section devoted to a review of the general properties of leucocytes, stands pre-eminently as a free-granule producer.

That the cell in migrating from the vessels and passing through connective-tissue interstices has for its purpose to reach the myelin-spaces of nerves is clearly suggested by the manner in which the lymphatic spaces are arranged even in the finer ramifications. "In its course Henle's sheath is not applied against the nerve-tube," writes Berdal[98]; "there is between it and the nerve-tube a space occupied by lymph-plasma which has for its purpose to supply the cylinder-axis with its nutrition." If this statement is interpreted from the standpoint of my views, it is more than nutrition, but myelin-granules, which insinuate themselves—through chemical affinity, doubtless—wherever there is need for them: *i.e.*, wherever their consumption has been greatest. "Medullated nerve-fibers, when examined, frequently present a beaded or varicose appearance," say Pick and Howden[99]; "this is due to manipulation and pressure causing the *oily* matter to collect into drops, and in consequence of the extreme delicacy of the primitive sheath even slight pressure will cause the transudation of fatty matter, which collects in drops of *oil* outside the membrane." Evidently we are not dealing with a fixed mass, but with one made up of extremely mobile particles, which to me, at least, represent as many basophile granules. If the space between Henle's sheath contains lymph supplied with myelin-granules, what is the difference between a nerve thus supplied with its primary source of energy and a "medullated" nerve? None, in my opinion. *Such a nerve as a non-medullated nerve does not exist,* therefore, since a nerve deprived of myelin, if interpreted from my viewpoint, would become a mere plasma-channel.

The pathway to all nerves becomes greatly simplified down to their terminal ramifications, it seems to me, in the presence of Gulland's observation concerning the passage of a basophile leucocyte "through a narrow hole between two bundles of connective tissue." Indeed, "the lymphatic vessels do not exist as distinct channels in the interfascicular connective tissue," says Berdal. "There is no lymphatic vessel in the thickness of the

[98] Berdal: *Loc. cit.*, p. 152.
[99] Pick and Howden: *Loc. cit.*, p. 1117.

nervous bundles nor in the sheath surrounding them (Ranvier). The circulation of the lymph in the interior of the bundles is insured by the arrangement of the interfascicular connective tissue, the meshes of which represent lymphatic cavities communicating with the vessels of the interfascicular tissue through *holes* in the lamellar sheaths." On the whole, therefore, it seems to me permissible to conclude that:—

1. The physiological function of the basophile leucocyte is to convert fats derived from the intestinal foodstuffs into myelin-granules, and to distribute the latter to all parts of the nervous system, including the brain.

2. The basophile leucocytes thus supply the entire nervous system with the lecithin-containing compound which combines with the adrenoxidase of the blood-plasma of axis-cylinders, neuroglia fibrils, etc., in the production of nervous energy.

The different varieties of leucocytes reviewed so far represent, it seems to me, the only three *adult* functional types, the lymphocytes and hyalines being, as stated, immature cells. This does not mean, however, that the latter are functionless; indeed, we have seen that when an active process is initiated these younger cells rapidly increase in the blood and intestinal tract, to replace the large number of their elders that have disappeared to take part in this process. Their development must be extremely rapid, therefore, and their number commensurate with the number of adult leucocytes brought into action, whether this be to distribute (1) the neutrophilic peptones, myosinogen- and fibrinogen- granules, (2) the eosinophilic hæmoglobin granules or (3) the basophilic myelin-granules.

THE FUNCTIONS OF THE LEUCOCYTES IN IMMUNITY.

As the study of the functions of these cells is continued and amplified in the second volume, I will merely incorporate in this section a few brief facts based on the data submitted in the foregoing pages and conclusions based on a study of the subject, which study cannot for want of space be reproduced here.

Metchnikoff terms "phagocyte" any cell deprived of a cellular membrane and capable of incorporating bacteria and other

substances, and of disintegrating them. In the blood, certain leucocytes, particularly the mobile or wandering neutrophilic or polymorphonuclear forms (the "microphages"), the fixed

EXPLANATION OF PLATE.—Fig. 1.—Pfeiffer's phenomenon occurring in the exudation taken from an untouched guinea-pig, the exudation having been withdrawn ten minutes after the injection of 1 cubic centimeter of bouillon containing one loopful of a culture of Constantinople cholera and 0.04 cubic centimeter of preventive serum (of the strength of 1/5 milligramme). Staining with methylene-blue. *l*, Lymphocytes. Ocular 3. 1/18 Zeiss.

Fig. 2.—Mass of granules placed around a collection of leucocytes. Exudation of a guinea-pig withdrawn nine minutes after the injection of 1 cubic centimeter of bouillon to which had been added a third of a culture of Oriental-Prussia cholera and 0.04 cubic centimeter of preventive cholera serum of goat (strength, 0.0002). Ocular 2. D. Zeiss.

Fig. 3.—Granular leucocyte surrounded by a zone of vibrionic granules. The exudation was withdrawn twenty-five minutes after the peritoneal injection of one-tenth of an agar-agar culture of Massowah vibrio. Ocular 2. 1/18 Zeiss.

Fig. 4.—Two mononuclear leucocytes surrounded by granules; a lymphocyte (*l*) and a red blood-corpuscle (*h*) from the same exudation. Same power.

Fig. 5.—The same cells after remaining for three and one-half hours at 26°.

Fig. 6.—Five polynuclear leucocytes from the exudation withdrawn four minutes after the injection into the peritoneum of a guinea-pig (highly vaccinated and prepared with 3 cubic centimeters of bouillon) of 1 cubic centimeter of bouillon with one-third of an agar-agar culture of Oriental-Prussia cholera. *n*, Nucleus of a crushed macrophage. Staining with methylene-blue. Ocular 3. 1/18 Zeiss.

Fig. 7.—Mononuclear leucocyte filled with Courbevoie cholera vibrios. Peritoneal exudation of a guinea-pig. Ocular 3. 1/18 Zeiss.

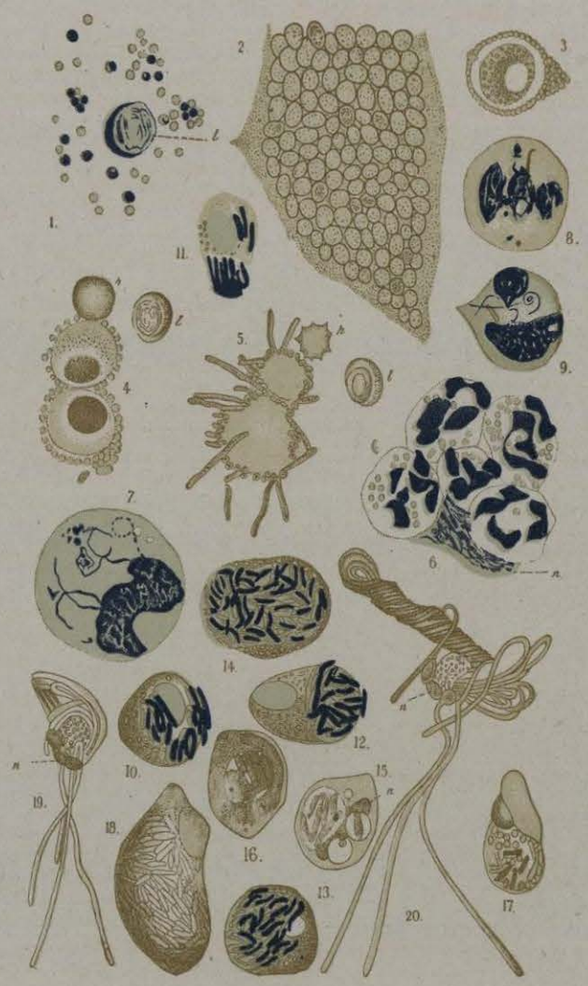
Figs. 8 and 9.—Two polynuclear leucocytes from the same exudation. The vibrios stained gray in the plate are vibrios in the eosinophile stage. Ocular 3. 1/18 Zeiss.

Figs. 10 to 14.—Various phases in the formation of cultures of cholera vibrio (Oriental Prussia) within leucocytes. Hanging drop, stained with methylene-blue, of the exudation of a guinea-pig hypervaccinated for almost six months and prepared with 3 cubic centimeters of bouillon. The exudation was withdrawn four minutes after the peritoneal injection of one-third of an agar-agar cholera culture placed in 1 cubic centimeter of bouillon and kept at 38°. Ocular 3. 1/18 Zeiss.

Figs. 15 to 18.—Various phases in the formation of cultures of the Kiel red bacillus within leucocytes. The hanging drop was kept for twenty hours at 17° and was made with the exudation from an hypervaccinated guinea-pig prepared with an injection of 3 cubic centimeters of bouillon. The exudation was withdrawn four minutes after the introduction into the peritoneum of the Kiel bacilli. Ocular 3. 1/18 Zeiss.

Figs. 19 and 20.—Two consecutive phases of a culture of Kiel red bacilli grown from within a polynuclear leucocyte in a hanging drop of peritoneal exudation. The drop was prepared from the exudation of an hypervaccinated guinea-pig, withdrawn three hours and fifty minutes after the injection of Kiel bacilli into the peritoneum. *n*, Nucleus. Ocular 2. 1/18 Zeiss.

endothelial and connective-tissue cells, those of the splenic pulp, and the large lymphocytes of the blood ("macrophages") are endowed with this property. Precisely as do the familiar amœbæ, so do these phagocytes ingest bacteria and assimilate them. An



INTRAPHAGOCYTIC DESTRUCTION OF BACTERIA. *[Metchnikoff.]*

[Annales de l'Institut Pasteur.]

animal is immune, according to Metchnikoff, as long as its phagocytes freely take up and destroy pathogenic organisms. In proportion, on the other hand, as the functions of the phagocytes are impeded, so is the animal susceptible to disease. That living and dead bacteria are thus disposed of seems to have been satisfactorily shown, while chemotaxis fairly accounts for the affinity which phagocytes show for certain germs in preference to others. Metchnikoff's doctrine as regards the power of certain leucocytes, migrating and fixed, to act as phagocytes is sustained by experimental evidence; the process can easily be followed visually and the leucocytes be seen to ingest micro-organisms, to which they are drawn by chemotactic influence. In 1862 Haeckel witnessed the ingestion of indigo by leucocytes; in 1863 Recklinghausen observed that pus-cells were endowed with amœboid motion, and, having injected cinnabar grains in the dorsal lymph-sac of frogs, saw that they were ingulfed by cells floating in the lymph.

Cohnheim, as long ago as 1867, noted that the smaller vessels of the mesentery became dilated and saw leucocytes range themselves along the vascular walls, plunge their pseudopodia through the mural stomata, and penetrate beyond them, thus migrating and becoming "pus-cells." These pus-cells, in the light of Metchnikoff's theory, are the remains of protective microphages which have succumbed after migrating through vascular walls to meet offensively the pathogenic organism. Dead material, pigment-granules, fragments of tissue, dust-particles, indigo, ivory (in the osseous medullary canal, according to Kölliker), in fact, almost any foreign substance capable of invading the living organic structure, seems to become their prey. An aseptic catgut ligature, a fragment of bacilli-laden tissue, etc., soon becomes coated with an exudate filled with leucocytes which first ingulf the bacilli and then the disintegrated tissue. Let any inhibiting cause appear, however,—an excessively virulent germ, an abnormally high temperature, for instance,—their powers cease, and at once the bacilli multiply, causing death of the animal used for the experiment. The rapidity of multiplication of pathogenic organisms is an additional factor operating against successful phagocytic action. When such is the case the phagocytes are themselves destroyed.

Successful phagocytes may be traced from their working field by staining the latter, as was done by Rosenberger; long lines of colored cells may then be seen to radiate in various directions from the stained area. The pathogenic germs, once ingulfed, usually cease to multiply, and, either through a toxic action or starvation, soon die and disappear. That organisms are ingested alive Metchnikoff has shown. Spermatozoa, for instance, ingested by macrophages were seen to continue their motile activity until the tail had also been taken up. Begun in 1865 with the digestive epithelium of *Gedesmus bilineatus*, the cellular elements of which were shown to digest various extrinsic substances, Metchnikoff's labors developed in 1883 into his present doctrine of phagocytosis, which, notwithstanding much adverse criticism, has maintained its ground.

The rapidity with which the protective process is carried on in cases of general infection is well illustrated by Cantacuzene:[100] "Immediately after injecting anthrax bacteria in a vein of a rabbit's ear," says this author, "the organisms are taken up by phagocytes. At the end of seven minutes in the liver, eight minutes in the lungs, and one hour in the spleen none of the germs are free. Their destruction in the phagocytes is at first very rapid, but soon some of the latter are overcome, and the bacteria, by multiplying within them, cause them to become centers of pullulation. Still, the bacteria that escape from the dead phagocyte are seized by others; but, the number of the former becoming greater as the battle progresses, their protective powers are correspondingly reduced, and the bacteria finally invade the entire blood-stream. In the liver . . . practically all the bacteria are destroyed and digested within a few minutes after the injection. This superiority of the hepatic phagocytes in the fray lasts almost throughout the disease; but the activity of the phagocytes finally decreases; the bacteria multiply within them and become generalized. In the lungs there is rapid destruction of bacteria by polynuclear cells, then intracellular development of bacteria and generalization."

The phagocytes just referred to, the microphages, are wandering or migrating cells—free to respond and travel more

[100] Cantacuzene: Quoted by Marcel Monnier: Gazette médicale Belge, July 13, 1899.

or less promptly toward pathogenic bacteria, in virtue of the chemotactic attraction possessed by the latter. The process is graphically illustrated in the annexed colored plate.

What is the nature of the intraphagocytic process?

I have shown that the germicidal phagocytes, the neutrophiles, absorbed trypsin not only in the intestinal canal while ingulfing foodstuffs, but likewise in the portal vein, the ferment in the latter being, from my viewpoint, the splenopancreatic secretion. This intraphagocytic trypsin is capable of digesting not only certain poisons: toxic albuminoids, toxins, vegetable poisons, venoms, and even drugs, as will be shown in the second volume, but also bacteria. In other words, all materials, poisons, germs, etc., find their doom in the digestive vacuoles of the phagocyte. "Just as amœbæ digest their prey with the aid of amibodiastase, a soluble ferment belonging to the group of the trypsins," writes Metchnikoff, "white corpuscles submit the foreign bodies they inglobe to the action of cytases. These cytases (the alexins or *complements* of other authors) are the soluble ferments which also belong to the category of *trypsins*."[101] The two italicized[102] words should be carefully noted, as they indicate that the antibody Ehrlich has termed "complement" is Metchnikoff's "trypsin." As shown in the second volume, this is confirmed from various directions.

As to the rôle of this cytase or trypsin-like body, it is also clearly defined in another sentence by Metchnikoff: "In blood removed from the body the white cells allow plasmane, which causes coagulation of fibrin and the formation of the clot, to pass into the liquid. But at the same time these abandon a portion of their cytase, which communicates to the serum its *hæmolytic* and *bactericidal* qualities." Briefly, when bacteria are ingested by phagocytes or phagocytic cells of any kind (for these include the finely granular oxyphiles and the hyaline and giant cells, all wandering cells) they are actually digested in their vacuoles by the trypsin-like cytase precisely as they would be in the alimentary canal of highly organized animals.

It becomes a question now as to *how* the phagocytes become supplied with this trypsin, and how their protective activity to

[101] L'Immunité dans les Maladies Infectieuses, p. 573, 1901. See vol. ii, p. 907, for additional evidence.
[102] The italics are my own.

the system at large is awakened. Considered from my viewpoint, *i.e.*, with the structure and functions of leucocytes, as described in the foregoing sections, those cells which are phagocytic are influenced in the following manner, when pathogenic bacteria, their toxins, toxic albuminoids, etc., provoke an autoprotective reaction in the body:—

Stimulation of the adrenothyroid center by the toxic increases the production of adrenoxidase and thyroiodase, which in turn enhance correspondingly oxidation and, thereby, the functional activity of all tissues. Among these tissues are (1) those which produce phagocytes (lymphatic structures mainly), thus causing leucocytosis, and also (2) the spleen and pancreas, which jointly (from my viewpoint) produce Metchnikoff's trypsin-like cytase (Ehrlich's complement). This substance, being secreted (as an internal secretion, see p. 367) into the splenic and portal veins, is taken up therein by the newly created phagocytes and stored in their perinuclear vacuole,—their stomach, so to say. The cells, after traversing the liver, penetrate into the general circulation and carry on therein their function of scavengers, *i.e.*, that of ingesting and digesting the pathogenic substance or the bacteria whose toxin had excited the adrenothyroid center—the sentinel whose mission was to start the defensive reaction.

How Bacterins (Vaccines) Act and how Opsonins Enhance Phagocytosis.—We have seen that in 1907 I advanced the view that the thyroparathyroid secretion corresponded in its chemical, physiological, and clinical properties with Wright's opsonins, and that the labors of Marbé, Malvoz, and Stepanoff have sustained me. I have also submitted evidence to the effect that it was by increasing the sensitiveness of the *phosphorus* of all cells, and particularly their nuclei, to the oxidizing action of the adrenoxidase that functional activity was enhanced. We have now seen that leucocytes, including the phagocytes, are supplied, like other cells, with a nucleus rich in phosphorus. When, therefore, an excess of thyroparathyroid secretion, *i.e.*, opsonin, appears in the blood, through the above-described mechanism, and through the red corpuscles, the phagocytes become unusually active and aggressive.

Simultaneously, the pathogenic organisms themselves

undergo a process which renders them vulnerable to the phagocytic host. The thyroparathyroid secretion, we have seen, powerfully excites metabolism, but particularly the catabolic phase of the process; hence its potent action in the reduction of obesity; it influences bacteria precisely as it does adipose tissue, and with especial activity those that are rich in phosphorus, the tubercle bacillus, for example. It renders the germ more vulnerable to digestion by the trypsin of the phagocyte. This effect on them modifies the surface of the germs, softens it and causes them to adhere together, *i.e.*, to agglutinate.

This suggests that opsonin and *agglutinin* are one and the same thing. That such is the case is shown by many experimental facts. We have seen that the thyroparathyroid secretion, which is the opsonin, and the adrenoxidase are contained in the red corpuscles; Nolf[103] noted that the addition of red corpuscles to serum gave it agglutinating properties. Indeed, Arthur Klein[104] found that agglutinin could be dissolved out of the red corpuscles by means of salt solution or distilled water. It will be recalled that I traced the thyroparathyroid secretion, *i.e.*, the opsonin, to the lungs; Ruffer and Crendiropoulo,[105] in a study of agglutinins, remark: "Strangely enough the lungs of immunized guinea-pigs were the only organs which in the majority of cases possessed agglutinating properties greater than the serum." As is well known, Bordet's "sensibilisatrice" is derived from the red corpuscles; now, Savtchenko[106] has pointed out that this substance was endowed with specific opsonic properties, acting both on bacteria and on leucocytes, as I explained above. Finally, agglutination is evidently a feature of opsonins. Bulloch and Atkins,[107] for example, were led experimentally to conclude that opsonins were "simple substances resembling agglutinins."

This involves the necessity on the part of the opsonin of leaving the red corpuscle to influence morbidly the bacteria. Nolf[108] showed that this was due to an action of the complement (the phagocytic trypsic cytase) on the red corpuscles which

[103] Nolf: Ann. de l'Inst. Pasteur, xiv, p. 297, 1900.
[104] Arthur Klein: Wiener klin. Woch., Apr. 17, 1902.
[105] Ruffer and Crendiropoulo: British Medical Journal, Apr. 5, 1902.
[106] Savtchenko: Annales de l'Inst. Pasteur, xvi, p. 106, 1902.
[107] Bulloch and Atkins: Proc. Royal Soc. of London, lxxiv, p. 379.
[108] Nolf: Annales de l'Inst. Pasteur, xiv, pp. 297, 492, 1900.

caused "the contents of the latter to leave them," and that "the injection of the corpuscular contents incited hæmolysis." In other words, the trypsin of the phagocytes being secreted into the blood, it causes the red corpuscles to secrete their opsonin (with the adrenoxidase—amboceptor), and digestion entailing destruction of the germ can thus occur in the plasma and prove so active, in fact, when there is hyperpyrexia, for example, as to destroy the blood-cells besides, *i.e.*, produce hæmolysis.

The bacteria being softened by the opsonin and rendered more inert, while simultaneously the phagocytes, their enemies, are rendered more active and aggressive, the former are attacked and ingested and they are submitted to digestion in the phagocytic vacuole and destroyed.

On the whole, as viewed from my standpoint, the functions of the leucocytes in general immunity, including Metchnikoff's conception of phagocytosis, may be summarized as follows:—

1. When bacteria appear in the blood, their toxins—or bacterins or vaccines injected into the tissues which ultimately reach the blood—awaken a defensive reaction in the body at large by exciting the thyro-adrenal center, oxidation and metabolism being increased in all tissues, the production of phagocytes is activated and their aggressiveness is intensified.

2. The thyroparathyroid secretion (opsonin and agglutinin) and the adrenoxidase (amboceptor) stored in the red corpuscles are then secreted by these cells under the influence of the phagocytes (Nolf), to sensitize and soften the bacteria.

3. The bacteria are then ingested by the phagocytes and digested by their cytase (complement), a trypsin-like ferment, and this process continues as long as there are bacteria to produce toxins capable of exciting the adrenothyroid center.

This completes the process as far as the phagocytes are concerned. As their functions are to co-operate with the plasmatic defensive process described under the preceding heading, the nature of this co-operation should also be ascertained. This is described in what I believe to be:—

A SIMPLIFIED THEORY OF IMMUNITY.

There occurs, at first, what might be termed the "preparatory" stage, the purpose of which is to increase the defensive

constituents of the blood and other body fluids. On the whole, it seems permissible to conclude that:—

The toxic (certain toxins, wastes, drugs, vaccines, etc.) excites the immunizing center. This center, in turn, stimulates the thyroparathyroid glands and adrenals, thus causing them to supply the blood (and to a certain extent the lymph and serous fluids) with an excess of THYROIODASE *and* ADRENOXIDASE. *Metabolism being enhanced in all tissues by these substances, the pancreas also secretes an excess of* TRYPSIC FERMENT, *while the leucocytogenic tissues (bone-marrow, lymph-glands, etc.) produce an increased number of leucocytes, mainly* FINELY GRANULAR OXYPHILES *and* PHAGOCYTES.

The blood and other body fluids being now provided with all its defensive agents, the following process is started:—

The thyroiodase (opsonin, agglutinin) sensitizes and softens the pathogenic agent, while the adrenoxidase (amboceptor) oxidizes the phosphorus of the nucleo-proteid granulations, liberating heat; the activity of the trypsic ferments (plasmatic and phagocytic complement) being correspondingly increased the pathogenic agent is converted into benign and eliminable products.*

The whole process imposes protective properties upon each ductless gland involved, *i.e.*, the thyroparathyroid apparatus, the adrenals and the pituitary. As to the two first named their power to antagonize intoxication is now generally recognized. As to the pituitary body, its rôle in this connection has only, so far, been studied by myself. Suggestive, however, are the remarks of J. A. Flexner,[109] in reference to the after effects of removal of the pituitary for tumor of this organ. "I wish to call attention," writes this observer, "to the extreme susceptibility of these patients to morphine and to autointoxication, especially of purin origin." Then, referring to a personal fatal case, he states that "one twenty-fourth of a grain kept her narcotized for over a day," and he rightly concludes that "this sudden and unexpected end was due to stopping of her glandular feedings and to the severe autointoxication which resulted from her careless dietary." The rôle I have ascribed to the pituitary body alone explains this phenomenon.

* This relationship of the nucleo-proteid granulations with functional efficiency of the cytase is studied in the second volume, to which the reader is referred.

[109] Flexner: Louisville Monthly Journal of Medicine and Surgery, May, 1912.