be obtained as to the nature of the differences which I observed by experimenting with bacteria which from their properties could never have been capable of growing in the mammalian body. Large numbers of bacterial species cannot grow at all at the temperature offered by warm-blooded animals. These are termed "psychrophil" bacteria by Fischer. Could it be shown that there were differences in the bodily response to the presence of different sorts of such psychrophil bacteria it would suggest that these variations depended upon some fundamental differences in the chemical nature of the

bacterial proteins, and not upon any acquired properties on the part of the body, derived from past ancestral experience of infection.

The response of the animal body to the presence of non-pathogenetic bacteria is practically a virgin field of research, yet it is one which is full of promise as to the light which may be shed upon the more directly important problems of immunity. I have experimented with only two psychrophil bacteria and it may have been a happy chance that I chose two against which I found the animal body to respond in a fundamentally different manner. I possessed a strain of a coccus which I had isolated from the air many years before and which I identify with the organism described by Menge as "micrococcus citreus agilis." It is a coccus, positive to Gram's stain, and provided with one or two very long flagella—i.e., it belongs to Migula's genus "planococcus." My strain was totally incapable of growth at body temperature. The other species with which I have worked is a motile organism isolated from water. It is a rather long bacillus provided with one or two terminal flagella, negative to Gram's stain, and forming a feeble orange pigment. I identify it with the organism described as "bacillus fluorescens aureus." My strain is incapable of growth at body temperature.

These two psychrophil bacteria, the first two which chanced to present themselves, were inoculated in large living doses into rabbits, and the succeeding phenomena studied just as I should have studied those due to an important pathogenic species. The following differences at once became apparent. The injection of micrococcus citreus agilis into the circulation caused no leucopenia, but was followed by a moderate leucocytosis. Nothing approaching anaphylactic shock was ever seen with this organism. B. fluorescens aureus, on the contrary, caused a definite though delayed leucopenia even on the first injection; the total leucocyte count fell from 9200 per cubic millimetre before inoculation to 1800 in two and a half hours; the subsequent leucocytosis was slight. And on each of the two occasions on which I injected the bacillus into the rabbit there was a marked dyspnœa in a few hours.

I wished to know whether these differences were associated with the presence of a lytic antibody in the serum in one case and not in the other, and Dr. Gordon very kindly undertook this investigation for me, carrying it out with all the details and controls prescribed by the classical method of performing Wassermann's reaction. I furnished him with sera from an animal twice immunised against B. fluorescens aureus, and from one three times immunised against M. citreus agilis, together with serum from a normal rabbit as control; at the same time I gave him suspensions of the respective micro-organisms in normal saline. His results were unequivocal. The animal immunised against B. fluor-

escens aureus had in its serum a lytic amboceptor against this organism; that immunised against M. citreus agilis had none.

I next instituted experiments on the bactericidal power of normal and immune rabbit's serum against these two bacteria, using Wright's method. A preliminary experiment showed that B. fluorescens aureus perished completely when an emulsion was incubated for 17 hours at 37° C. without the addition of serum, whereas M. citreus agilis survived, though in somewhat reduced numbers. I therefore modified Wright's technique by carrying out the experiments in a 20° C. incubator, in addition to a similar set at 37° C. Neither at 20° C. nor at 37° C. was there any evidence of bactericidal power against micrococcus citreus agilis in normal or immune serum. With B. fluorescens aureus no result could naturally be obtained at 37° C., since the temperature alone killed, but at 20° C. there was evidence of a very marked bacteriolytic effect, both on the part of normal and of immune serum, no definite difference being found between the two.

I now turned to the opsonic effect of normal and immune serum upon these two bacteria and found differences no less striking. I tested them as regards spontaneous phagocytosis—i.e., in the absence of serum—and also as regards opsonic action with both normal and immune rabbit's serum, using the same human leucocytes, washed four times, for all the experiments. Micrococcus citreus agilis proved wholly insusceptible to spontaneous phagocytosis; not a single coccus was found ingested in a hundred consecutive polynuclear leucocytes. In presence of normal serum 232 were found ingested by 100 leucocytes, and in presence of immune serum 358. The latter number was swelled by the presence of several leucocytes containing 40 and even 50 cocci apiece. If no cocci over ten were counted in any given leucocyte (and none such were found with normal serum) the number found with immune serum fell to 269.

B. fluorescens aureus, on the other hand, was readily susceptible to spontaneous phagocytosis. In absence of serum 116 bacilli were found ingested by 100 polynuclear leucocytes, and by a curious chance the numbers obtained with normal and immune serum were equal—274 in each case.

These results are very striking. I was aware that differences of this kind existed amongst pathogenic bacteria, but I was surprised to find them so well marked in harmless species. In the absence of any possibility of aggressins or specially evolved toxins in such psychophil bacteria it is natural to refer the differences in the reaction of the body to some fundamental diversity in the chemical nature of the bacterial protoplasm in the two cases, or at least to the presence of some constituent of the protoplasm in the one which is absent in the other. The subject is one which seems well worth further investigation, and I should judge it possible to approach it from the chemical side.

Turning now to the reactions of the body against the better known pathogenic species of bacteria we find that differences exist such as I have just described, but even more strongly marked. It is worth while to consider these differences in some detail.

Pyogenic Cocci.

The pyogenic cocci form a group which may be taken together, since the response of the body to their presence seems similar in all cases. Putting together the well-established clinical facts as regards human infection by these organisms, and the numerous experimental observations which have been made by animal inoculation, and adding the various observations which I have brought before you in these lectures, it seems possible to lay down the law with some confidence as to the means of defence employed against them by the body.

This defence is not only essentially phagocytic and dependent upon the activities of the polynuclear leucocytes, but it is also, in the main, opsonic. The arguments in favour of this view may be summarised as follows. Dealing first with negative evidence, it seems clear that the pyococci form no soluble toxin, and that antitoxic defence, in the sense in which we see it in diphtheria and tetanus, is out of the question. That endotoxins are present in the bacterial protoplasm is likely enough, but we are not in a position to affirm the provision of antibodies to neutralise them. Again, there is good evidence that the body deals with the pyogenic cocci by lytic action feebly or not at all. Experiments with serum *in vitro*, even with highly immune serum, show little or no bactericidal action upon these cocci. Wright's method of measuring bacteriolytic power has, in his and in all other hands, yielded negative results. Gordon and I have attempted to show by another method that in the early hours of contact between serum and staphylococci there is a distinct and often a marked decrease in the numbers of the cocci: it is possible that some minor degree of defence is thus explained, but we have never affirmed that this could be the main mechanism upon which the body relied for its protection. In the experiments as regards anaphylaxis, which I have detailed in preceding lectures, I have shown that initial leucopenia is almost absent with the pyogenic cocci, except in the highly immune animal. Even in such an animal the symptoms of anaphylactic shock are trivial or absent, and if the views I have expressed as to the nature of this phenomenon have any truth in them, this fact may be held to negative the idea of a lytic antibody as of serious importance in defence. Agglutination also, for what it is worth, is so capricious a phenomenon in relation to the pyogenic cocci as to be practically negligible.

On the other hand, the positive evidence which can be brought forward as regards phagocytic defence against these cocci is overwhelming. In almost every infection in which they are concerned, even when it is a secondary infection, we see a prompt polynuclear response. The only exceptions are grave septicæmias without local lesion: the strains of pyococci capable of producing such a result are of exceptional virulence. The visible polynuclear response seen in almost all infections by pyogenic cocci is in the first place *local*—i.e., there is an accumulation of these cells at and around the seat of bacterial invasion as a factor in the process of acute inflammation. In the second place, it is *circulatory;* in all but the most trivial infections we find a polynuclear leucocytosis. Lastly, it is *medullary;* the bone marrow undergoes a neutrophil leucoblastic change within a day or two of serious pyococcal invasion. It is inconceivable that the polynuclear leucocytes and their mother tissue should behave thus unless their functions lay at the foundation of bodily defence.

At the same time there is irresistible evidence of the coöperation of a humoral element in the defence. With all pyogenic cocci spontaneous phagocytosis is practically absent in experiments *in vitro.* It is abundantly proved by the labours of Wright and his disciples, and confirmed by every one who has worked at the subject, that the opsonic action of the serum is needful before phagocytosis can take place. It may be that in immune serum there is an opsonin differing in its nature from that of normal serum, as Muir and Martin have maintained. It is one of the difficulties which we encounter, in endeavouring to explain opsonins as lytic antibodies in another guise, that opsonic action is most signally manifest in the case of the pyogenic cocci, against which lytic action is feeble or absent.

I am therefore ready to accept the opsonic index in these infections as an important indication as to the degree of immunity in any given case. It is true that it measures only one factor in a complex process, but it is a very essential factor. I have already stated my reasons for not attributing exclusive importance to the opsonic index; I assign also much importance to the anatomical substratum of immunity.

The Tubercle Bacillus.

The mechanism of defence against the pyogenic cocci has been seen to present no apparent difficulty in explanation. It is otherwise when we approach the micro-organisms concerned in the causation of the infective granulomata. Some of these affections, such as actinomycosis, are largely suppurative, and here the polynuclear leucocytes are presumably concerned, much as they are in infections with the pyogenic cocci. But in regard to the two commonest and best known

infective granulomata this is by no means the case. Syphilis and tubercle are almost non-suppurative affections: the so-called "pus" of chronic lesions is mostly, though not entirely, necrotic and softened tissue débris. True suppuration in these diseases is usually due to secondary infection with pyogenic organisms.

I do not propose here to consider the nature of the bodily defence in syphilis. The disease is not one which lends itself to laboratory experiment in this country, where the higher apes are hard to procure. I will only say that the clinical and histological evidence obtainable from cases of human syphilis suggests in the strongest possible way that the polynuclear leucocytes play no part whatever in defence. Apart from accidental complications polynuclear leucocytosis is a phenomenon practically unknown in syphilis. In the histology of syphilitic lesions the polynuclear leucocyte takes no part; this cell seems to stand aside from the conflict during the whole course of syphilis, and to play no part in the manifestations of the disease at any stage. If any leucocyte plays a part it would seem to be the lymphocyte.

Tubercle, on the contrary, lends itself well to investigation, and there is a considerable body of experimental, as well as of clinical, evidence to guide us in framing our views as to the nature of the defence offered by the body against the bacillus. I will consider first the clinical facts. It is habitually asserted, and I believe it to be true, that in acute tuberculous affections, without secondary infection, polynuclear leucocytosis is absent. This is the opinion of most of the observers who have studied the subject, amongst whom I may mention von Limbeck and Cornet. In pulmonary tuberculosis secondary infection can, of course, rarely be excluded; even in tuberculous meningitis a secondary streptococcal infection of the meninges is not infrequently found post mortem, when it had not been suspected during life; the high polynuclear counts occasionally found in meningitis may thus perhaps be explained. It is now part of the ordinary routine of clinical pathology to make a cytological examination of fluids withdrawn from the various cavities of the body for purposes of diagnosis, and the student is rightly taught that tubercle, whether of the peritoneum, pleura, or meninges, is associated with a lymphocytosis rather than with an increase in the polynuclear leucocytes.

The histological study of tuberculous lesions is emphatic in disclaiming any participation of the polynuclear cells; they form no part of the structure of a miliary tubercle, and even in a tuberculous "abscess" they are habitually sparse. The cells of a miliary tubercle are essentially fixed tissue cells—endothelial and connective tissue in nature, with a certain number of lymphocytes. The clinical evidence, as a whole, offers no suggestion that the body relies upon its

polynuclear leucocytes in defence against the tubercle bacillus.

The experimental evidence connecting the polynuclears with defence against tubercle is not abundant or convincing. It is true that these cells will swallow tubercle bacilli *in vitro* if they are presented in company with a suitable opsonin, but they will swallow almost anything. It is also true that a similar phagocytosis occurs *in vivo* when tubercle bacilli are introduced into the body, but this seems an initial phenomenon only. I have seen it in the rabbit after the intravenous injection of bovine tubercle bacilli; the polynuclear leucocytes impacted in the lung may contain the bacilli after 15 minutes. Kisskalt has described it in mice cutaneously inoculated with human tubercle bacilli,[2] but, though he found phagocytosis of the bacilli by polynuclear leucocytes in the earlier stages, these cells were in a few days supplanted by large mononuclear cells which took up the work of phagocytosis. I have never succeeded in producing more than a trivial leucocytosis with killed tubercle bacilli in the rabbit, and even a prolonged course of intravenous vaccine treatment failed in this animal to excite any leucoblastic reaction in the marrow. It is stated that after injection of tuberculin there is a circulatory leucocytosis (in which the eosinophils take part) during the height of the reactive fever, but it appears to be a transitory phenomenon. On the other hand, there is abundant histological evidence that in established experimental tuberculosis in animals the polynuclear leucocytes play no more part in the lesions than they do in human tubercle.

A dispassionate survey of the preceding facts must, I think, from the strong contrast which they offer with those seen in the case of infections with the pyogenic cocci, convince us that in tubercle, as in syphilis, the polynuclear leucocyte takes little part in bodily defence. This seems essentially a function of the fixed tissue cells. The first reaction which appears to occur when a tubercle bacillus lodges in a lymphatic gland or elsewhere is a proliferation of the local endothelium, and when, in a favourable case, a local infection is overcome it is the connective tissues which wall in the caseous focus and reduce it to impotence.

What, then, is to be said of the tuberculo-opsonic index? Here I find myself confronted with a paradox which I cannot pretend to solve, and which has long been a stumbling-block to me. I cannot resist the evidence that the opsonic index is of value in the diagnosis of tubercle, and although I confess that I cannot see eye to eye with those who claim it as indispensable in controlling the dosage with tuberculin I freely admit that here, too, it has proved of use. But the tuberculo-opsonic index measures immunity by the behaviour

[2] Zeitschrift für Hygiene, Band xlv., No. 1.

of a cell which plays no apparent part in serious defence against tubercle. It may be that opsonin is essential to the ingestion of bacilli by the fixed tissue cells. I do not know that proof of this has ever been brought forward, nor do I know that bacilli engulfed by the fixed tissue cells are destroyed; more often it would seem to be the cell which perishes.

This difficulty has not, I think, received sufficient consideration by the opsonic school; the admitted usefulness of the opsonic index requires some explanation in view of the fact that nearly all that we know of pathological cytology points away from the polynuclear leucocyte as a factor in defence against tubercle. There is a possibility, at which I have before hinted, that some antibody which is evoked in immunisation against tubercle possesses, as an accessory property, the power of opsonic action, and that the opsonic index thus measures immunity indirectly.

Bacillus Coli and its Allies.

In this group, including not only B. coli but the typhoid bacillus, the dysentery bacilli, Gärtner's bacillus, and the paratyphoid organisms, we find a scheme of bodily defence which seems to differ from both those already discussed. This group of bacteria shares with the cholera vibrio the faculty of evoking the production of lytic antibodies to a degree which has no parallel amongst the pyogenic cocci. Even normal serum may possess well-marked bacteriolytic power against these organisms and in immunity the power is greatly increased. The existence of lytic antibodies probably explains why anaphylactic phenomena are especially well marked in animals immunised against the members of this group.

But although lytic action is readily witnessed in the test-tube, there is reason for the belief that in the living body, even though the serum be richly furnished with lytic antibodies, phagocytosis and intracellular destruction also take place freely. In the serous cavities of an immune animal lysis is doubtless seen, but it has been questioned how far it occurs in the tissues, even in the immune—much more in the normal animal.

If we look at the clinical evidence we find that most of the bacteria belonging to the colon group have some power of exciting suppuration. In acute infections with B. coli itself pus may be completely absent, as is occasionally seen in cystitis; but in chronic coli infections suppuration is common. I will not speak of perforations of the gut, because the infection is here a mixed one, at least at the outset. But suppurations due to an unmixed infection with B. coli or its near allies are commonly seen in chronic

cystitis, in chronic inflammations of the bile passages, and elsewhere; the cells in the pus are in such cases true polynuclear leucocytes, and phagocytosis can often be demonstrated. Again, in typhoid cystitis a true pyuria is present, and from the purulent lesions of post-typhoidal periostitis one may obtain pure cultures of the typhoid bacillus. I need not multiply examples; it is clear that the polynuclear leucocytes play some part at least in defence against this group of organisms, though perhaps chiefly in chronic infections.

Nevertheless there is a great difference between these infections and those due to the pyogenic cocci as regards the changes seen in the blood and in the bone marrow. It is well known that in typhoid fever there is no circulatory leucocytosis. It is indeed asserted that in the very earliest stages of this disease the polynuclear leucocytes are increased, but I have never had an opportunity of verifying this. In practically every case of uncomplicated typhoid fever that one sees the circulatory polynuclears are not only not increased but actually lowered in number—often to 1000 or 2000 per cubic millimetre. Again, in chronic urinary infections with B. coli, though pus may be passed in large amounts, I have never found any marked polynuclear leucocytosis in the blood.

In experimental rabbits treated with intravenous doses of living and dead colon bacilli, Gärtner's bacillus, and B. typhosus, I have indeed regularly noted a polynuclear leucocytosis, often a high one, following the anaphylactic leucopenia, but it is a transient affair, and I believe it to be a reaction following the leucopenia rather than a serious attempt at phagocytic defence. I have never found a leucoblastic reaction in the marrow of animals thus treated.

The colon group of bacilli would thus appear to differ from the pyogenic cocci in several important respects as regards the defensive mechanisms they excite. Certain humoral responses, notably agglutination and bacteriolysis, are better marked than with most other bacteria, and in spite of the fact that it is difficult to prove that these actually play their part in the tissues, it is equally difficult to believe that they have no meaning. The evidence as regards opsonic response is difficult to appraise, but I am disposed to attach less importance to it than in the case of the pyogenic cocci. And although these bacteria may excite suppuration in certain cases the absence of a constant polynuclear leucocytosis in the blood, and of a leucoblastic reaction in the marrow, forbids us to believe that the polynuclear cells are of more than subsidiary importance in defence.

We have thus in the pyogenic cocci, in the members of the colon bacillus group, and in the microbes of tubercle and probably also of syphilis, examples of micro-organisms against which the body seems to defend itself in very different

fashion. So far as the polynuclear leucocytes are concerned, we seem entitled, from the facts I have reviewed, to conclude that these cells are of fundamental importance in defence against the pyogenic cocci, of merely secondary importance in the case of the colon group, while in tubercle and syphilis they seem to play almost no part at all. These three groups of bacteria by no means include all those of serious pathogenic importance, but they are those with which I have chiefly worked, and the short time at my disposal prevents me from dealing with such groups as the pre-eminently toxic bacteria in which yet other means of defence come into play.

At the present day it is almost superfluous to urge that no one theory of immunity can meet all the facts. The reactions between the body and its invaders are manifold and complex, and must be viewed from many sides if their meaning as a whole is to be fully grasped. Truth is a gem, cut by many different workers, each intent upon the facet on which he is himself engaged, striving to smooth and polish it that he may gaze into the interior. The view thus gained is too often but a partial one. Another, working at a different facet, may gain a glimpse of the same facts, but in an altered light, and may formulate a very different theory to explain what he sees. Yet little by little the crystal becomes cut and polished till in the end we may hope to view it as a perfect whole. My humble effort in these lectures has been to peer through only one facet in the crystal of truth, that at which I have lately been engaged in working. I am not blind to the fact that there are other facets of equal importance, but I shall have succeeded in my aim if I have attracted renewed attention to the significance and value of the anatomical element in immunity presented by the leucocytes.