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THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

OPSONINS AND OTHER ANTIBODIES[1]

LOOKING back, we find that only fifty years ago the conception of the nature of the processes that run their courses in the animal body in infectious diseases, generally speaking, were hazy and still often even mystical. Preceded by occasional brilliant anticipations, notably by Bretonneau in regard to the specificness of infectious processes and by Henle with respect to the interaction between parasite and host, the tireless, unmeasurably fruitful investigation of the modern era, introduced by Pasteur and Koch, has brought to light not only the actual causes of many infectious diseases, but a great deal also in regard to the means and reactions in the infected body whereby they are overcome. Of these defensive processes phagocytosis and the action and formation of that remarkable group of bodies known as antibodies have received and still receive the greatest amount of attention. Indeed, the discovery of the wonderful power of the animal organism to respond to the effects of certain substances by the production of new antibodies must be reckoned as one of the great events, not only in medicine, but in general biology. We feel best acquainted with the antitoxins, the lysins, the agglutinins, the precipitins and the opsonins, but this does not exhaust the list, which is a growing one. The estab-

[1] Address of the vice-president and chairman of Section K—Physiology and Experimental Medicine, American Association for the Advancement of Science, Baltimore, 1908.

lishment of curative serum therapy by Behring when he demonstrated that antitoxic serum protects healthy animals against fatal doses of the corresponding toxin and may even cure the already sick, served to turn to the common good this innate faculty of the animal body to develop in so marvelous a manner its own resources.

Let it be noted once more that serum treatment, which has robbed diphtheria of its former terrors and the benefits of which are being extended to other diseases, is the direct outcome of scientific animal experimentation, and that without such experimentation it would not have been discovered and could not be maintained and extended.

The new methods and principles developed by the study of the animal reactions to infection did not long remain the exclusive property of the workshop of the bacteriologist. Ehrlich's side-chain theory of toxic and antitoxic action proved most heuristic, leading directly to fruitful investigation of other fields, and to-day biology, clinical and legal medicine, and physiological chemistry are using *immunological* methods to solve important problems.

According as stress was placed on the part of phagocytosis, on the one hand, and on the rôle of antibodies, antimicrobic as well as antitoxic, on the other hand, investigators until but recently were largely partisans of either the phagocytic or the humoral theory of healing and immunity. But the sharp antagonism between the adherents of these theories has subsided, because it has been made clear that neither mode of action is accomplished without the cooperation of cells and fluids. This is particularly true and easy of demonstration in the case of phagocytosis. Metchnikoff, the genial founder of the phagocytic theory, by broad comparative studies established the general occurrence and the significance in health and disease of phagocytosis in the higher as well as in the lower animals, and Denys and others have shown that the fluids of the blood play an essential part in the phagocytic process by so acting on microbes and other elements that they are made susceptible of phagocytic action. This property of the blood-fluid is now ascribed to definite substances, the opsonins of Wright and Douglas, and the tropins of Neufeld, both in all probability the same substances, and destined, I believe, to bear the name of opsonins, at least in the English language. While our acquaintance with the opsonins dates back only four or five years, they have been the subject of many researches, and much has been written about them, and it is to some more or less final results and certain general bearings of this work, fruit of the phagocytic theory as modified and perfected by the opsonic theory, that I wish to direct your attention.

It is generally accepted that phagocytosis of many bacteria—and also of red blood corpuscles, which are highly serviceable objects for the study of certain problems—is dependent upon substances—opsonins—which become attached to the bacterial cells or corpuscles, as the case may be, and so alter them that they are readily taken up by the leukocytes. The chief reasons for this conclusion are that leukocytes, carefully freed by repeated washing in salt solution, from the fluids in which they naturally exist, have but very little or no phagocytic power with respect to certain bacteria or corpuscles suspended in salt solution, while the same bacteria or corpuscles, after having been treated with suitable opsonic serum and then freed from the serum, are taken up by serum-free leukocytes. A few bacteria, however, *e. g.*, influenza bacilli, are readily phagocytable without the presence of opsonic serum.

Bacteria or corpuscles are not necessarily altered in opsonic serum and many bacteria, notably streptococci, pneumococci, anthrax bacilli, as well as others, grow freely in such serum. Heretofore the belief that phagocytes may cause destruction of bacteria rested largely upon more or less convincing morphological appearances. By means of the plate method for demonstrating bactericidal action it now has been shown conclusively that certain bacteria that do not suffer demonstrable injury by blood serum alone, such as those just mentioned, undergo intraphagocytic destruction when put into mixtures containing living leukocytes and opsonically active serum. In serum alone and in suspensions of serum-free leukocytes active growth occurs, but when the two are mixed destruction takes place, other factors being equal, in proportion to the number of leukocytes present. The actual demonstration of phagocytic annihilation of bacteria, formerly so often demanded by the opponents of the phagocytic theory, is here furnished.

The indications are that various opsonins with more or less well-marked specific affinities occur in all animals down to and including the echinoderms, being, like other antibodies, present to a variable extent in normal blood and other fluids.

In the course of his studies on lymph formation Professor Carlson[2] finds that opsonins and related bodies are more concentrated in the serum than in the lymph, that their concentration varies in the lymph from different organs, and that their apparent relative concentration in different lymphs also varies. The fact that the relative concentration is not the same in all lymphs speaks of course strongly in favor of the antibodies being distinct substances, a point concerning which there is still difference of opinion, some believing that it concerns different modes of action of the same body, others that each action is dependent upon a distinct body.

At first opsonins were regarded as substances of a relatively simple structure, quite easily destroyed by heat (60° C. for 15 to 30 minutes) and other agents. But it has been found that in most cases the total opsonic effect of fresh serum is the result of the combined action of two bodies, one relatively resistant to heat, the other easily destroyed by heat. The heat-resistant element is capable of opsonic action by itself and seems to unite quite firmly with the object upon which it acts; the opsonic effect as measured by the resulting phagocytosis is, however, greatly promoted on the addition of the other, thermolabile element, which alone has no opsonic power. In other words, opsonins, as a rule, seem to have the same duplex constitution as the lysins with which they are held by some to be identical.

The heat-resistant opsonic element appears to attach itself firmly to the bacterium or corpuscles upon which it acts because, in some instances at least, it is not detached even after many washings of the opsonified bacteria or corpuscle in large quantities of salt solution. Consequently opsonification is to be regarded as the special action of a distinct unit and not as the result of the influence of plasma or serum as a whole. The thermolabile, activating element, however, according to the results of recent experiments, probably remains free in the fluid of the phagocytic mixture, and there seems to me to be good room still for question as to whether its effect is exercised upon the phagocytable object or upon the phagocyte. Years ago Metchnikoff expressed the view that serum may stimulate leukocytes and other cells directly to phagocytosis, while, on the other hand, bacteria or red blood corpuscles that

[2] Personal communication.

take up what he and his followers then called "fixateur" thereby are made phagocytable. It is not impossible that further analysis of the mechanism of phagocytosis, under the guidance of the opsonic theory, will lead to this as the final result. At all events the failure to recognize the interaction of the two elements in the opsonic function of serum and the great difference in their combining properties is responsible for many of the divergent results of various investigators.

While normal blood contains only comparatively small amounts of heat-resistant opsonic substances, each unquestionably possessed of more or less well-marked specific affinities, the blood in conditions of acquired immunity may be richly charged with newly formed thermostable opsonic substances with marked specific affinity for the object against which the immunity is directed. Injections of suitable animals with bacteria or with alien red corpuscles cause specific opsonins to form; in human beings new opsonins arise as the result either of spontaneous infections or of the artificial introduction of killed bacteria and various bacterial products.

The opsonin content of the blood may be measured more or less accurately, either by means of the opsonic index or by determination of the highest dilution of the serum at which opsonic effect is still obtainable and comparing it with some normal standard. Speaking only in general terms, the opsonic index of Wright with respect to a given bacterium is obtained by comparing the number of bacteria taken up under the influence of the serum of the person or animal in question with the number taken up under the influence of the corresponding standard of normal serum under conditions that are as comparable as they possibly can be made.

By following the fluctuations of the opsonin content at frequent intervals important facts have been learned in regard to the laws of opsonin production. In the language of immunology any substance capable of giving rise to antibodies in suitable animals is called an antigen. Microbes and various microbic derivatives, cells, red corpuscles and serum may contain several antigens and incite the formation of more than one kind of antibody so far as indicated by the usual modes of antibody effect. Thus the proper single injection in a suitable animal of typhoid bacilli or of alien red cells is followed usually by the appearance in the blood of increased amounts of lysins (lytic amboceptors), agglutinins and opsonins for the particular cells injected. Usually all three of the bodies mentioned are not increased in the same proportions so far as determinable by our present methods of measurement, but they all commonly follow the same general course, which seems to hold good for antibodies in general: For the first day or two or three there is often, but apparently not always, a fall below normal in the amount of the specific antibodies in the serum; this period is called the negative phase and is succeeded by a steady rise above the normal, which, as a general rule, reaches its maximum about the eighth to twelfth day when there is a fall, the apex of the curve being sometimes quite sharp, at other times more rounded, and then begins a gradual return to the normal.

It is important to note that the fall below normal, the negative phase, is specific, that is, affects only the normal opsonin, and by inference the other antibodies, for the particular bacterium or corpuscles injected, a clear indication, it strikes me, that there are several normal antibodies, each with specific affinities and probably not different from the corresponding body formed when the machinery of immunization is set in motion. The cause of this interesting negative phase is not well understood, but it lies closely at hand to ascribe it to neutralization of the normal antibodies by the antigen, or to its effect on the antibody-forming cells. There is good reason to believe, especially on clinical grounds, that the general resistance to the specific infection is lowered in the negative phase, although certain experimental results indicate that the opposite may be the case.

Blood serum may contain antigens causing the production of antibodies for its homologous corpuscles; thus, the injection of antidiphtheric horse serum is followed by a wave-like rise and fall of the lysin, agglutinin and opsonin for horse corpuscles in the blood of the patient, the highest point being reached usually about the tenth day. Undoubtedly these antigenic substances are derived from disintegration of the corpuscles.

Serum and other protein mixtures also induce the formation of specific precipitating substances in suitable animals. Whether the specific precipitin test for protein material, now extensively used for the identification of blood and in the solution of allied problems, will prove of service also in the study of pure proteins, remains to be seen.

In several acute infectious diseases the course of the formation of new opsonin for the infecting agent, in the typical attack, terminating promptly in recovery without complications, shows a marked general resemblance to the opsonin or antibody curve after a single antigen injection in the normal animal. It also bears definite and constant relations to the clinical phenomena. During the early stages when the symptoms are pronounced there is a negative phase, and then as the symptoms begin to subside the opsonin curve rises above normal, reaching the highest point several days after the onset, followed by a gradual subsidence. This is true of the pneumococcus opsonin in pneumonia, of the opsonin for the diphtheria bacillus in diphtheria, of the streptococcus opsonin in erysipelas, and also of the opsonin for the diplococcus of mumps in that disease. The curve is typical as well for the streptococcus in scarlet fever, indicating clearly that this organism unquestionably plays a definite rôle in scarlet fever whatever its actual causative relation to the disease may be. In pneumonia the greatest rise in the leukocytosis appears to precede somewhat the highest rise of the opsonin. In all these diseases the typical wave-like opsonin curve is modified by the development of complications of various kinds and at the onset of which it commonly undergoes a distinct depression. In rapidly fatal cases, for instance of pneumonia, the opsonic curve or index may not return from the primary depression but sink lower and lower. In prolonged infections, general as well as local, there occur irregular fluctuations and in chronic, more or less stationary cases, the opsonic index is often subnormal. At this time further details can not be given. My chief point is to make clear the close association between recovery and the wave-like rise of the opsonin and, as a result of the immunization, in all likelihood also of other antibodies in the typical attack of acute so-called self-limited infections. In some of the diseases the opsonin is the only antibody that we can measure readily with our present means. As I have stated, intraphagocytic destruction of pneumococci and streptococci takes place in the presence of fresh leukocytes and opsonic serum, whereas either alone constitutes a good medium for these bacteria. Taking these facts into account, it seems to me that the wave-like course of the opsonin in pneumonia and in acute streptococcus infections is a strong point on the side of the importance of phagocytosis in their healing, whatever other measures, of which at present we know less or nothing, may be in operation also.

Whether the opsonic action of serum is caused by distinct and independent substances or by antibodies with other actions as well, has been an interesting question concerning which there is still difference of opinion. The question now seems to be narrowed down to whether the opsonins and lysins are the same, some claiming that opsonification merely is the result of an early stage of lysis before actual solution takes place. Opsonins would appear to be distinct from other antibodies because a given serum may be opsonic, but not lytic, while the reverse probably also occurs. But here certain difficulties arise. While it is well established that serum may be strongly opsonic without being lytic and without even containing lytic amboceptor so far as our present methods indicate, the suggestion is made that in such cases the failure to obtain lysis may be owing to the state of the object tested and not to the absence of lysins. This consideration applies with most effect to instances in which we know the bacterium or corpuscle is susceptible both to lysis and to opsonification, and in which lysis might not take place either because the serum was not active enough or because of some special resistance to lysis. The explanation falls short, however, when applied to bacteria like pneumococci and streptococci, which, while readily opsonified, are yet insusceptible to lysis. In this case the claim that lysis does not take place because of the physical state of the bacteria is merely an assumption.

If opsonification and lysis depend upon the same body the opsonic and lytic powers of the serum of an animal in the course of immunization should always run parallel. If they do so that fact does not of itself prove that it concerns one body, but failure to run parallel would indicate the existence of separate bodies with different functions. Actual observations show that in certain animals single injections of alien red corpuscles may increase the opsonic power of the serum for that corpuscle a hundred times or more above normal, while the lytic power for the same corpuscle may be increased comparatively much less and in some conditions not at all. On this account, then, as well as for other reasons, the view that opsonins, meaning thereby the thermostable opsonic substances, constitute a distinct class of antibodies, seems to me to be correct.

That the activating or complementing opsonic substance is closely related to the complement of lysis is indicated by a number of considerations: Both are sensitive to the action of heat, being destroyed by an exposure of thirty minutes to 58–60° C.; both appear to be split up into two distinct components by water, and both are neutralized by a number of ionizable salts. As stated before, the opsonic complement, however, seems to remain free in the phagocytic mixtures, whereas the complement of lysis is regarded generally as bound by the amboceptor.

We come now to a most interesting part of our subject, namely, the resistance offered by microbes under different conditions to antibodies and more particularly to opsonins.

Since the discovery of the chronic microbe carrier the adaptation of microbes to the defensive mechanisms of the animal body is no longer merely of academic interest. Under the conception that phagocytosis and bacteriolysis form the basis of healing and immunity in perhaps most of the infectious diseases, the infecting microbes should disappear at the time of recovery. This is probably the general rule, but there are many striking exceptions illustrated well by the now familiar "*bacillus carrier.*" The body may overcome the disease but not the cause, which may persist in spite of the increase in antibodies. The disease subsides, the disturbances are smoothed away, and yet the germ lives on in the host, apparently harmless and unharmed, sometimes for remarkably long periods. But the equilibrium is not always a stable one; the immunity of the host may give way and recurrence develop; or the resistance of the germ may weaken and eventually complete destruction and final elimination take place.

Germs isolated from typhoid and cholera carriers have been found in some cases to offer special resistance to antibodies, including opsonins, but the mechanisms of this mutual immunization of microbe and host are still obscure, and on account of the self-evident and tremendous importance of the carrier in spreading disease they invite special study.

At this point I may recall that the relapses in relapsing and related fevers are now ascribed to the survival in each attack of a few spirilla which, having become immune to the antibodies of the host, give origin to new "serum-fast" strains that continue the relapses.

Exceedingly interesting conditions are found in certain chronic infections of the urinary tract with bacilli of the colon group, the indications being that the infecting bacillus may partially immunize itself, in one case to the lysin, in another to the opsonin, in the patient's blood, or that the amounts of different antibodies vary greatly in the different cases.

In Metchnikoff's original doctrine of phagocytosis in infectious diseases a fundamental tenet reads that as a microbe grows in virulence its resistance to phagocytosis increases. Recent experiments give results in complete harmony with this teaching. On analysis the resistance of certain highly virulent bacteria to phagocytosis is found to depend on insusceptibility to opsonic action, owing apparently to lack of affinity for the opsonin. As pneumococci, streptococci and other bacteria on successive passages through suitable animals become more and more virulent for these animals, they at the same time acquire a parallel increase in resistance to phagocytosis. When cultivated outside the body reversion readily takes place to less virulent states, associated with a returning affinity for opsonin and an increasing susceptibility to opsonic action. Investigating this property of pneumococci to develop such strong defense against phagocytosis, Rosenow found that extraction or autolysis of virulent pneumococci brings into solution a substance or group of substances that neutralize the pneumococco-opsonin in human serum, but not other opsonins. After extraction of this substance, which is thermostable and insoluble in alcohol or ether, virulent pneumococci unite with opsonin and become phagocytable, while avirulent pneumococci on treatment with extracts of virulent strains not only become resistant to phagocytosis in the test-tube, but also to some degree virulent for animals.

Entirely independently, Tchistovitch and Yourevitch appear to have reached identical results on all points, except that they did not study the virulence of avirulent pneumococci after treatment with extracts of virulent strains.

We may say then that the properties called *virulence* in pneumococci appear to depend, to a very large extent, if not wholly, on the formation of an actual substance—"virulin"[3]—which may be extracted and studied by itself. It is hoped that this demonstration may prove a basis

[3] "Antiphagin"—Tchistovitch and Yourevitch.

of departure for new and fruitful work in pneumococcus and similar infections.

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THE FIRST ANNUAL CONFERENCE OF THE GOVERNORS IN NEW ENGLAND

In these days of conventions and association gatherings, it is difficult to select one on the grounds of special importance or significance. Among those which I have had the privilege of attending in recent years, the conference held in Boston, November 23 and 24, easily surpassed all others of material import when measured by the importance of the subjects considered and the vast possibility of bettering existing conditions. This was the first annual conference of the Governors of the New England states, called for the purpose of considering certain natural industries and utilities common to all, and legislation affecting them.

The conference was presided over by Governor Curtis Guild, Jr., of Massachusetts, and attended by every governor in office and governor-elect with one exception, namely, Governor Higgins, of Connecticut, "who had married a wife and therefore could not come."

Subjects Considered.—There were three sessions, and each session was devoted to the consideration of one subject, or a correlated group of subjects. The first session was devoted to tree planting interests, and this was divided into two parts: (1) Forest trees and (2) orchard trees.

The forest-tree side was discussed by Mr. Gifford Pinchot, United States forester, who showed impressively how rapidly the forest supplies of the country were decreasing; how vast areas of lands in New England, of little or no value for farming, might be utilized; the profitableness of forests as a commercial enterprise; and then finally urged the passage of uniform legislation in New England providing adequate protection of forest lands against fires. This subject aroused a lively discussion, and drew attention to the reasonable opportunities for safe investment of capital.

Orchard Trees.—The planting of apple trees on the hilly lands in New England, not in the valley farming lands, was urged by the professor of horticulture of Cornell University. He did this on the ground that New England was the natural home of the apple in the United States, for it was here that the leading commercial varieties of to-day originated; that the land was cheap, that labor was abundant, and markets both foreign and domestic convenient. Moreover, the quality of the New England apple was unsurpassed by that produced in any other section. The demand was keen, and for fruit of fine quality New England markets were the best in the country.

What was needed to improve the situation was reorganization of ideas and practises in relation to orcharding. The slipshod methods of generations and the opening of new irrigated fruit lands in the west discouraged on the one hand the would-be planter, and on the other attracted his attention to the opportunities in distant lands. What in his opinion was now needed was the redirection of capital to apple-growing as a staple and safe industry. We should have illustration orchards, planted and conducted either by men of faith in the business with sufficient capital to back up the enterprise, or by the state governments themselves. Such work should be conducted with the energy characterizing western enterprises, and guided by intelligence and up-to-date methods. Certain legislation was required in order to secure a uniform grade, uniform methods of packing, and certain standard packages.

The second session of the conference was devoted to a consideration of the fast-disappearing lobster and the much-preyed-upon mollusks.

These subjects were discussed by experts and aroused much interest. They proved their contention that these staple sea foods were in a fair way to be exterminated within a measurable length of time, and that without intelligent protection a great natural re-