THE CROONIAN LECTURES

ON

THE PROGRESS OF DISCOVERY RELATING
TO THE ORIGIN AND NATURE OF
INFECTIOUS DISEASES.*Delivered before the Royal College of Physicians of London.*By J. BURDON SANDERSON, M.D., D.C.L., LL.D., F.R.S.,
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LECTURE III.—MITIGATION AND IMMUNITY.

Mitigation.—Methods of Producing it.—Pasteur's Protective "Vaccination."—Relation between Vitality and Pathogenic Properties of Microphytes.—Acquired Immunity.—Definition of "Virulence" and of "Immunity."—Naegeli's "Contest" Theory.—The Phagocyte Theory a Teleological Theory.—Comparison of Phagocytes to Policemen.—Hypothetical Endowment of Phagocytes with Consciousness.—The Doctrine of Phagocytes tested in its Application to Erysipelas and Relapsing Fever.—Chemical Theories of Immunity.—The Antibiotic Properties of the Blood and its Constituent Parts.—Conclusions.

WE have now, in continuing our study of the struggle between the organism and disease, to pass from the phlogogenic to the specific infections, and with this view I must begin by asking your attention to certain terms which represent the two great divisions of the subject. These words are "mitigation" and "immunity."

PART I.—MITIGATION.

For the word "mitigation" (in German *Abschwächung*) the word "attenuation," borrowed from M. Pasteur, has come to be very generally used. There is no objection to it, provided it is remembered that what is designated is not what M. Pasteur originally meant by attenuation. For "insusceptibility" we have also taken to the use of the word immunity, which is inconvenient, because the states to which we have most frequently to refer are neither states of immunity nor of the contrary, but somewhere between the two. What is chiefly important to bear in mind is that the word attenuation relates to a variable quality of the virus, the word immunity to a variable quality of the infected organism. These two qualities are respectively virulence and susceptibility. They are closely related to each other, and the bond which links them is the relation between the growing power and toxic activity of the microphyte to the vitality of the organism. And inasmuch as these two are antagonistic, the relation between them is one of contest, that is, of struggle between organism and microphyte—a contest in which preventive medicine strives to promote the victory of the organism either by strengthening its prophylactic power or by weakening the growing power of the microphyte. In 1880 M. Pasteur not only discovered that the virus or contagium of certain communicable diseases can be mitigated by artificial means, but was able to demonstrate in the most triumphant way that the virus so mitigated can be applied to the protection of animals valuable to man.

In this great discovery M. Pasteur took as his point of departure, not the infected organism but the microphyte itself. He found that the micrococcus¹ which produces the so-called chicken cholera could, like the bacillus anthracis be made to grow in bouillon, and the fresh cultures so obtained preserved their activity of growth and their morbid power for some time. But eventually the liquid, which was at first opalescent or even turbid from the number of "microbes" which it contained, became clearer and clearer. This process M. Pasteur called attenuation, recognising its resemblance to the change in fermenting liquors, to which that term has long been applied by brewers. He attributed it partly to the exhaustion of the nutrient material, partly to the accumulation in the liquid medium of products excreted by the microphytes contained in it. This, however, was not

the main point of the discovery. The essential point was that *pari passu* with the attenuation of the culture there was mitigation of its effect, for he found that whereas the fresh culture produces a characteristic disease which terminates fatally in twenty-four hours, a cultivation which has vegetated for several weeks gives rise to nothing more than a temporary disturbance of health. Pasteur further discovered that in animals inoculated with the mitigated virus, the micrococcus really multiplied in the body and that it was thereby rendered so completely insusceptible that it could not be infected by any virus however active.

The inference which M. Pasteur drew from his discovery of the attenuation of chicken cholera was that there is an intimate and essential relation between the virulence of a contagium and the vegetative vigour of the microphyte which produces it. The only way in which we can judge how far this is or is not the case is by the observation that the relation holds good by whatever means the attenuation, that is the diminution of organic activity, is brought about. In M. Pasteur's experiment it seemed to be produced by mere exhaustion of the soil, but the same result has been attained since in a variety of ways, of which I must shortly describe the most important.

METHODS OF PRODUCING MITIGATION.

Pasteur's Method.—One of the most generally applicable consists in cultivating the microphyte at a temperature which, although not incompatible with its growth, applying this method to the bacillus anthracis, M. Pasteur found that the growth of this organism, vigorous at the temperature of the body, gradually diminishes in vigour as the temperature is raised to 42.5° C. (108° or 109° F.), and above this ceases; but in this case the most marked effect of the higher temperature is not attenuation, but diminished tendency to the formation of spores. Below 100° F. the constant habit of *B. anthracis* is to form spores wherever it has access to air or oxygen; at 109° F. it grows into a labyrinth of filaments, which, except that they form no spores, do not differ in appearance, or scarcely differ, from those grown at lower temperature. To this change M. Pasteur also applied the term attenuation, not meaning thereby that the filaments had undergone any diminution of size, but because he had found that a sporeless growth which has been cultivated for a length of time at 108° F. loses its disease-producing powers more or less completely. At first it fails in infecting more refractory animals, but is still potent enough for the susceptible. Soon these cease to react, and a point is reached at which the culture can neither be used to start a new crop in a nutrient medium to which it is transferred, nor to communicate the disease. These were the facts which led M. Pasteur to his great practical discovery—the use of attenuated virus for protective vaccination. If the mitigated condition of the anthrax bacillus, which I have described, could be made permanent, if crop on crop of virus of any desired degree of mitigation could be produced, it would be possible at once to use it on a large scale for the purpose of rendering animals insusceptible. Guided by the purely biological conception that those characteristics of plants and animals which are transmitted from individual to individual through the germ are less subject to subsequent modification—that is, have more permanency—than those which result from the action of environment or successive crops propagated by continuous vegetation, Pasteur, although no biologist, was led to anticipate that, having to do with a microphyte which is capable of propagating itself by spores, the most effectual way of accomplishing his purpose would be to act on it rather in its spore-producing than (as in his first experiment) in its vegetating form or condition; and this he carried out by the simple expedient of transporting cultures which had been attenuated, as above described, by cultivation at 110° to another warm chamber in which the temperature was 98° F. At the lower temperature spores were produced which yielded crops of a permanently inferior degree of virulence.

Chauveau's Method.—A second method is that of Chauveau, which is based on the peculiar relation which exists between disease-producing microphytes, that is, of microphytes capable of inhabiting the living tissues of man or of the higher animals, and the quantity of oxygen supplied to them, that is, the tension of free oxygen in the medium which they inhabit. Those which are anaerophytes require the feeblest dose which can be imagined, and die if they have too much. Others, like the *B. anthracis*, grow differently according to the supply afforded to them, so that oxygen is clearly a powerful agent of modification. Even in the attenuation of the chicken cholera dumb-bells, M. Pasteur has shown that, in a certain sense, the determining cause of the modification is oxygen. For if this plant, which is an aërophyte, is freely exposed to air with a limited supply of food, it vegetates vigorously, uses up its resources, and "attenuates" itself by exhaustion. Restrict both its supply of air and nutriment, it goes on growing at a vastly diminished rate, but—as M. Pasteur has shown—retains its virulence for an almost indefinite period. In this case the attenuating cause is too much oxygen, which permits the plant to exhaust itself. Now it was long ago found by the late M. Paul Bert that *B. anthracis*, although it, as we have seen, rejoices in oxygen and produces spores most vigorously in free air, yet may have too much. It can actually be killed by subjecting it to an oxygen pressure of over thirteen atmospheres. Profiting by this discovery of Bert, and anticipating that if oxygen at high pressure kills, inferior pressure would "attenuate," M. Chauveau devised a method which, although technically more difficult to carry out than that of Pasteur, is quite as effectual in producing an attenuated virus. The difficulty was the mechanical one of constructing a cultivation chamber which could be charged with oxygen at any desired pressure. This surmounted, he was able at will, by the regulation of time and pressure, to bring about any

¹ Duclaux, *Le Microbe et la Maladie*, p. 157.

desired degree of mitigation. In this way "vaccines"—in the peculiar sense in which this word is used by the Pasteur school—were obtained, which yielded what is called *immunité solide*, with this drawback only—that cultures attenuated by Chauveau's method are less permanently mitigated, that is, more capable of regaining virulence by cultivation under favourable conditions, than those of Pasteur.

The Chemical Method.—A third mode of attenuation which has been successfully employed in M. Pasteur's laboratory may be distinguished as the chemical method, for it consists in adding to the medium an ingredient of which the unfavourable influence is not strong enough when used in extremely small percentage to arrest the growth of the microphyte, but is yet capable of impairing its activity. For this purpose, either the organic bodies which are recognised as disinfectants have been used, or recourse has been had to metallic salts—particularly potassium bichromate—the use of which is alleged to have yielded mitigated cultures as permanent as those of either of the other methods.

Relation between Vitality and Pathogenic Properties of Microphytes.

The fact that by such different methods as the action of an unsuitable temperature, a too abundant supply of oxygen, and finally the introduction into the nutrient medium of such a body as potassium bichromate, such a deterioration or mitigation of a virus can be produced as to fit it for the purposes for what M. Pasteur calls a vaccine, and that each of these methods has been used on the large scale for the protection of cattle from anthrax is one of great practical importance; but irrespectively of its utility, it involves a fundamental principle relating to the etiology of infective diseases—namely, that there is an essential relation (though as we shall see a very complicated one) between the pathogenic property of a microphyte and its viability—between its vital activities and its power of producing disease. In the discussion of this subject we shall find that much obscurity has arisen from the confusion of the botanical (phytological) fact of attenuation with the pathological fact of mitigation. In all the researches relating to the subject which were published in France during the first half of the last decade, the two were assumed to be identical. We shall have occasion as we proceed to see that it is not merely a logical necessity to separate the two ideas—attenuation and mitigation—in our minds, but that, in fact, the two qualities do not even vary in the same direction.

In Germany, the Pasteurian doctrine that virulence varies in the same direction with vigour has not been accepted without limitations. German pathologists talk of "Abschwächung," but mean thereby not attenuation, but mitigation—that is, diminution of virulence, not diminished vigour of growth. So far from attenuation being the necessary condition of mitigation, it is shown by Flügge² (one of the most accurate and acute of German bacteriologists) that as regards the less specific pathogenic microphytes it is just the contrary—namely, that the state of mitigation is not one of atrophy, but, on the contrary, of luxuriant vegetation. There are some microphytes which, although they grow readily in nutrient media, in doing so very rapidly lose their virulence. This appears to be the case, for example, with the chain coccus of erysipelas and with the dumb-bell coccus of pneumonia; in both the microphyte, while doing the work of a saprophyte, grows vigorously, but loses the disease-producing power it before possessed. We can best conceive how this may be by attributing it to a sort of variability (in the Darwinian sense). We may suppose that the microphyte exists in two modifications or moods; that in one mood it is capable of multiplying in the living body—that is, is virulent—that in the other mood it grows more luxuriantly in nutrient media, but is unadapted to the conditions which exist in the organism. Or we may suppose, with Flügge, that under all conditions there are two symbiotic forms of the microphyte, of which one gets the better when the medium is non-living, the other when the organism has to multiply, as best it can, in the living body.

CLASSIFICATION OF PATHOGENIC MICROPHYTES ACCORDING TO THEIR SAPROPHYTIC PROPERTIES.

Guided by these considerations we might, therefore, arrange pathogenic microphytes in a series, placing at one end those which grow vigorously as saprophytes, but are also capable of multiplying in the organism under favouring conditions, that is, staphylococcus, and near the other the bacillus of tubercle, which grows readily in the organism, with difficulty in nutritive media, but is always virulent in proportion to its vitality and to its activity of growth. In such a classification in line, B. anthracis would occupy an intermediate position. It grows vigorously both in dead nutritive media and in the living body. While much less constant pathogenically than the bacillus of tubercle, it is much more so than the streptococcus of erysipelas, but still admits of

² Flügge, Studien ueber die Abschwächung virulenter Bacterien, *Zeitsch. f. Hygiene*, vol. iv, p. 208.

being permanently modified; for as we have already seen, it is capable not only of vegetating but of producing spores in a state of mitigated virulence. It is probable that these spore-producing mitigated cultivations obtained by Pasteur's method are also attenuated, for it was found by Chauveau that they had less power of resisting high temperatures (80° C.) than ordinary spores. As regards cultures which do not contain spores, it has been shown experimentally that "resistance," as measured by the dose of disinfectant required to kill the bacilli, is a very accurate criterion of virulence.

PART II.—ACQUIRED IMMUNITY.

Definition of "Virulence" and of "Immunity."—Every disease-producing microphyte must possess the power of multiplying under such conditions as prevail in the living body of man or other animals of similar organisation to man, and of causing therein poisonous products. It is this power which pathologists now express by the term "virulence," which I must now try to define. The word may be used in a general way as the equivalent of morbid activity, but more strictly it means the relation between two sets of activities—those of the microphyte on the one hand and those of the infected organism on the other—the influence by virtue of which the microphyte, with all its specific endowments, produces the morbid process. It is evident that this influence depends (1) on the power of the microphyte of growing in the living body; (2) on its power of developing chemical products which are toxic; and, lastly, (3) on the specific adaptation of the organism. It is to the last of these, under the term susceptibility and its contrary insusceptibility, or as it is now commonly called immunity, that we have now to direct our attention.

It is a matter of common experience that to each microphyte different animals have different susceptibility. As this word stands, not for a positive quality of the organism, but rather for the want of a power, which the animal body always possesses in very variable degree, of resisting or protecting itself from the invasion of contagia, it is in general more convenient to use a word which more expresses this protective power, namely the word immunity, than one which suggests its absence.

Naegeli's "Contest" Theory.—Any one who considers what would happen if the golden cluster coccus, the properties of which we had before us in a previous lecture, were allowed to spread in the organism without restraint is led to recognise that such a protective power must exist, and that in relation to the microphyte in question it must be exercised either in restraining its development or in destroying its vitality; and consequently that there must be something like a contest between the living body which is invaded and the invading enemy.

To express this notion, which of late years has been present to many minds, the great botanist whose loss we have so lately had to deplore, Naegeli, used the word *Concurrenzvorgang*, comparing the battle between cells and microphytes with the struggle for existence between ferment organisms of different kinds in the same nutritive medium. The analogy is suggestive, but too incomplete to be useful, for what we have before us is not an encounter between opponents on an open arena, but an effort made by the organism as a whole to defend itself against invaders.

The contest theory, however, was of great value at the time it was promulgated; for when twenty years ago we first began to think of microphytes as causes of specific diseases, we were too apt to assume, and I willingly admit that I thought and said so myself, that all that was necessary for the production of a disease was the sowing of the seed. It is not surprising that even after Naegeli and others had drawn attention to this mistake the importance of the defensive reaction of the organism was still underrated. Now it is entirely different. The vast accumulation of exact knowledge as to the morphological characters and physiological endowments of microbes is valued not for its own sake, but because it enables us to understand the reactions which they evoke in the living body; reactions of which some are beneficial and protective, though they are called into existence by abnormal conditions, while others are of such a nature as to indicate that the defences of the organism have been forced, that the invaders are in possession and that disease has begun.

It is with the first of these two classes of reactions exclusively that we have for the moment to concern ourselves. For what we mean by immunity is the power which the

human or animal organism possesses of so resenting the presence of a disease-producing microphyte as to protect itself from the disease.

Further Consideration of the Theory of Phagocytes.

We have already had before us, in relation to the etiology of inflammation, one of the explanations which has been given of the nature of this protective reaction—that which attributes it to the action of phagocytes. In its more special application to the question which occupies us to-day, it must, I think, be freely admitted that the general acceptance of the notion that protective reaction is the essence of immunity was very materially promoted by Metschnikoff's earlier publications and by the experimental facts which he adduced, and that his more recent investigations have exercised a very remarkable influence in the same direction. But, unfortunately, we have to encounter the doctrine of Metschnikoff, not as a result of experimental inquiry to be freely discussed on its merits, but as a dogma which has acquired extraordinary popularity among the large class of persons outside of the profession who take an intelligent interest in pathological questions without possessing any acquaintance with pathology.

Phagocytes resemble Policemen.—In my last lecture I drew attention to the difficulty of supposing that in the spread of traumatic erysipelas the invading myriads of streptococci can be effectually opposed by the leucocytes which follow in their rear. A similar difficulty exists as regards the more specific infections. If phagocytes are to be looked upon as policemen, whose business it is to watch over the safety of the organism, they ought to present themselves in force wherever and whenever there is danger. Now, Metschnikoff's own observations, confirmed as well by his supporters as by his opponents, show that that is precisely what does not happen, that is, that although it is perfectly true that leucocytes take part in the local reaction to which the entrance into the organism of many pathogenic microbes give rise (they are like policemen in this respect, that) they neither appear at the place nor at the time that they are wanted.

Never Present when most Wanted.—For if we compare the reaction which a mitigated culture produces in the subcutaneous tissue of a healthy animal with that which is evoked by a virulent one, we find that the main point of difference is that whereas in the former case, where there is no danger, our policemen assemble in force, they are either entirely absent or appear in scanty numbers where danger is imminent.

Effect of Local Injury on Power of Growth of Pathogenic Microphytes.—Now I am well aware how, if Dr. Metschnikoff were here, he would triumphantly explain this difficulty. He would say that the very fact of the absence of leucocytes is the reason why the microphyte is able to display its quality of virulence, and if we were to proceed to ask why they are absent we should probably be told that the reason is that the toxical influence of very virulent microphytes is so intense that the leucocytes are promptly overpowered by it and unable to act; and in support of this explanation reference would be made to experiments which show, if the organism is "prepared" beforehand for the reception of the morbid microphyte by a previous localised poisoning, the result is the same as if the virulence of the microphyte were increased—that is, that under these conditions a mitigated culture acts as if it were virulent. I have had the opportunity of seeing the striking experiments which have been made on this subject by Dr. Ruffer, and fully appreciate their value and importance; they afford conclusive evidence that an organism or part may be so weakened by the action on it of a poison that the power of protective reaction is abolished; but what, I think, they fail to show, is that that protective reaction depends exclusively on the presence or absence of leucocytes, or that the swarming of these corpuscles around bacilli derived from mitigated cultures may not be otherwise accounted for than by attributing it to a protective purpose.

Whence come the Phagocytes?—Even if we went so far as to admit leucocytes to be possessed of such a degree of consciousness as would enable them to judge of the degree of virulence of microphytes when in their immediate neighbourhood, the advocate of the protective agency of phagocytes would still be in a difficulty, from which, so far as I can see,

there is no escape. The leucocytes which we meet with at a focus of irritation have, we most of us believe, migrated from the blood vessels, but, as we know, there are some people who hold to the old doctrine of proliferation or tissue germination. Let us admit that they may originate either way. If leucocytes are emigrants the theory involves the monstrous notion that a leucocyte, as it is being carried along by the blood stream, is so affected by the fact that virulent microphytes have been introduced into some part of the body that of its own spontaneity it stops short, makes its way out into the normal tissue, through normal blood vessels, seizes on as many of the offenders as come within its reach, swallows and digests them, and returns whence it came. If they are not emigrants, the difficulty which presents itself is quite as great, for it must be supposed that the dissemination of virus from the focus of infection is intercepted by the agency of cells, which, at the moment of invasion, are as yet unborn, and which have to be brought into existence and activity to meet the emergency.

The Doctrine of Phagocytes as Tested in Erysipelas and Relapsing Fever.

Among the specific diseases in which the advocates of phagocytosis attribute importance to the protective action of leucocytes, the most remarkable is relapsing fever. In this disease, as everyone knows, spirilla are present in the blood in great numbers during the paroxysm, and disappear at the crisis. Metschnikoff,³ availing himself of the fact first discovered by Dr. Carter, of Bombay, that this disease can be communicated to the quadrumana for the purpose of thoroughly investigating it from the bacteriological side, has given us a full account of all that happens. In agreement with other observers he has found that although the spirilla are in constant relation with the blood leucocytes they are never incorporated by them; and he has further made the important observation that when at the critical defervescence they disappear, they are deposited in the spleen and incorporated in its cells. Strange as it may appear, Metschnikoff gives no explanation of the indifference or phagocytic incapacity of the blood leucocytes in this case, but regards incorporation in the spleen cells as the cause of the defervescence. That it is a phenomenon of very great interest no one will dispute, but we can scarcely make up our minds to believe that it is the cause of an event already accomplished.

CHEMICAL THEORIES OF IMMUNITY.

The considerations I have brought forward are, I think, sufficient to justify the assignment of a very secondary place to phagocytosis in the mechanism of immunity. What I have now to do is to state what advances have been made by pathologists towards a more rational explanation.⁴ Eight or ten years ago the notion that immunity was due to chemical unfitness of the living body to serve as a nutritive medium was accepted with much less question than it is now. There were two opposed theories on the subject, that of exhaustion and that of retention.

The Theory of Exhaustion.—According to the exhaustion theory—that of Pasteur—it was thought that, just as in cultivation liquids, bacteria undergo attenuation and eventually die if inadequately supplied with nutrient material, so in the body some special material necessary for the development of the microphyte, and consequently of the morbid process with which its growth is associated is used up, and cannot be replaced. And so, for want of this material, the organism becomes insusceptible.

The Theory of Retention.—The theory of retention was founded on the fact that in nutrient media outside of the body, in which microphytes have lived for a long time, certain products of their vital-chemical processes accumulate which are more or less colytic. And it was thought that in the living body a first attack might in like manner leave behind metabolic products—as they are called—which, taking their abode in the organism, might guard it against further attacks. In the crude forms

³ Metschnikoff, "Phagocytenkampf beim Rückfallyphus," *Virch. Arch.*, vol. cix.

⁴ I will not occupy time with proving what I conceive to be the most elementary fact relating to the subject, namely, that the organism, that is the living human or animal body, possesses means of arresting the development within itself of pathogenic microphytes, and of eventually destroying them. I will also take it to be admitted that this function resides both in the blood and in the tissues, both in organised protoplasm and in intercellular liquid, and consequently that the inhibitory or destructive action is chemical. In accordance with this principle we should expect that susceptibility would consist in the impairment or suspension of this function and the acquirement of immunity in its development.

which it originally took, the hypothesis that the chemical compounds of known constitution, and particularly those belonging to the aromatic group which are known as products of sepsis could produce such effects, became untenable so soon as it was proved that the colytic activity of those bodies is so feeble, and the quantity in which they are produced so small, that they cannot have any appreciable action.

Immunity Produced by Chemical Products of Bacterial Action.

But the following out of the same idea, the idea, namely, that in the vegetation of pathogenous microphytes, products come into existence which are inimical to their own development, eventually led, through a succession of steps in experimental research, to the better knowledge which we now possess. The first of these steps was, I think, the discovery by Messrs. Salmon and Smith,⁵ that pigs can be "protected" against pig typhoid by inoculating them with the liquid in which the microphytes of that disease have been cultivated. This was immediately succeeded by the discovery of Chamberland and Roux that in guinea-pigs immunity from malignant cedema was acquired by injection into the peritoneal cavity of cultures of bacillus anthracis, in which the microphyte had been effectually destroyed by exposure to heat. This method had the disadvantage that the high temperature which it was necessary to use in order to ensure the thorough devitalisation of the microbe was more or less destructive to the chemical products which it was desired to separate. The recognition of this drawback led to the use of a new expedient, or rather to the reapplication of an old one in a much improved form—that of filtration under pressure through porcelain, by means of which it was found that a germ-free filtrate could be obtained which was much more active than the liquid obtained by heat devitalisation.

Mode in which Immunity is thus Produced.—As to what happens in the particular cases we have before us, it is sufficient for the moment to say that the protective action of the filtrate is not due to its direct action on the bacilli, for it has been shown experimentally that these organisms grow and multiply in the very liquids which serve as vaccines, and that consequently we must look for our explanation to their action as the infected organism rather than as the infecting microphytes. In accepting this inference it is obvious that we at once abandon the very clue which guided us to it; for if it can be shown, as we shall see to be the case, that these chemical products of bacterial life, although they have no such power outside of the organism, are actually able to exercise it within the organism, that is, when used as vaccines, the only view which we can take of the matter is that when used as vaccines they so act on the organism as either to awaken in it an inhibitory or destructive power which was before latent or too feeble.

The Bactericidal Power of the Blood.—It has been long known (specially from the research of Moritz Traube in 1874) that the blood possesses an extraordinary power of destroying septic organisms, but no one recognised its importance in relation to infection until, in 1887, v. Fodor published his paper on the power of the blood to destroy pathogenic bacteria. V. Fodor showed first that B. anthracis, when injected into the blood, is destroyed with extraordinary rapidity. Then followed the much more elaborate research of Dr. Nuttall, made under Flügge's directions, which showed that the bacterium-killing power which v. Fodor had assigned to the blood in its uncoagulated state was possessed both by defibrinated blood and by serum, and was destroyed by subjecting it to a temperature of 55°. This research was succeeded by another of equal importance, also conducted in Flügge's laboratory. It was found that defibrinated blood could be diluted with sterilised salt solution without losing its antibiotic power, that it acted much more readily on some microphytes than on others, and particularly on the bacillus of typhoid, that it belongs as much to plasma as to serum, and is not destroyed by those products of peptic digestion of proteids which deprive blood of its coagulability. Finally this subject was more fully investigated by Buchner,⁶ who confirmed and extended the results of his predecessors, while correcting some mistakes.

Methods.—The method of testing consists in very thoroughly mixing

⁵ Salmon and Smith, Experiments on the Production of Immunity by Hypodermic Injection of Sterilised Cultures: International Medical Congress, Washington, 1887. A preliminary communication had been made at an earlier period to the Washington Biological Society.

⁶ Buchner, Untersuchungen ueber die bacterienfeindlichen Wirkungen des Blutes, etc., Arch. f. Hygiene, vol. x, p. 85.

with (say) 5 cubic centimetres of serum a measured quantity (say) 5 cubic millimetres (that is, one-thousandth part) of culture; then in taking the same quantity (5 cubic millimetres) of the mixture and diluting it with warm gelatine solution, which is immediately poured out on a levelled glass plate, and subjected to such a temperature as will just admit of its gelatinising. In a couple of days the surface of the gelatine is beset with dots of opacity—colonies of typhoid. These can be counted by placing the plate over a plate of glass divided into squares, the area of the whole being some simple multiple of that of each square. By counting the number of colonies contained on several squares a mean numerical result is obtained which represents the original number of bacilli contained in the culture used, and thereby serves as a unit with which the results of other enumerations may be compared. The next step is to repeat the process with samples of the same liquid (that is, serum) containing 1 per cent. of culture taken two hours and a half and five hours respectively after mixture, the result being that for every hundred microphytes originally existing not more than one or two are forthcoming after five hours in serum. By applying the same method of testing to serum or blood under other conditions it is learnt that serum may be kept for a week or more at a low temperature without losing its antibiotic power; that this, however, is not diminished by freezing and thawing, even when this is several times repeated, whence it is abundantly evident that the property in question is not due to the stray corpuscles which serum always contains, for these are effectually killed by a single freezing. From a variety of circumstances Buchner is led to infer that the antibiotic power is associated with the proteid constituents of the serum. The fact first observed by Flügge, and confirmed by Buchner, that the action is destroyed by a temperature of 55°, agrees best with the properties of a proteid: so also does the observation that when serum of dog's blood is repeatedly frozen it divides by subsidence into two layers, of which the lowest is active and the upper sterile. But the most decided indication of the albuminous nature is given by the effect of diffusion. If a plate of gelatine is soaked in serum the gelatine swells and charges itself with the water and salts of the blood, and it is found that the antibiotic power is entirely lost, both serum and diffusate being now favourable media for the growth of the bacillus.

Specific Alexeterics.

New facts have been very recently brought to light which give the discovery of v. Fodor its true meaning. So far as I know, no pathologist supposed that the specific insusceptibility which, as we have seen, may be conferred on animals in so many different ways (and I have by no means mentioned all) is due to the general property possessed by the blood of destroying pathogenic microphytes. It was from the first understood that the whole value of Buchner's research consisted in this (and it was abundantly sufficient to give it great importance as a step in the progress of knowledge), that it brings before us in the clearest way the possible existence of a chemical agency in the living organism—an agency in which cells as such take no part—capable of destroying infective microphytes as rapidly and effectually as corrosive sublimate. For the proof of the existence of such an antibiotic power and of its embodiment in a chemical substance afforded good ground for the anticipation that the specific antibiotic power which was already believed to be resident in the organism of specifically protected animals would be in like manner chemical and independent of the direct co-operation of living cells. Consequently the question came to be one of a specific chemical agency analogous to that proved to exist by Buchner's experiments.

No sooner did the question present itself than it was seen that in each case the specific agent might act in one of two ways, or both at the same time but in different degrees. It might act either antitoxically or antitoxically, or both at the same time. It might protect either by destroying or inhibiting the development of the microphyte, or by antagonising its chemical action. Now we have seen, as regards phlogogenic microphytes, that the weapon by which these exercise their power of injury is a chemical one; and from the experiments of M. Bouchard and Dr. Ruffer we learn that this same weapon is employed by the microphyte as a means of promoting its own victory in the struggle for existence. If, therefore, we can deprive it of this weapon, we not only make it harmless, but so impair its viability in the organism as to put a stop to its multiplication. In other words, the specific chemical agent by which the organism protects itself against a specific infective process is at the same time antibiotic and antitoxic, but of these two the antitoxic carries with it or includes the other.

Now it is known that certain infections are biotic, others toxic—that is, that in some rapid multiplication of the organism is the potent element, in others the production by a relatively small number of microphytes of a relatively intense toxic action. Of the former class, splenic fever, or a similar disease of pigs—pig erysipelas—is the type, of the latter tetanus. If, therefore, we assume that the specific alexeteric

agent may possess the antibiotic and antitoxic properties in different proportions, according to the disease against which it serves as a defence, we should expect that the specific alexeteric against tetanus should be chiefly antitoxic, the specific alexeteric against swine erysipelas should be chiefly antibiotic.

Tetanus.—As regards tetanus, this has been proved to be so by the recent researches of Behring and Kitasato.⁷ In tetanus it has been conclusively proved, as we shall see more fully in the next lecture, that the whole complex of symptoms produced by the tetanus bacillus also results from the introduction into the organism of an almost infinitesimal dose of the toxical product. It has further been proved (1) that it is possible by a variety of methods to induce in an animal a state of complete insusceptibility to this poison—so complete that it is not affected by doses several hundred times greater than are sufficient to kill an unprotected animal; (2) that the blood of such an unprotected animal has the power of antagonising the poison, though not of inhibiting the growth of the microphyte which produces it; and (3) that when introduced into the circulation of a susceptible animal it confers immunity upon it.

These facts speak for themselves so plainly that they do not need comment, but it is essential that we should carefully guard against the assumption that because in tetanus the protective agent is primarily antitoxic, this is also true as to other diseases. To prove that it is not so let me refer to what has been ascertained as to the specific protective agent against swine erysipelas, or (in French) *rouget*.

Rouget.—*Rouget* is a disease which kills rather more than half of the animals which it attacks, and is characterised by its rapid progress and the spreading erythema which accompanies it. The bacillus which causes it was discovered by Loclier in 1882, and so closely resembles that of another disease, "mouse septicæmia," that they can scarcely be distinguished. It is found in great abundance in the blood of infected animals, and continues to multiply after death. It derives a special interest from the investigations of Pasteur, who discovered first that its virus could be mitigated, though not attenuated, by transmission of the virus through the body of the rabbit, and that it could be aggravated by transmission through the pigeon, and that pigs which had received the disease through the rabbit were protected against the pigeon virus. *Rouget* has a further importance as one of those in which it is alleged that the agency of leucocytes can be best observed as destroyers of bacilli. On this ground, as well as from the circumstance that the relation between virulence and morbid process which it occasions is quite as obvious as, for example, in splenic fever, we take *rouget* as a disease specially adapted for the discussion of the mechanism of acquired immunity. In *rouget* it has been proved that the multiplication of the bacilli in the blood of a susceptible animal is astonishingly rapid, but that the bacillus is as rapidly destroyed in an animal of the same species in which immunity has been conferred by Pasteur's method. In other words, the antibiotic property of the blood, namely, its power of destroying morbid bacilli, is in that disease associated with the acquirement of immunity.

It is well known that the protection afforded by Pasteur's method for *rouget* is not absolute, that is, that a certain number of the vaccinated animals are attacked by the disease, and some die, and that for this reason it has not come into use like Pasteur's other modes of vaccination. Emmerich⁸ succeeded better by adopting a different plan, which consists in first introducing *per venas* a very small dose of a virulent culture, and then some ten days later injecting a full dose. As the second injection produced no symptoms, the immunity obtained might be regarded as absolute; but in order to ensure its thoroughness, larger quantities were injected subsequently. In this way a number of healthy rabbits were prepared, the whole process requiring about four weeks for its completion, and the next step was to compare them as regards the antibiotic properties of their blood and tissues with normal rabbits. The table exhibits the experiments, the gist of which is that whereas in the susceptible animal the effect of introducing half a centimetre is to produce a process in which the blood is crowded with microphytes, and they are also found in the tissue, the bacilli introduced into the circulation of the protected animal have almost disappeared in three hours, and are absolutely destroyed in less than eight hours.

Having got thus far—having proved that what I may venture to call the antibiotic state can be produced in an animal by a process of gradual habituation, it was evident that to Emmerich, as regards the particular disease and the particular animal under observation, he had the rest of the problem in his hands. It was proved that the antibiotic effect was produced in the organism—that the bacilli were effectually killed, both in the blood and tissue of the immune animal, whereas they multiplied in the susceptible, but it could not as yet be positively stated whether the agent by which immunity was conferred was an essential product of their metabolism, such as there might be expected to manufacture in any nutrient medium, adapted to their growth, or a result of their action on the living tissues of the infected body. And the way to do this was evidently not to operate on the bacillus, but on the blood and tissues of the immunised rabbit, with reference to which two facts of great moment were

already known, namely, that no bacilli were forthcoming, and that the antibiotic property resided in them.

Method.—This purpose was accomplished by the method of filtration through porcelain, to the immense value of which as a method of pathological research I have already adverted. By subjecting the previously comminuted tissues of an immunised rabbit to extreme pressure, and collecting the expressed juice in sterilised flasks, and filtering through porcelain, a liquid is obtained free from all structural elements, which when injected into the body of a normal, that is susceptible, rabbit produces no disturbance of any function, but confers upon it an antibiotic state, so that if a sufficient quantity of the liquid is injected immediately after a fatal dose of the virulent culture of *rouget*, the development of the disease is averted. In such an animal a slight febrile reaction immediately follows the injection, whereas in an animal in which the development of the disease is left to itself the body temperature of the infected animal culminates on the fifth day with a temperature of 100°. On testing the condition of the blood by plate cultures it was found that, as in the immune animal, there was no multiplication of bacilli. In other experiments, instead of introducing the virus and its antidote simultaneously, the injection of the former (the virus) was deferred for twenty-four hours. The result was much the same, showing that the effect continues for some time after the material itself has been discharged from the system, and this inference was confirmed by observing that even after eleven days the effect of a fatal dose was much mitigated, for the disease ran a favourable course ending in recovery.

These experiments show that the introduction of a soluble product into the organism of an animal susceptible to *rouget* restrains or inhibits the development in it of the bacillus. A few years ago this would have been attributed (in accordance with the "retention" theory) to the direct action of the product on the microphyte, but this we can no longer admit now that we know that in several instances pathogenic microphytes grow and multiply without check in the bodies of infected animals, and that this growth continues after death, until the pathogenic microphyte is overpowered by saprophytes, and for several other reasons. Consequently we are led to adopt the only possible alternative, namely, that although the agent by which the pathogenic microphyte is destroyed or its development arrested is a chemical one, it is not identical with or contained in the substance which has been introduced, but is what in physiology we call a metabolic product of the living protoplasm of the infected organism.

CONCLUSIONS.

We have learned on the one hand from the experiments of Behring and Kitasato, on the other from those of Buchner and Emmerich, that a substance is contained in the liquor sanguinis, that it acts in relatively small doses, that it is capable of counter-infecting the whole organism, that by combining antibiotic and antitoxic properties in varying proportions it adapts itself to the requirement of the particular infection against which the organism is to be protected.

How does all this bear on practical medicine? In medicine we have heretofore depended and still depend on the *vis medicatrix nature*, as the means by which diseases may be cured. It has been proved that the organism possesses the power of bringing into existence certain chemical agents which are the embodiment of the particular *vis protectrix* required, by which the organism guards itself against some of the most important of the specific infections to which it is exposed.

To have learned this is to have entered on the beginning of a new progress, to have turned over a new leaf in pathology. It seems to me to require no prophetic power to foresee that we are on the very threshold of discoveries in medicine such as will eclipse in their splendour all that have preceded them. Happy are they who are young enough to hope to see this anticipation realised.

DR. G. E. YARROW, M.O.H., St. Luke's, Middlesex, was called to the Bar at Gray's Inn on November 17th.

DR. ROBERT BARNES has been elected an Honorary Member of the Leipzig Obstetrical Society.

SIR EDWIN SAUNDERS, F.R.C.S., has been elected Foreign Corresponding Member of the Royal Society of Public Medicine in Belgium.

CHARITY FOOTBALL MATCH.—The annual football match between members of the Dublin hospitals clubs and the County of Dublin, took place on Saturday, November 7th. Unfortunately the weather was very bad, and prevented many from attending. The play was, however, very good, and resulted in a win for the hospitals team by two goals and a try to *nil*. These matches have been the means of providing over £600 to aid the Dublin Hospital Fund.

⁷ Behring u. Kitasato: Ueber das Zustandekommen der Diphtherie-Immunität, etc., bei Thieren, *Deutsche med. Woch.*, 1890, p. 1113. Kitasato: Exper. Untersuchungen ueber das Tetanusgift, *Zeitsch. f. Hyg.*, vol. x, p. 267.

⁸ Emmerich u. Mastbaum, Die Ursache der Immunität, etc., *Arch. f. Hygiene*, vol. iv, p. 208.