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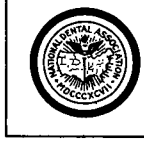

PHAGOCYTOSIS, AND ITS PRACTICAL APPLICATION IN DENTISTRY.

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AS EARLY as 1870 certain biologists noticed the presence of microorganisms within the cells of both animal and human tissues. A little later, several observers noticed that some leucocytes contained bacteria, but did not interpret it as a means of protection, but rather as a means of transporting the bacteria thru the infected body.

It remained for Metchnikoff in 1883 to give to the world the result of his observations, in which he claimed that the leucocytes acted as protectors of the animal organism, that they ingested, and digested bacteria which had invaded the animal economy, acting as phagocytes or scavengers. He compared this process of cell ingestion, or phagocytosis of foreign particles, to that taking place in the protozoa. In the Amoeba for instance, the simple jelly like mass of protoplasm moves by means of the pseudopodia toward its food, engulfs it, digests it, and moves on, leaving an indigestible portion. In Sponges, and in the Coelenterates, individual cells still perform the function of taking up food particles and digesting them, but as we come up in the scale of life, we find that cells become more specialized, and the digestion of food takes place in the cavity of the stomach and intestine, with the help of various digestive juices which are poured upon it. While certain specialized cells take care of the nutritive function, other cells develop the function of protectors of the animal from invasion by microorganisms which will produce disease unless overcome. It is to these cells that Metchnikoff has given the name of phagocytes.

From what has been said of the Amoeba, Sponges and Coelenterates, you can readily see that the phagocytic cells of man are descendants of cells whose normal function was to digest *intra-cellularly,* and thru them we still possess this method of digestion side by side with the *extra cellular* digestion which occurs in our stomach and intestine.

At the time when Metchnikoff began his study of this subject, pathologists held very complicated views of inflammation, which involved complex coordinated reactions of vascular and nervous systems, and his primary purpose

was to observe reaction to irritations on some animal of simple form that was devoid of specialized vascular and nervous apparatus, so he began his observations on a small fresh water crustacean, the Daphnea.

In this small animal he studied the reaction which took place upon the introduction of yeast cells. He found that a struggle took place between the ameboid leucocytes of the crustacean, and the infecting agents, and determined that complete enclosure of the yeast within the leucocytes assured protection to the animal, while a failure to do so either from fortuitous causes, or because of too large a quantity of the infecting agents, resulted in disease and rapid death.

Since this beginning, a tremendous amount of experimentation and investigation have taken place. The phagocytic theory was attacked by a number of noted pathologists, and this stimulated greater research,—on the one side in defense of the theory, and on the other to disprove it. While much still remains to be discovered as to the manner in which both humans and animals resist or overcome disease, yet enough has been proven to give us a good working knowledge to build upon, and the phagocytic theory is now generally accepted, altho some still maintain that various antibodies in the blood play the principle role, and that the phagocytes digest only dead bacteria. In this paper we cannot take up the claims of those who present opposing theories but rather adopt the view which Zinsser takes when he says "It seems reasonable to classify both the phagocytic action of body cells, and the formation of antibodies, in the blood plasma, primarily as emergency devices for the digestion of foreign materials, both formed and unformed, which, under abnormal conditions, penetrate into the physiological interior of the body (blood stream or tissue spaces,) and must be disposed of." (1)

Phagocytosis is not confined to the white blood corpuscles. The leucocytes of the circulating blood are known as motile phagocytes, while others are known as fixed phagocytes. These fixed phagocytes consist of certain connective tissue cells, endothelial cells, splenic pulp cells, certain cellular elements of the lymph nodes, neuroglia tissue, and all phagocytic cells which are ordinarily confined to some definite localization in the body.

Metchnikoff further classifies these phagocytic cells into *microphages* and *macrophages*. The *microphages* are the polymorphonuclear-leucocytes of the circulating blood, while the *macrophages* include the fixed cells, and the large mononuclear elements of the blood,—in fact all phagocytic cells except the microphages.

"While no *absolute* functional differentiation is possible between the two, it is true, in a general way, that the microphages are concerned primarily with the phagocytosis of bacteria, and especially of those which invade acutely, while the microphages are concerned especially with the resorption of cellular detritus, foreign bodies, and such bacteria as are more chronic in their activities or are peculiarly insoluble. On the other hand, microphages *may* take up foreign particles and bacteria of *all* kinds under suitable conditions." (1)

The small lymphocytes, and most-cells of the circulating blood are considered non-phagocytic, or possessing phagocytic, powers under exceptional circumstances only, but this does not mean that they may not play their part in defense of the body, for it has been noted that especially in tuberculosis, they generally form the majority of the cellular elements that accumulate at the site of the lesion.

Now let us see what takes place when an emulsion of pyogenic staphylococci is injected into an animal subcutaneously, for by noting the changes that take place

we can readily grasp the importance of the leucocytes, and see that they are the chief elements in overcoming an infection.

Very soon after the injection, the site of puncture will become reddened and swollen, and microscopic examination will, within a few hours, show a swelling and engorgement of the blood vessels.

"The injected cocci will be found to lie partly scattered in the tissue spaces, in part within polynuclear leucocytes and connective tissue cells, which have begun to digest them. The tissue spaces will be swollen and stretched by the exudation of blood serum from the vessels. This condition will begin in from 4 to 6 hours after injection, and increase during the next 24 hours in extent and severity according to the quantity and virulence of the cocci injected.

After from 12 to 48 hours there will be seen the results of an active and destructive struggle between the invading bacteria and the defending cells. In the center of the area of invasion, tissue has been destroyed and disintegrated. Amid the necrotic detritus, closely packed, lie leucocytes and cocci, and active phagocytosis has taken place. In some cases the intracellular bacteria appear swollen and disintegrating, in others, the leucocyte itself overcome by the larger number of bacteria it has taken in, becomes vacuolated, indefinite in outline, and apparently is being itself destroyed. The presence of blood serum, which is aiding in the destruction of bacteria, both by its bactericidal powers and its re-enforcement of the phagocytic process, renders this mass fluid or semi-fluid, and the whole mixture constitutes what is known as pus. Around the periphrey, cocci and leucocytes becomes more scattered and sparse, and bacteria, together with leucocytes loaded with cocci, may be seen lying within large mononuclear cells (macrophages.) Whether the process goes on to further extension, or is eventually walled off into a distinct abscess by the formation of granulation tissue and new connective tissue, depends upon the balance of forces between attacking agent and defensive factors.

If we inject a similar emulsion of bacteria into the pleural or peritoneal cavity of an animal, a process similar to that just described will be observed.

Normally the peritoneum contains a small amount of serous fluid and a moderate number of white blood cells, chiefly lymphocytes. When a bacterial emulsion is injected into the peritoneal cavity there follows a brief period, during which there is a diminution of the free cellular elements in the peritoneal fluid. At this time there is a clumping of the cells in the folds of the omentum and mesentery, a transient stage of flight away from the point of injury. This, however, is soon over. Within one to two hours, an active immigration of leucocytes into the serious cavity occurs, and if, during the next 12 to 24 hours small quantities of fluid are, from time to time, withdrawn with a capillary pipette, a rapid and constant increase of leucocytic elements, chiefly of the microphage or polynuclear type, is observed.

If the injected emulsion of bacteria has been small in quantity and moderate in virulence a rapid phagocytosis gradually rids the fluid of micro-organisms and within 24 hours after injection, few, if any, free bacteria are visible.

A little exudate taken at this time shows large numbers of microphages, varyingly crowded with well preserved and disintegrating bacteria. Some of the phagocytes having literally taken up more than they can digest, are vacuolated and disintegrating, but in general, the victory lies with the cells. A little later large mononuclear elements appear, and here and there will be seen to take up dead leucocytes together with injected cocci. In this way gradually a cleaning out of the peritoneum takes place,

the animal recovers and the peritoneum returns to normal.

If on the other hand the bacteria injected are in larger doses and of greater virulence, we shall find that after a period of active phagocytosis, there may be a gradual increase of bacteria over leucocytes. The phagocytic cells are found to be undergoing degeneration in larger numbers, the free bacteria increase and the impending death of the animal can often be foretold by the appearance of the exudate. Finally the peritoneal fluid may consist chiefly of free and rapidly mutilating bacteria, with a practical absence of phagocytic sells." (1)

The part played by these phagocytic cells in resisting invasion and defending the body, consists of two phases—First, the active motion of the cells toward the point menaced—Second, the act of ingestion of the invading organism.

It is only natural for us to ask what attracts the phagocytes to the point where they are needed? For they invariably turn towards it just as the branch of a plant turns toward the light or its roots toward the ground.

The action of these "leucocytes toward the invading substance indicates a sensibility on the part of the cell to changes in its environment incited by the foreign agents, and since the stimuli most likely to reach the leucocytes and bring about this alteration in the direction of their movements are chemical in nature, the phenomenon is spoken of as chemotaxis." (1)

If the changes in the direction of a moving cell is towards the foreign object, thus showing its attraction, it is said to exert a *positive chemotactic action.* If on the other hand it repels, it is said to be *negative chemotactic.*

This property of chemotaxis is of vital interest to us, because any agent which repels the approach of the phagocytes must be detrimental, while any factor which attracts them will act as a powerful means of defense.

Leber in his work on "Inflammation" found that powdered copper and mercury, actively attracted leucocytes and that dead bacteria exerted a similar positive chemotactic influence, while Buchner succeeded in extracting substances from various bacteria which possessed similar properties.

It appears from these, and other investigations, that the power of stimulating positive chemotaxis is a general property of bacterial proteins, equally evident in bacterial extracts, dead bacteria, or the living organisms. It is likely, therefore, that the attraction of leucocytes toward the point of bacterial invasion is, in part at least, due to the properties of the bacterial proteins themselves. That this, however, is not the whole story is evident from the work of Massart and Bordet, who showed that the products of cell destruction and disintegration possess similar positively chemotactic properties. This is true not only of the products of disintegrated tissue cells, but of those of the destroyed leucocytes themselves. Thus it appears that when any injury of tissue occurs, a stimulus which attracts leucocytes takes place, even when the injury is not accompanied by bacterial invasion. This would explain the participation of leucocytes in reactions to injury and in inflammations not of bacterial origin, and thru local accumulation following the injection of insoluble inorganic substances.

When bacteria are actually present, however, the added stimulus due to the diffusion of bacterial proteins probably increases the process to a degree often sufficient to meet the added requirements for protection. Following this, both the destruction of tissues, of bacteria, and of leucocytes, may together exert a cumulative chemotactic power which continues the process proportionately with the extent of the lesion. (1)

But we must not go away with the idea that bacteria attract their enemies

and have no means of protecting themselves, for observations made in some diseases "point strongly to the existence of substances of negative chemotactic influence which protect the bacteria, not merely from phagocytosis, but from that necessary forerunner of phagocytosis, the approach of the leucocyte itself." (1)

In order to make this information of practical benefit in our every day practice, we must, in treating any given case, try to determine what the condition is which now exists, and then determine what we can do to promote active phagocytosis. This must be our guide in the selection of any remedies used. I have sought for records of experiments tried upon animals in order to determine the chemotactic influence of various anticeptics upon phagocytosis, but have had little success. I find a record of work done in the Pasteur Institute by Massart and Bordet in which capillary tubes containing various substances were inserted under the skin of the frog. Two medicaments used in dentistry were among those tested, they were lactic acid, and glycerine, but both these substances repelled the leucocytes. (2)

The result of researches by Hamburger of the University of Gruningen, Holland, published this year, showed that iodoform in small quantities mixed with a 0.9% common salt solution increased phagocytosis. The method used in the investigation was this—white corpuscles from the blood of a horse are transferred to various media mixed with very small particles of carbon. After having stood some thirty minutes in an incubator at body temperature, a great many of the cells have eaten up carbon. Microscopical preparations are made and it is ascertained what percentage of the leucocytes have taken up carbon. The percentage is the measure of the degree of phagocytosis, and gives the value of the influence the various agents used, on that function of life.

It was found that when the leucocytes were placed in a 0.9% Na. Cl. solution that 43.8% of the leucocytes had taken up carbon. When iodoform was added in the proportion of 1 to 100,000 the percentage was raised to 58.2%. When added in the proportion of 1 to 500,000 the percentage was 61.4%. When added in smaller quantities the percentage began to drop again, but even when there was only 1 part of iodoform to 5,000,000 of Na. Cl. solution the percentage was still above that of the Na. Cl. solution alone. Therefore there seems to be no doubt but that iodoform in dilute solution promotes phagocytosis, but the question arises: How can this favorable effect be explained? The investigators say that it is not due to the iodine content, as they have found that iodine is noxious in a high degree. They suggest the following interpretation —that the outer layer of the cells consists of a fatty substance, a so-called lipoid surface. Iodoform is soluble in fat and it is obvious that such a surface will grow more soft and flexible after having absorbed iodoform, and consequently the plasticity and mobility will be facilitated. They say that if this theory is correct, then other substances soluble in fat, such as chloroform, alcohol, camphor and benzene should show the same results. Their experiments show that they do,—chloroform giving the best results when added to the salt solution in the proportion of 1 to 100,000. This warning is added, that these substances, which, when applied in very small doses show a stimulating effect, paralyze when given in greater quantities. (3)

The results obtained from these investigations, prove to us that if we wish to get the best results from our treatment of diseased conditions we must study to apply those remedies which will aid in promoting phagocytosis instead of hindering it. Evidently strong antiseptics have no place in accomplishing this end.

Let us see how surgeons are meeting

this problem. From ten to twenty years ago strong antiseptics were used to wash the part about to be operated upon, scrubbing with green soap, washing with alcohol, packing with a bichloride of mercury pack, sometimes the parts were washed with bichloride of mercury solution 1 to 1000 or in some cases even 1 to 500. I read recently in a text book on surgery published in 1891, where the author stated that he filled the abdominal cavity with a bichloride of mercury solution 1 to 18,000 removing it and refilling three times in succession. (4)

Do surgeons use these methods now? No. They have proven that it is easy to be far too liberal in the use of antiseptics, especially the stronger ones, and thus hinder phagocytosis rather than aid it.

In a report recently printed in the British Medical Journal by a consulting surgeon who is serving in France near the front, he says that he is compelled to acknowledge the inefficiency of antiseptics when used as preventive or disinfecting agents in badly infected, lacerated wounds, no matter what kind or strength of antiseptics used, but that the resisting agencies of a patient's own body are far more effective in dealing with a local infection than any antiseptic solution, powder, or paste introduced into it from without. That organisms, which have invaded the tissues are not killed by the antiseptic as such, unless the antiseptic is sufficiently strong to destroy the tissue which they have invaded, but all successful treatment of infected wounds depends on calling forth the physiological forces, the protective elements of the body whether fluid, or cellular, or both.

In place of antiseptics, they are irrigating the wounds with either isotonic or hypertonic salt solution, but these solutions must be brought into contact with every infected part of the wound.

The hypertonic solutions are said to stimulate a flow of lymph containing antibodies, thus bringing about what is known as lymph lavage, while the isotonic or physiological salt solutions, stimulate diapedesis, bringing a concentration of leucocytes into these tissues.

Should the infection be a streptococcus infection, the *isotonic* salt solution must be used, because the streptococcus thrives in lymph, but finds the phagocyte a deadly enemy. If it is a multiple infection, the hypertonic salt solution is used.

The hypertonic salt solution is a 5% solution of Na. Cl. and sometimes 0.5% of Sodium Citrate is added. Iodin is used on skin outside of wound. This is the method giving the best results, so he states. (5)

In view of these facts does it not appear to you that many in our profession are using antiseptics far too strong, and trusting to them rather than to nature's methods of resisting and overcoming infection?

In the treatment of antral infection where it is possible to wash thru from mouth to nostril, this should be the ideal place to use a salt solution and thus promote healing in nature's way.

After the extraction of teeth the salt solution is of great aid and I consider it far more preferable to any of the antiseptic mouth washes that are on the market.

In the treatment of pyorrhea it is high time for us to abandon lactic acid, trichloracetic acid, ammonium fluoride, etc.; which have been used to dissolve any calculus that had been overlooked upon the root. It may be that this result was achieved in some instances, but at what cost? The destruction of considerable soft tissue, and the etching action of the acid upon the cementum which must certainly destroy the life of the cementum superficially if any life still remained in it, and thus prevent healing. If it is absolutely necessary to irrigate these pockets after scaling the teeth, (and some hold that is is detrimental,)

it is only common sense to avoid the use of remedies which destroy animal tissue cells as well as the bacteria, and use the normal salt solution. I believe you will achieve better results in shorter time.

When it comes to treating alveolar abscesses we have a problem on our hands as to the best method of applying the knowledge we have on this subject.

We have the hard tissue of the tooth itself to disinfect, and here the phagocytes cannot help us. We have the tissue in the periapical space also infected. Here the phagocytes must play an important role. How shall we secure the disinfection of the tooth and yet do no damage to the tissues at the apex?

I wish that I could bring you a record of experiments tried, to determine the chemotactic influence upon phagocytes, of the various antiseptics which we use, but I cannot. I am inclined to think that all antiseptics will be found to possess negative chemotactic powers, but I cannot say that that has been proven yet.

We have tho, the experiments conducted by Drs. Black and Peck, some years ago, as to the effect of various antiseptics on the skin of the body. These experiments were repeated more recently, and were reported to us in Jan., 1916, by Dr. Arthur Black.

In these experiments it was proven that the formo-cresol solution took the lead in irritating effects and in causing death of the tissue, while Oil of Cloves showed the least amount of irritation or discomfort. Beachwood Creasote ranked next to Oil of Cloves in being non-irritating.

In view of these facts it is only reasonable to abandon whatever causes marked destruction of tissue, and in use that which is least irritating, provided we can destroy the bacteria in the tooth by that method.

Let us turn to the report of some other experiments conducted by Drs. Black and Peck in 1898 testing the potency of various antiseptics in preventing the growth of organisms obtained from the saliva of members of the class of 1898, and infecting broth, which was kept in the incubator and tested for three days. It was found that Formalin prevented growth when present in the strength of 1 part to 1400 parts of the broth. Beachwood Creasote was effective in 1 part to 1280, Oil of Cloves 1 part to 1150, Oil of Peppermint 1 part to 875. Black's 1-2-3 mixture 1 part to 454 2-7 parts, Phenol 1 part to 338 8-9 parts, Eucalyptol 1 part to 116 2-3 parts, Bichloride of Mercury was effective in 1 part to 36 2-3 parts of the broth.

These experiments show that Oil of Cloves being effective in a strength of 1 to 1150 should be sufficiently strong to destroy bacteria without doing very serious damage to the soft tissues surrounding the apex of the root. The fact that this remedy placed upon an irritated and exposed pulp, will soothe the pain, proves its non-irritating qualities. Oil of Peppermint which is only slightly irritating, and is effective in a strength of 1 to 875 can be used in the anterior teeth where there is danger of staining from the Oil of Cloves. Eucalyptol is less effective, requiring a strength of 1 to 116 2-3, but this should be sufficiently strong to destroy the bacteria present.

We may use strong antiseptics in the pulp chamber to disinfect putrid contents of a root canal, but one thing that we must remember in using these strong antiseptics in a tooth for disinfecting purposes is, that while we may not do any harm to the dentin by their use, yet we have a layer of cementum at the apex of the root, that varies in thickness from a mere line in a young person, to as much as a millimeter in thickness in elderly people. This layer of cementum may retain its vitality, or it may not. If vital, we should endeavor to conserve its vitality by not allowing agents to come in contact with it that we are sure will destroy it.

If now we have disinfected the hard

structure of the tooth, we must look to the phagocytes to clear up the infection in the soft tissues.

The amount of dead tissue in the apical region should have a positive chemotactic influence on the phagocytes and promote healing.

If the abscess has assumed the chronic form it will be found that the area is walled off and surrounded to a great extent by the lymphocytes and that very few microphages are present, so in this condition it is wise to destroy a small amount of tissue in order to promote an active phagocytosis. If the fistulous tract is thoroly irrigated by a normal salt solution in order to remove all detritus and pus, and then followed by a small amount of phenol carefully injected, and stopped as soon as it appears at the fistula, and the tooth sealed, this should destroy enough tissue to start phagocytosis.

I have secured excellent results by applying this treatment, letting the tooth rest about four days to a week, and then repeating it, then dry out root canal, and fill. What the final results of this method of treating chronic abscess will be, only time and the X-ray can tell, but I feel that I am proceeding along logical and scientific lines.

The treatment of blind alveolar abscess will call for some agent that is slightly irritating in order to break up its chronic tendency and promote active phagocytosis.

I have not been able to give you as definite information how to proceed in each specific instance that you will have to contend with, as I could wish to, for the data is not yet at hand, but I have tried to present in a general way how the forces of nature work to resist and overcome infection, and thus direct your thoughts and endeavors to work intelligently with those forces. You may be able to solve some of the problems that are still unsolved.

BIBLIOGRAPHY.

(1) Infection and Resistance—by Dr. Hans Zinsser.

(2) Bacteria and Toxins—by Bordet.

(3) Researches of Phagocytosis—by H. J. Hamburger. British Medical Journal, Jan. 8th, 1916.

(4) Text Book on Surgery—Wyeth 1891.

(5) General Treatment of Infected Gunshot Wounds from a Clinical point of View—by Col. H. M. W. Gray. British Medical Journal, Jan. 1st, 1916.