IMMUNITY TO INFECTIOUS DISEASES: REVIEW OF SOME CONCEPTS OF METCHNIKOFF[1]

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I. INTRODUCTION

Approximately 70 years have passed since presentation of the cellular (Metchnikoff, 1884) and humoral (Von Behring, 1890) theories of immunity to infectious diseases. That these were indeed great discoveries is attested by their prompt and lasting recognition, and by the highly important practical and theoretical developments to which they led. Both were honored by Nobel Prize Awards (Von Behring in 1901; Metchnikoff shared with Ehrlich in 1908) and together they formed the foundation for a new branch of medical microbiological science, immunology.

In this review concepts of one of these research giants, Elie Metchnikoff, will be considered in the light of present knowledge. In addition to historical interest, this revisitation of Metchnikoff should serve to orient perspective in the field of immunity to infection, and also to assess progress, or lack of progress, in understanding of immune phenomena during the twentieth century. No comprehensive review is intended; rather, the discussion will be largely limited to selected areas of immunology, primarily those dealing with mechanisms of host resistance to bacterial agents.

[1] Based on a presentation before the Symposium on Interplay Between Infectious Agents and Phagocytic Cells, May 13, 1959, at the 59th Annual Meeting of the Society of American Bacteriologists held in St. Louis, Missouri.

II. BIOGRAPHICAL ASPECTS[2]

Elie (Ilia) Metchnikoff was born in 1845 in the steppe region of the province of Kharkoff, Russia. His parents, middle class land owners, were apparently somewhat concerned with the sickly, nervous behavior of this, their youngest child. At a very early age he demonstrated unusual academic ability and special interest in natural science. Upon completion of schooling at the Lycée at Kharkoff, he expressed the wish to study medicine, but was dissuaded by his mother, who thought him too sensitive to withstand the sight of human suffering. Further studies at the Universities of Kharkoff and Petersburg were then in the fields of embryology and zoology.

During the next twenty years Metchnikoff taught and did research in zoology, but his life was most unsettled. He seemed always to be on the move, switching from one university post to another and spending considerable time studying or vacationing in Italy, France, or Germany. Personal tragedies—his own ill health, the death of his first wife and of his parents—along with deterioration of academic freedom at the Russian universities, combined to produce a most unhappy picture. In 1882 he resigned his professorship at Odessa and went to Messina so that he might pursue research on the shores of the Medi-

[2] Taken largely from the biography of Metchnikoff written by his wife (reference 1).

terranean. It was here that the "jobless" wandering zoologist made discoveries leading to the phagocytic theory.

His own words provide a vivid description of the conception of this theory: "I was resting from the shock of the events which provoked my resignation from the University and indulging enthusiastically in researches in the splendid setting of the Straits of Messina.

"One day when the whole family had gone to a circus to see some extraordinary performing apes, I remained alone with my microscope, observing the life in the mobile cells of a transparent star-fish larva, when a new thought suddenly flashed across my brain. It struck me that similar cells might serve in the defence of the organism against intruders. Feeling that there was in this something of surpassing interest, I felt so excited that I began striding up and down the room and even went to the seashore in order to collect my thoughts.

"I said to myself that, if my supposition was true, a splinter introduced into the body of a star-fish larva, devoid of blood-vessels or of a nervous system, should soon be surrounded by mobile cells as is to be observed in a man who runs a splinter into his finger. This was no sooner said than done.

"There was a small garden to our dwelling, in which we had a few days previously organised a "Christmas tree" for the children on a little tangerine tree; I fetched from it a few rose thorns and introduced them at once under the skin of some beautiful star-fish larvae as transparent as water.

"I was too excited to sleep that night in the expectation of the result of my experiment, and very early the next morning I ascertained that it had fully succeeded.

"That experiment formed the basis of the phagocyte theory, to the development of which I devoted the next twenty-five years of my life" (1, pp. 116–117).

On the return journey to Russia from Messina, Metchnikoff stopped in Vienna to see Professor Claus, a well known zoologist. During their discussion the question of an appropriate name for these wandering cells was considered. Metchnikoff suggested a Greek translation of "devouring cells" and thus was born the term phagocyte.

This initial observation in star-fish larvae was promptly confirmed and extended by Metchnikoff. Studies on daphnia, a tiny transparent fresh-water crustacean, revealed that the outcome of natural infection with a fungus, *Monospora bicuspidata*, was intimately related to the interaction between these parasites and phagocytic cells of the daphnia. Certain strains of the fungus were seen to attract phagocytes and to be engulfed and destroyed by them with resulting recovery of the animal, whereas in other instances the phagocytes were indifferent to or incapable of handling the fungi, a situation leading to disseminated infection and death of the host.

Studies on the anthrax bacillus revealed that susceptibility of this microbe to phagocytosis varied with virulence; phagocytes were incapable of attacking fully virulent strains of anthrax, whereas attenuated strains were readily ingested and killed.

These observations thus laid the foundation of the phagocytic theory of host resistance to infection. Their publication initially received a mixed reaction, with some prominent workers giving the concept enthusiastic support while others were vociferously critical of it.

Metchnikoff returned to Odessa but the academic situation there again soon became intolerable. On a vacation in 1887 which included Paris in the itinerary, he took the opportunity to visit the newly constructed Pasteur Institute and to meet the Great Man. Metchnikoff described this meeting as follows:

"On arriving at the laboratory . . . I saw an old man, rather undersized, with a left hemiplegia, very piercing grey eyes, a short beard and moustache and slightly grey hair, covered by a black skull-cap. His pale and sickly complexion and tired look betokened a man who was not likely to live many more years. He received me very kindly, and immediately spoke to me of the question which interested me most, the struggle of the organism against microbes.

" 'I at once placed myself on your side,' he told me, 'for I have for many years been struck by the struggle between the divers micro-organisms which I have had occasion to observe. I believe you are on the right road' " (1, p. 132).

Pasteur's warm reception included the offer of a laboratory in the Institute to Metchnikoff. This offer was soon accepted; in 1888 Metchnikoff resigned for the third and final time from the University of Odessa and moved to Paris. For the next twenty-eight years he led a settled and productive life at the Pasteur Institute. The early part of this period was spent extending and firmly

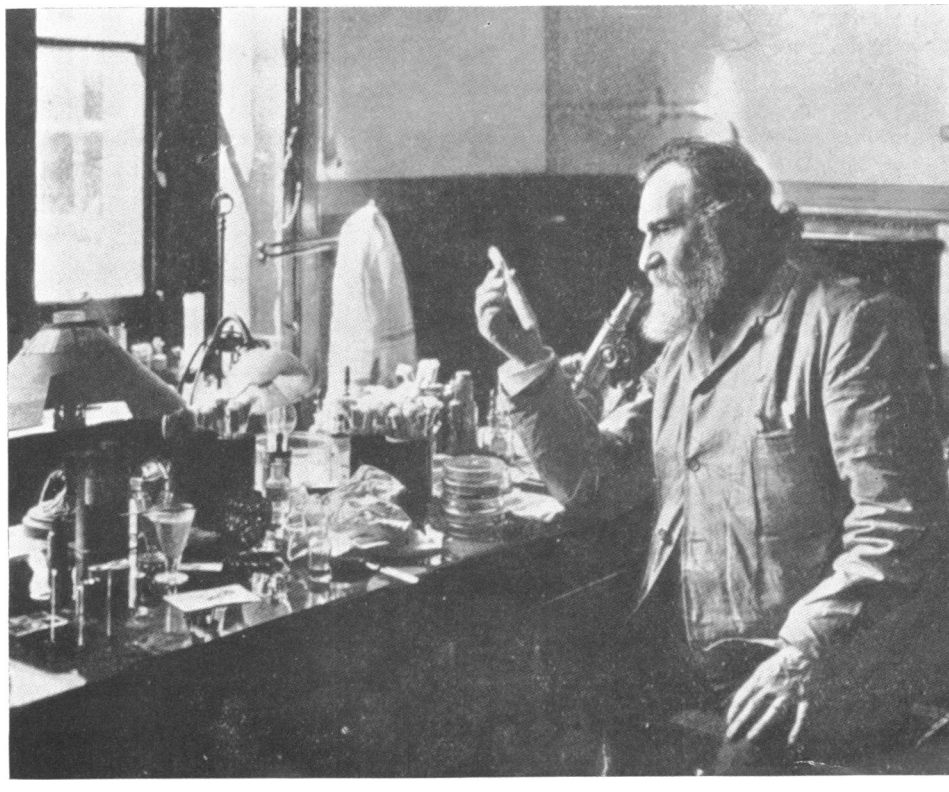

Figure 1. Metchnikoff in his laboratory at the Pasteur Institute, Paris. (From Metchnikoff, Olga 1921 *Life of Elie Metchnikoff.* Houghton Mifflin Co., Boston and New York.)

establishing the phagocytic theory. When this had been accomplished, he turned to studies on immunity in general, then briefly considered mechanisms of fever production, and finally worked on problems of aging, and especially on possible noxious effects of the normal intestinal bacterial flora. Figure 1 shows Metchnikoff at work in his laboratory at the Pasteur Institute.

III. DEFINITION OF IMMUNITY

Metchnikoff defined immunity to infectious disease as follows:

"Immunity against infective diseases should be understood as the group of phenomena in virtue of which an organism is able to resist the attack of the micro-organisms that produce these diseases. It is impossible, at present, to give a more precise definition, and useless to insist upon it. Some have thought it necessary to distinguish between immunity properly so called, that is to say a permanent refractory state, and 'resistance,' or a very transient property of opposing the invasion of certain infective micro-organisms. It is not possible to maintain this distinction, for in reality the limits between these two groups of phenomena are far from being constant.

"Immunity may be inborn or acquired. The former is always natural, that is to say, independent of the direct intervention of human art. Acquired immunity is also often natural, from the fact that it is established as the result of the spontaneous cure of an infective disease. But in a great number of cases acquired immunity may be the result of direct human intervention as in the practice of vaccination.

"For a long time all the phenomena of immunity against infective diseases were collected into a single group. Later, it was recognized . . . that it is necessary to distinguish sharply between immunity against the pathogenic micro-organisms themselves and that against microbial poisons. Hence the idea of antimicrobial and antitoxic immunities" (2, p. 10).

This definition appears entirely satisfactory today, more than fifty years after it was written. The essential criteria of immunity, and the vari-

ous types thereof, are clearly and succinctly set forth, requiring no further elaboration or alteration to bring them up to date.

IV. ROLE OF HOST IMMUNITY IN DETERMINING WHETHER OR NOT INFECTION PROCEEDS INTO INFECTIOUS DISEASE

In recent years, Dubos (3) and others have emphasized the predominant part played by the state of host resistance in pathogenesis of many of the currently prevalent infectious diseases. Potentially pathogenic microbes, for example staphylococci and tubercle bacilli, are widely distributed in the human population, yet produce disease in only relatively rare persons in whom mechanisms of immunity have broken down. Thus, the presence of microorganisms (infection) is a necessary but usually not a sufficient cause for production of infectious disease. Since in this situation the state of host resistance determines directly whether or not disease ensues, the importance of understanding immune mechanisms is obvious.

Over half a century ago, Metchnikoff commented in a similar vein:

"As soon as he is born, man becomes the habitat of a very rich microbial flora. The skin, the mucous membranes ... the intestines and the genital organs offer a feeding ground for Bacteria and inferior Fungi of various kinds. For long it was thought that in healthy individuals all these micro-organisms were inoffensive and sometimes even useful. It was supposed that when an infective malady was set up a specific pathogenic micro-organism was added to this benign flora. Exact bacteriological researches have, however, clearly demonstrated that as a matter of fact the varied vegetation in healthy persons often includes representatives of noxious species of bacteria. Besides the diphtheria bacillus and the cholera vibrio, which have repeatedly been found in a virulent form in perfectly healthy individuals, it has been demonstrated that certain pathogenic micro-organisms, *e.g.*, the *Pneumococcus*, staphylococci, streptococci and the *Bacillus coli*, are always, or almost constantly, found among the microbial flora of healthy persons.

"This observation has necessarily led to the conclusion that in addition to the micro-organism there exists a secondary cause of infective diseases—a predisposition, or absence of immunity. An individual in whom one of the above-mentioned pathogenic species is present, manifests a permanent or transitory refractory state as regards this specific organism. As soon however as the cause of this immunity ceases to act, the micro-organism gets the upper hand and sets up the specific disease. It is thus in diabetic persons that boils make their appearance as the result of the development of *Staphylococcus pyogenes*, a micro-organism that is almost always found in abundance on the skin and mucous membranes of the human subject. The diabetes is, in most cases, the cause of the suspension of the immunity which exists in the healthy individual.

"People who carry the *Pneumococcus* on their mucous membranes may remain for long without being attacked by fibrinous pneumonia or any of the other maladies due to this micro-organism. But often, in consequence of some special circumstance, a cold for example, the refractory state gives way to a more or less marked susceptibility.

"It is unnecessary to multiply the number of such examples; they demonstrate in the clearest fashion that, in addition to the causes of disease which come from the outer world and which are represented by the micro-organisms, there are yet other causes which lie within the organism itself. When these internal factors are powerless to prevent the development of the morbific germs, a disease is set up; when, on the other hand, they resist the invasion of the micro-organisms properly, the organism is in a refractory condition and exhibits immunity" (2, pp. 7–8).

As is seen in the above quotation, Metchnikoff appreciated clearly the role of altered host immunity in determining the outcome of encounters with potentially pathogenic microbes. Furthermore, he illustrated this situation with two specific examples, the susceptibility of diabetics to staphylococcal infection and the occurrence of bacterial pneumonia following "colds" (influenza ?), both of which are to this day widely quoted. The precise mechanisms of host resistance which are altered in these and many other conditions associated with increased susceptibility to infection, remain as ill-defined today as they were at the turn of the century.

V. NATURAL IMMUNITY TO INFECTION

A. Cellular Mechanisms of Natural Immunity

The phenomenon of phagocytosis was described by many workers in the sixth and seventh decades of the nineteenth century. Before Metchni-

koff presented his observations, it was generally held that phagocytic cells were simple scavengers whose function it was to pick up and carry to suitable disposal sites any foreign material which they chanced to encounter. Ziegler had, in fact, stated that infections might be spread as a result of transportation of microbes by the motile phagocytic cells. Metchnikoff's contribution, then, was not the discovery of phagocytosis, but rather discovery of the crucial role of phagocytic cells as a host mechanism of defense against invading microorganisms. He commented on cellular mechanisms of immunity to infection as follows:

"The facts ... afford us a general picture of the phenomena exhibited in natural immunity against micro-organisms. The dominant feature is represented by the phagocytic reaction that is observed throughout the animal series and that is exercised against parasites belonging to all the microbial groups" (2, p. 206).

"Phagocytosis is a phenomenon of considerable complexity. When it is exhibited by leucocytes, these cells are in the first place affected by various substances which possess an attraction for them. They proceed towards these substances by means of their amoeboid movements and then englobe them. Intracellular digestion may afterwards occur. Here then we have phenomena of sensibility, contraction, ingestion, and production of digestive fluids.

"It cannot be denied that the phagocytic reaction is far from representing a perfect mechanism, as is evidenced by the frequency of many diseases. But there is also no doubt that the generalisation of bacteria takes place much more rapidly in the case when they are not englobed by the phagocytes. ... The importance of the leucocytes as carriers of infective bacilli has been very much exaggerated" (4, pp. 203, 206).

That phagocytic cells play an essential role in host resistance to bacterial infection remains undisputed today. The function of the microphage (polymorphonuclear leucocyte) in this regard is clearly illustrated by a condition called agranulocytosis, in which these cells are severely reduced in number or even absent. Persons afflicted with agranulocytosis are notoriously susceptible to bacterial infections. No similar naturally occurring condition is known to demonstrate the antimicrobial function of fixed and wandering mononuclear phagocytic cells, but such a role for them is widely acknowledged today, especially in certain chronic infections. Modern concepts of interactions between phagocytic cells and microorganisms have recently been thoroughly reviewed by Suter (5).

Metchnikoff made the following comments on the various types of phagocytic cells and their respective function:

"In the *Vertebrata* we meet with two great categories of white corpuscles, of which one group resembles those of the *Invertebrata* in that they also possess a single large nucleus and an amoeboid protoplasm. These are the macrophages of the blood and of the lymph, and are intimately connected with the macrophages of such organs as the spleen, lymphatic glands, and bone marrow. Another group of white corpuscles in the *Vertebrata* is made up of small amoeboid cells which are distinguished by having a nucleus which, although single, is divided into several lobes. These are the microphages (2, pp. 547–548).

"Phagocytosis is exhibited not only by the macrophages but also, in a high degree, by the microphages which stand out as the defensive cells *par excellence* against micro-organisms (2, p. 206).

"Just as the *Amoebae* and the *Infusoria* make a choice from amongst the small organisms that surround them, so the leucocytes choose bodies which are best suited to their use. The macrophages seize by preference animal cells such as the blood corpuscles, the spermatozoa, and other elements which are derived from animals. Among the infective microorganisms the macrophages have a predilection for those that set up chronic diseases such as leprosy, tuberculosis, and actinomycosis and also for those which are of animal nature. Into this last category come the amoeboid parasites of malaria, Texas fever and the *Trypanosomata*. The macrophages can also ingest the bacteria of acute diseases, but, save in exceptional cases, their intervention is of little moment.

"The microphages, on the other hand, appear to play their part specially in acute infections. Their intervention against animal cells is *nil*, or almost so. Thus, they rarely seize the red corpuscles of the same or of a foreign species of animal" (2, pp. 548–549).

For purposes of study and discussion, Metchnikoff divided the phagocytic process into component parts or phases: ameboid movement, chemotaxis, engulfment, and intracellular digestion. Let us then compare knowledge of these phases

of phagocytosis as it existed in 1900 and as it stands today.

Ameboid motion as a property of phagocytic cells was recognized in the 1860's. No one has yet succeeded in discovering the fundamental mechanism by which movement takes place. Observations in recent years have established the speed of polymorphonuclear leucocyte migration as approximately 30 μ per minute, and also have demonstrated that complement and calcium ions in serum increase motility of phagocytes (see reference 5).

In addition to the motion *per se*, Metchnikoff commented at length on a property of phagocytic cells which is termed tactile sensibility, this term connoting the ability and inclination of these cells to crawl along fibrin strands, through small openings, etc. In this connection he also presented an intriguing explanation, in an evolutionary sense, for the unique polymorphous character of the granulocyte nucleus.

"... the lobed and polymorphous shape of the nucleus of the pus corpuscles has long been remarked. This particular shape is peculiar to the polynuclear leucocytes, which represent the vast majority (75 per cent) of the total number of white cells. ... The shape of their nucleus may be ... explained as a special adaptation for passing through the vessel-wall. If the process of diapedesis be watched, the difficulty experienced by the nucleus in getting through will at once be noticed. Directly this has occurred, the rest of the protoplasm follows rapidly. It is obvious that a nucleus divided into several lobes can pass through the wall more easily than one not so separated. Hence in pus the polynuclear leucocytes are more numerous than the mononuclear leucocytes, and hence the lobed shape of the nucleus is found only in the leucocytes adapted for diapedesis and does not occur among the *Invertebrata*" (4, pp. 185–186).

Early observations on chemotaxis were made by Stahl on slime mold. These organisms were shown to respond in a reproducible fashion with movement towards or away from chemical substances placed near but not in contact with them. Examination of this phenomenon in quantitative manner demonstrated that chemotactic response was in some ways similar to sensory reactions in higher forms; for example, chemotaxis followed Weber's law, *i.e.*, the reaction varied as the logarithm of the excitation. Again quoting from Metchnikoff:

"We owe to Leber, however, the first clear exposition of the ... chemiotactic sensibility of the leucocytes. In his experiments on keratitis produced by a crystalline substance extracted from cultures of *Staphylococcus aureus*, he showed that the leucocytes at a distance were attracted towards the point where this substance had been introduced. On putting some small glass tubes filled with this substance, into the anterior chamber of the eye, they became filled with a mass of leucocytes, although the tubes were so placed that the cells had to move against gravity in order to effect an entry into them. This important discovery was the starting-point of a series of researches which proved beyond question the existence in leucocytes of chemiotactic properties absolutely analogous to those of plasmodia and other lower organisms. Gabritchevsky ... pointed out moreover that whereas leucocytes are strongly attracted by sterilized or living cultures of most pathogenic and saprophytic bacteria ..., they are repelled by the most virulent bacteria ..., and by lactic acid, ten per cent solutions of sodium and potassium salts, alcohol, chloroform, glycerine, jequirity, bile and quinine" (4, p. 117).

Active research on the intriguing phenomenon of chemotaxis continues at present, and the observations resemble those made in the "old days" except that investigators no longer employ such colorful substances as jequirity (poisonous seeds of a plant native to India). Modern concepts of chemotaxis have been recently reviewed by Harris (6). We still have no explanation, and in fact no good hypothesis, to explain this action at a distance.

The actual process of engulfment of foreign particles by phagocytic cells is astonishing to behold in living specimens under the microscope. With suitable preparations the phagocyte is seen to pursue the microbe; on contact the bacteria rapidly pass through the phagocytic cell wall with no detectable disruption in the continuity of the cellular membrane. That microorganisms are engulfed in a living state was established by Metchnikoff in direct observations on suitable motile microbes.

"It has been often thought that the leucocytes which are gathered together in an inflamed area may only serve to effect the absorption of dead cells and microbes, and they have been looked upon as simple 'scavengers.' We have already seen that this hypothesis is not justified by facts, and that from the very onset of infection the

leucocytes wander towards and englobe the parasites in a living condition (4, p. 190).

"That the bacilli are in a living condition when swallowed, is shown by the fact that they perform active movements although enclosed in the nutritive vacuoles of the leucocytes" (4, p. 115).

It was recognized by Metchnikoff that certain bacteria, even though they exerted an attractive force on phagocytes, seemed nevertheless capable of resisting engulfment. Research in the present century has contributed considerably to understanding of this phenomenon. Largely as a result of the beautiful studies of Avery and his associates (7) on pneumococci, it has been established that virulence is in general correlated with possession by these microorganisms of a complex polysaccharide capsule which renders them resistant to engulfment by leucocytes. As will be discussed subsequently, the phagocytosis-resisting effect of this capsule can be neutralized by the presence of specific antibody. In addition, recent studies of Wood and collaborators (8) have demonstrated the importance of the physical nature of the environment in determining whether or not engulfment is accomplished. Provided a suitable surface or structure in which bacteria may be "cornered," encapsulated microorganisms may be taken in by phagocytes even in the absence of antibody. It is seen then that in recent decades most emphasis has fallen and, probably as a consequence, most advance has been made in relation to this particular phase of the over-all phagocytic reaction, namely the act of engulfment.

The final stage of the phagocytic process in relation to immunity is concerned with intracellular fate of engulfed bacteria. Bacteria taken in by phagocytes may be retained, or in some instances may pass through the cell wall from inside out. This phenomenon of egestion was recognized long ago in amebae and other simple organisms, and its occurrence in leucocytes of mammals has recently been demonstrated and emphasized by Wilson (9). The role played by egestion in influencing or determining immunity has not yet been established.

Three fates are possible for bacteria ingested and retained by leucocytes: (a) death, (b) survival without multiplication for variable periods of time, and (c) multiplication. Metchnikoff recognized clearly and gave specific examples of the second possibility, that phagocytic cytoplasm may exert a bacteriostatic rather than a bactericidal influence on some microorganisms.

"In several cases where the leukocytes have not the power of killing the microbe, they can nevertheless hinder it from growing and exerting its toxic influence. The best examples of this are afforded by bacterial spores, which are endowed with great powers of resistance. Anthrax spores are readily ingested by the leukocytes of many species of animals, and among others, by those of immune animals such as the frog and fowl. In spite of the fact that the spores germinate and grow easily in the lymph-plasma of the last-mentioned, they are incapable of doing so in the bodies of the leukocytes themselves, where they cannot exert their injurious effects, although they preserve their vitality and their virulence often for a considerable length of time. Once, however, that the vitality of these cells has been extensively lowered, as may be effected in fowls by cooling them or in frogs by heating them artificially, the spores germinate within the dead or enfeebled leukocytes, and infect the whole body" (4, p. 128).

This description reveals Metchnikoff's awareness of the phenomenon of latent or attenuated infection. His examples demonstrate that microbes can survive in a state of hibernation within host cells without producing disease, only to be provoked into multiplication and resultant disease by disturbances in the physiology or environment of the host. Needless to say, innumerable instances of latent infection have now been uncovered in plants, mammals, and even in bacteria.

Metchnikoff pointed out that some bacteria survived and multiplied inside of phagocytic cells:

"It is undeniable then that leukocytes possess digestive powers, and that in particular they are able to digest microbes. But it does not therefore necessarily follow that these cells kill and digest all the microbes they englobe. In certain diseases the leukocytes take in a number of bacteria, such as tubercle bacilli or the bacilli of swine erysipelas or mouse septicaemia, a few of which may be digested while the others resist the digestive action of the leukocytes, multiply in the cells and finally invade the whole organism" (4, p. 128).

Many investigations conducted since 1900 have dealt with intracellular fate of microorganisms (see reference 5). Some nonpathogenic bacteria and several microbes which produce acute infections appear to be rapidly killed within phagocytes. Group A streptococci, for example, are no longer viable after a 15 to 30-min sojourn within granulocytes. On the other hand, the growth of

mycobacteria and brucellae within polymorphonuclear leucocytes has been repeatedly demonstrated, and observations suggest that some staphylococci and *Haemophilus influenzae* may also survive in granulocyte cytoplasm. Somewhat surprisingly, the intraphagocytic fate of certain bacteria of importance to man seems never to have been adequately studied (*e.g.*, *Neisseria*, *Corynebacterium*, and some *Salmonella* species).

Of central importance in understanding the susceptibility or resistance of microorganisms to killing by leucocytic cytoplasm is discovery of the biochemical intracellular substances or conditions which exert antibacterial action. Metchnikoff commented on this problem as follows:

"We are at present ignorant of the precise manner in which this digestive and destructive action is accomplished, and do not even know whether the substance which kills microbes is a ferment or not. The fact that the ferments of the higher animals, such as pepsin and trypsin, do not kill bacteria, is no reason for assuming that there may not be other ferments which are capable of exercising a bactericidal action (4, p. 127).

"I have always openly acknowledged that the question as to what substances within the phagocytes harm and destroy the microbes is still quite undecided. They may be ferments, digestive or otherwise, or they may be substances, acid or alkaline, completely different from ferments. We shall have to find new and more perfect methods before being able to solve this delicate problem" (4, p. 208).

Metchnikoff observed that the reaction about ingested microbes in leucocytes was often acid.

"I have already brought forward arguments in favour of the view that the staining of the ingested elements indicates a feebly acid reaction inside the phagocytes. Sometimes this reaction manifests itself in the digestive vacuoles; in other cases it is exhibited only in the micro-organisms directly lodged in the protoplasm. Whilst the phagocyte is still living, the acid juice which fills the vacuoles or permeates the ingested organisms does not mix with the protoplasm which is always alkaline. But shortly after the death of the phagocytes this mixture is effected without difficulty, and the alkalinity of the protoplasm is then amply sufficient to neutralise or even render alkaline the feebly acid juices. This interpretation of the facts is in complete harmony with all the data, collected up to the present, on the staining by neutral red of phagocytised micro-organisms" (2, p. 182).

These observations were later extended by Rous and others (reviewed in 3). From experiments employing indicator supravital dyes, the pH in the vicinity of engulfed particles has been shown to reach distinctly acid levels; values ranging from approximately pH 3 to pH 6 have been obtained by various investigators in diffierent systems. Studies performed in recent decades have furthermore shed light on the origin of intracellular leucocytic acid. Both granulocytes and macrophages have been shown to utilize glycolysis rather than respiration as their main source of energy (5); the lactic acid produced during glycolysis undoubtedly is responsible, at least in part, for the low intracellular pH characteristic of these cells. That the lactate production and acid reaction can exert antimicrobial action is supported by studies of Dubos which demonstrated that many microorganisms, and especially bacteria of the gram-positive type, are suppressed in their growth or even killed *in vitro* by relatively low concentrations of lactate, especially when the medium is acid (see reference 3).

In addition to the acid content of leucocytes, Metchnikoff discussed experiments done by Gengou which indicated that other antibacterial agents might be present in white cells.

"In order to obtain exudations very rich in microphages Gengou injected gluten-casein by Buchner's method into the pleural cavity of dogs and rabbits. Usually at the end of 24 hours he was able to collect a large quantity of fluid containing numerous leucocytes, almost exclusively microphages. To obtain macrophagic exudations Gengou injected washed red blood corpuscles of the guinea-pig into the pleural cavity of his animals; two days afterwards he withdrew from the pleural cavity a very viscid fluid, containing, as regards formed elements, macrophages almost exclusively. After isolation of the leucocytes by centrifugalisation of the exudations, Gengou washed the cells with physiological salt solution and then added to them an equal volume of broth. This mixture was frozen by Buchner's method, and was then submitted to a temperature of 37°C. Under these conditions the leucocytes, killed by cold, gave up to the fluid their bactericidal substance.

"Studied in this way, the bactericidal power of the extract of microphages showed itself always superior to that of the corresponding blood serum. The greatest difference was observed in the dog, where . . . the serum of the blood has no bacteri-

cidal property as regards the anthrax bacillus, whilst the extract of microphages manifests this property very strongly. The microphagic extract of the exudations of rabbits was more active in the destruction of the bacilli of anthrax and typhoid, *Bacillus coli* and the cholera vibrio than was the blood serum. . . .

"The experiments of Gengou with the extracts of macrophages have demonstrated, on the other hand, that this fluid exerts no bactericidal power. Let it be understood at the outset that this fact is in no way an indication of the absence of the bactericidal ferment in the macrophages. Direct examination of the phenomena which are manifested inside these cells demonstrates most clearly that the macrophages kill and digest micro-organisms. But this process usually goes on much more slowly in the macrophages than in the microphages, owing probably in the former to the presence of a smaller quantity of the bactericidal substance. Under these conditions we can readily understand that this substance does not pass, or passes only in small amount, into the extracts. There is nothing remarkable in the fact that, with so imperfect a method of preparing the extracts, the greater part of the bactericidal substance should remain in the bodies of the cells" (2, pp. 186–187).

The present state of knowledge about antimicrobial factors in normal fluids and tissues, including leucocytes, has recently been reviewed by Skarnes and Watson (10). Considerable progress has been made in this area, but much remains to be learned. The most clearly defined and thoroughly studied of these bactericidal substances of phagocytes is lysozyme, an acetylamino polysaccharidase discovered by Fleming. This enzyme lyses promptly the few bacterial species whose cell wall is composed of a susceptible substrate, and in addition exerts inhibitory or lethal action on a wide range of microbes in combination with other materials such as acids and chelating agents, which might be present inside leucocytes. Large amounts of lysozyme (about 1 mg per g wet weight of cells) are contained in polymorphonuclear leucocytes, whereas, in agreement with Gengou's old observations, none of this antibacterial substance can be found in mononuclear phagocytes (11).

Other less well defined materials, protein or peptide in nature, also can be extracted from granulocytes (reviewed in detail in reference 10). These agents fall into two general classes, the one of which, called leukins, acts primarily on gram-positive bacteria, whereas the other, called phagocytin, seems to be more effective in killing gram-negative organisms. What has been called leukin in various studies may in fact consist of several different agents; there is much to suggest that some leukins are similar or identical to the basic proteins histone and protamine. Phagocytin may likewise turn out to be more than a single agent. This material also has properties suggesting that it is at least in part protein, and, like lysozyme, it appears to be present in large amounts in granulocytes but absent in macrophages.

Thus, although many studies have been made since the turn of the century, our knowledge remains grossly incomplete of the precise antimicrobial substances of phagocytes and of how these substances act.

B. Humoral Mechanisms of Natural Immunity

Metchnikoff was for many years engaged in a polemic, largely waged with German workers, as to the relative role of cellular and humoral factors in natural immunity to infection. His strong position in this controversy is illustrated by the following quotation:

"The phagocytes enter into a struggle against the micro-organisms and rid the animal organism of them without requiring any previous help on the part of the body fluids. Phagocytosis, exercised against living and virulent micro-organisms, is sufficient to ensure natural immunity. The bactericidal power of the serum, which for a long time served as the basis for a humoral theory of immunity, represents merely an artificial property, developed in consequence of the setting free of the microcytase of the leucocytes that have become disintegrated after the blood has been drawn. The agglutinative power of the normal fluids of the body plays no important part in natural immunity" (2, p. 206).

Observations had been made in the late 1800's of the lethal effect of "normal" serum and of the fluid portion of exudates on certain microbes. Metchnikoff pointed out that such effects were the exception rather than the rule; the vast majority of bacteria, both virulent and nonvirulent, were not killed by exposure to serum. Further studies, done by Gengou and Bordet in Metchnikoff's laboratory, indicated that the lethal effect of serum on some microbes was, in a sense, an artifact.

"Blood was drawn into paraffined tubes and

centrifugalised at once in other tubes whose walls were also lined with a layer of paraffin. The fluid thus prepared is certainly more allied to circulating plasma than is the blood serum obtained after coagulation of the blood. Nevertheless, it is still far from being identical with true normal plasma; it still coagulates, though tardily. Gengou compared, in their bactericidal action, the blood serum and the serum, decanted after the tardy coagulation of the fluid analogous to plasma. He carried out a great number of experiments with the two fluids, obtained from dogs, rabbits and rats, making a comparative study of their bactericidal power as regards the anthrax bacillus, the typhoid bacillus, and the cholera vibrio. I have closely followed all these experiments and can confirm the results described by Gengou, namely, that the fluid, in this plasma serum, possesses an insignificant bactericidal power or none at all, whilst the blood serum almost always exhibits this property to a marked degree" (2, p. 190).

These experiments, and others along similar lines, led Metchnikoff and his followers to conclude that the extracellular fluid of blood and exudates manifested bactericidal properties only when conditions were such that antimicrobial substances from polymorphonuclear leucocytes ("microcytases") leaked out.

The controversy over relative importance of cellular and humoral factors in natural resistance to bacterial infection continues today. Additions to our knowledge since the turn of the century consist, in the main, of further definition of natural serum components (complement, properdin, cations) which exert, at least in some situations, an antibacterial action (reviewed in reference 10). Furthermore, it has been clearly established that certain serum factors (complement, divalent cations, phagocytosis-promoting factor) greatly facilitate the phagocytic process (see reference 5). As with other controversies of long standing, it appears that each of the protagonists was, at least to a degree, correct. Cellular and extracellular agencies acting in consort appear to be required for maximum natural resistance to infection.

Almost all studies done in recent decades on various activities of the blood fluids have employed serum as the test material. Metchnikoff's concept that serum might contain cellular products released during clotting has been largely neglected by most modern workers. It is thus possible that many of the lysins and bactericidins demonstrated in serum may be in reality of leucocytic or platelet origin. And even in the case of complement, usually considered to be a true serum constituent, there is evidence suggesting that one of the essential components, C'4, is derived from white blood cells (12).

VI. ACQUIRED IMMUNITY TO INFECTION

A. Humoral Factors

Whereas Metchnikoff denied vociferously the role of serum components in contributing to native host resistance, he admitted freely their function in acquired immunity, emphasizing, however, that participation of phagocytes remained crucial for effective elimination of the parasites.

"The agglutinative substance . . . in the . . . fluids of the body becomes much more developed in those of immunised animals. It is truly humoral, as it circulates in the plasmas and passes into the fluid exudations and transudations. . . .

"The protective and fixative properties, most often closely connected with each other, are very markedly developed in an animal enjoying acquired immunity. They may act upon the micro-organisms which become permeated by the fixative substance, or upon the infected animal by stimulating its defensive reaction. . . . The two properties (protective and fixative) reside in the fluids of the body, but they are functions of the cell products. The elements of the phagocytic organs (spleen, bone-marrow, lymphatic glands), or phagocytes, produce the specific protective and fixative substances which pass thence into the plasmas.

"The phagocytic reaction is very general in acquired immunity. The phagocytes which have a very imperfect antimicrobial function or none at all, become, as the result of vaccination, much more active . . . (and) exhibit a very marked positive chemiotaxis . . . (2, pp. 295–296).

"The micro-organisms which can be deeply injured by the direct action of the specific serum are few in number. In most cases this action is a feeble one and needs, for its completion, effective co-operation on the part of the phagocytes" (2, p. 316).

Although Metchnikoff thus expressed a vague concept of antibacterial antibodies and their role, little was known of their nature or mechanism of action. In recent decades there has been an enor-

mous amount of research on antibody formation and function. The general chemical nature of antibody molecules, the site of their production, and various qualitative and quantitative aspects of the interaction between antigens and antibodies have been elucidated. The concept of a specific interaction between antibody and the bacterial surface with resulting increase in susceptibility to phagocytosis has explained definitively one important role of antibodies in host resistance. With certain exceptions, Metchnikoff's statement that specific antiserum does not exert a direct bactericidal action remains uncontroverted. Little information is available concerning possible effects of immune serum other than those mentioned above, *i.e.*, increasing susceptibility of microorganisms to phagocytosis (widespread) and direct killing (limited for the most part to certain gram-negative microbes). Theoretically, acquired immunity may also influence the intracellular fate of bacteria in one of two ways: (a) microbes ordinarily resistant to killing within leucocytes might be rendered susceptible as a consequence of interaction with immune serum prior to engulfment, a possibility frequently mentioned but never clearly demonstrated experimentally, and (b) the phagocytic cells might develop more effective antibacterial systems, specific or nonspecific, in their cytoplasm in the acquired immune state, as will be discussed subsequently.

In addition to antibacterial effects, whether direct or indirect, the very important activity of immune serum in neutralizing microbial toxins is mentioned but will not be further discussed here.

Serum antibodies are now held in the minds of many to be the mainstay, or even the entire structure of host resistance to infection. Resort to the older literature and the broad picture of immunity helps put the antibody mechanism in its rightfully important, but not all important, place.

B. Cellular Acquired Immunity

As demonstrated by the following quotation, Metchnikoff believed that acquired immunity was associated with an enhanced digestive capacity of the phagocytic cells.

"In certain infective diseases terminating fatally a very marked phagocytosis is observed even in susceptible animals. The most typical example of this is furnished by swine erysipelas and mouse septicaemia.... A method of vaccinating animals against the microorganism of swine erysipelas was worked out by Pasteur and Thuillier and was afterwards studied by many observers. Thanks to this method it has been possible to demonstrate the phenomena which may be observed in vaccinated animals (especially rabbits). Here also a phagocytosis takes place, even more rapid and more complete than in susceptible animals. What is more important, the intracellular digestion of the ingested bacilli is followed by the total destruction of the microorganisms in the vaccinated animals, though in the normal animals this digestion is very imperfect.

"The acquisition of immunity against microorganisms is, therefore, due not only to the change from negative to positive chemiotaxis, but also to the perfecting of the phagocytic and digestive powers of the leucocytes—a general superactivity and adaptation of the phagocytic reaction of the immunised animal is produced" (2, pp. 283–284).

Surprisingly little has since been learned about possible acquired cellular immunity. The studies quoted by Metchnikoff indicate that polymorphonuclear leucocytes develop, as a result of immunization, an enhanced capacity to destroy swine erysipelas bacteria. However, numerous subsequent attempts to demonstrate specific, acquired cellular immunity in granulocytes have met with failure; there exists no convincing evidence that these cells develop new or more efficient bactericidal mechanisms in their cytoplasm as a result of natural infection or vaccination.

On the other hand, in recent years numerous studies have shown that mononuclear phagocytes from immunized animals are more capable than those from normal subjects in handling tubercle bacilli and brucellae, irrespective of serum factors (5). The specificity and durability of this apparently acquired immunity of macrophages has not yet been established; it thus may represent a specific cellular alteration or may be more closely related to nonspecific factors as discussed below.

C. Nonspecific Acquired Immunity

On this topic Metchnikoff made the following comment:

"According to Issaeff's researches, the protective substances used by him must be arranged in the following order as regards their action against the cholera vibrio. Tuberculin is the most effective; then comes a 2% solution of nuclein, followed by normal human serum, broth, and urine, whilst physiological saline solution is the least

active. All prevent infection by the vibrios, but the protection is effective for some days only; this protective action is exerted against various kinds of bacteria, being in no sense specific (2, p. 320).

"These experiments served as the starting-point for several works on the vaccination of animals against anthrax by means of various micro-organisms, as well as by their products. Pawlowsky, Watson-Cheyne, and Bouchard have proved that bacteria not very pathogenic and even saprophytes, such as the *Cocco-bacillus prodigiosus*, Friedländer's bacillus, and the *Bacillus pyocyaneus*, were also capable of preventing infection by the anthrax bacillus. Freudenreich showed that not only did the bacillus of blue pus exert an antagonistic action but that the same effect could be obtained with sterilised cultures of this organism. Woodhead and Cartwright Wood studied the vaccinating action of these products on rabbits inoculated with virulent anthrax bacilli. The animals resisted completely or survived for some time. Analysing the phenomena produced under such conditions, these two authors came to the conclusion that the action of sterilised cultures of *Bacillus pyocyaneus* is 'indirect' and as 'taking place either by opposing itself to the action of the poison upon the tissues, or by stimulating certain tissues and increasing their functional activity.'

"In this action of foreign micro-organisms upon micro-organisms against which we wish to protect the animal we have to deal with something analogous to the condition we obtain when immunising with normal serums or with any other kind of fluid. In both cases immunity is rapidly established, but it is very transient and is confined to a stimulation of the phagocytic resistance" (2, p. 323).

The preceding is a clear description of nonspecific acquired immunity, based on experiments done in 1880–1890. These phenomena have been observed and investigated in more detail many times since then. Current studies in the same vein involve changes in resistance following administration of heterologous microbes, dead or alive, of bacterial endotoxin, etc. Effects of environmental factors (nutritional, emotional, climatological, and such) may well influence host resistance *via* similar mechanisms. Although many additional studies have been done, our understanding of the *modus operandi* of these nonspecific factors remains as vague as it was in 1890; they must alter ability of the host to handle either the microbes or the microbial toxins.

VII. CONCLUSION

A consideration of concepts of immunity to infection held by Metchnikoff has provided a means for appraising this field from the broad and long-term points of view. It is astonishing to find how much was known about mechanisms of host resistance to bacteria in 1900, and, in many regards, how little fundamental knowledge has been added in the twentieth century.

Bacterial infectious disease has gradually undergone a change from epidemic to endemic type. The era of the great plagues has largely past, but endemic infection continues to be a source of much illness and death. Potentially pathogenic microbes are widely spread among the population but in the vast majority of instances do not cause disease. This common state of infection without illness is converted into an infectious disease when host resistance mechanisms are upset, permitting invasive multiplication of the parasite. Thus the factor which determines whether or not disease occurs frequently is not contact with the parasite, but rather the state of host resistance. Impaired host resistance to bacterial infection may occur as a result of disease (diabetes, influenza, etc.), of therapy (use of adrenal steroids), from environmental factors (irradiation, malnutrition, stress), or in many instances for reasons unknown.

In order to deal with this problem effectively, additional knowledge is required, concerning over-all genetic and environmental factors which influence host resistance, and especially about the precise mechanical and biochemical mechanisms which operate to control infectious agents *in vivo*.

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