terior of these rhizopods, the more easily does it stain with aniline dyes." When we consider that aniline dyes include methylene-blue, we have evidence, in view of Ehrlich's observation, that the "conditions essential to the methylene-blue reaction" are "oxygen saturation and alkalinity," that the prototype of amœba, the leucocyte, must owe its nuclear functional activity to the plasma as exogenous reagent.

Metchnikoff further says: "We may often see flagellated monads taking up filaments of leptothrix several times as long as themselves, and finally inclose them in their digestive vacuoles." The process of ingestion is beautifully shown in the plate opposite page 692, in Figs. 19 and 20, the organisms here being spirilla of Asiatic cholera. "It is sometimes possible to follow all the changes undergone by the bacteria within an infusorium," continues the same investigator, "as in the case of the digestion by stentor of the sulphobacterium thiocystis, observed by le Dantec."[44] . . . "It is evident that the digestive function of the protoplasm of the protozoa must hinder the invasion of these animals by the lower organisms, and it is only in certain special cases that the latter can live as parasites within the rhizopoda and infusoria."

The true identity of the perinuclear vacuole seems further emphasized by the following lines, also quoted from Metchnikoff's text, that is to say, as interpreted by his translators: "The sponges are of such undifferentiated organization that they were long considered to be colonies of protozoa, consisting, like the protospongia, of separate flagellated and amœboid individuals. Later on, it was, however, ascertained that they bore a certain relationship to the polypi and their allies (cœlenterata)." . . . "There are a few species, such as the siphonochalina coriacea, whose mesodermic cells alone inclose all foreign bodies, so that the cylindrical cells of the endoderm merely serve to keep up the continuous passage of the fluid through the sponge. The *phagocytes* of both layers have the power of rejecting insoluble matters, which collect in the larger efferent canals." . . . "We are, however, chiefly concerned here with the fact that the mesodermic phagocytes

[44] Le Dantec: "Recherches sur la digestion intracellulaire," Lille, 1891.

are able to *digest* the substances as well as to inglobe them, and to *reject* the insoluble residue."

The nature of the digestive process has, however, remained obscure. "The bacilli which have been inglobed by leucocytes," continues Metchnikoff, "are much more rapidly digested in the case of mammals that are either naturally refractory, as the dog and fowl, or have been rendered artificially immune against anthrax by vaccination, as the rabbit. This fact is shown by the researches of Hess, as well as my own. It is easy to follow the digestion of many other microbes within the leucocytes. Vacuoles are often seen to form around the bacteria that have been swallowed, just as we have noticed in the digestion of nutrient material by the protoplasm of the protozoa and the myxomycetes. I have been able to observe the changes undergone by the spirilla of recurrent fever in the leucocytes of monkeys, as well as those undergone by the vibrio septicæmiæ in the leucocytes of immunized guinea-pigs, and those by erysipelas streptococci in the leucocytes of man, etc. 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 the microbes is a ferment or not."

Before submitting this question to analysis the manner in which the products of digestion, both the nutritional elements and the excrementitious products, are disposed of must be ascertained.

In the sponge the materials rejected by the phagocytes "and which collect in the larger efferent canals," says Metchnikoff, are eliminated "through large apertures of crater-like shape, the walls of which, according to some authors, are furnished with muscular fibers." What have we in the leucocytes to fulfill this function: *i.e.*, to represent what in the higher forms constitutes the intestinal canal? This appears to me particularly well shown in several of the figures in Gulland's illustration. In Fig. 10, for instance, a few "fibers"—our canaliculi—may be seen to project from the inner aspect of the line which to me represents the practically empty side of the vacuole. The same arrangement is clearly to be seen in Figs. 11, 12, 13, and 16.

If all the foregoing features are considered collectively, they suggest that:—

1. Leucocytes can ingest solid, semisolid, and liquid bodies through their cell-substance in two ways: (1) by projecting pseudopodia which infold or inglobe them; (2) by absorbing them without projecting pseudopodia.

2. Solids and semisolids are mainly ingested by infolding, and semisolids and liquids by absorption; but all substances, with what plasma accompanies them, are collected in a vacuole that surrounds the nucleus and in which the latter lies free; and, at times, in the smaller vacuoles in the cytoplasm.

3. What physiologically useful bodies are formed in the cell are mainly elaborated in the nuclear canaliculi and the perinuclear vacuole, and are collected in the form of granules in the canaliculi.

4. All the functions of the cell are probably governed by the astrosphere.

THE GRANULES AS SECRETORY PRODUCTS.—Gulland refers to granules or microsomes in the following words: "Ehrlich regarded the seven varieties of granules which he described as being all formed by the cells, and as being either reserve material or products for excretion. Hankin [1892-93] took the view that the acidophile granules were secretory products, containing 'alexins,' and destined to be secreted into the blood or lymph. Kanthack, Hardy, and Keng have taken much the same view of these special granules. Sherrington has thrown doubt upon it, and Metchnikoff disputes it and regards the eosinophile granules as reserve material." As viewed from my standpoint, the granules simultaneously represent reserve material *and* products of excretion. These processes are not the only ones, however, with which leucocytes are concerned.

Bail, to whose investigations I have already referred, is also stated by Stokes and Wegefarth[45] to have observed that, after the vacuole "in the nucleus" had formed, "the granules generally disappeared." Furthermore, he noted that upon destroying the staphylococci by adding ether, and diluting the centrifugalized sediment, the granules showed a dancing motion, and were seen to *leave the periphery of the cell* and enter

[45] Stokes and Wegefarth: *Loc. cit.*

the surrounding medium. Evidently at least *some* of the granules must have been dropped or ejected by the leucocytes, and their canaliculi thus freed of the impediment their presence constituted.

This is sustained by a closer examination of the question, —the purpose of Stokes and Wegefarth's paper, who used in their researches blood taken from about five hundred persons. The granules, when observed by them with the aid of artificial light, "resembled those of the eosinophilic or neutrophilic leucocyte." Kept at the temperature of the room, the latter showed no activity, but exposure for an hour to a temperature of 35° C. caused them to become active. The following lines are quoted from their article: "At times the granular leucocytes become actively amœboid, and the granules *within* the neutrophile exhibit a characteristic activity which might be compared to the swarming of bees around a hive. The number of fine granules free in the plasma is perceptibly increased. The eosinophilic granulations also show a less vigorous tremulous motion, and both varieties follow the changes in the direction of the pseudopodia, the protoplasm being thrown out first, and the granules following. The characteristic dancing motion of the granules in the neutrophilic leucocyte can be brought out very plainly by simply mixing the drop of blood with an equal amount of distilled water containing 1 per cent. of alcohol. The granules become very active and present a characteristic picture." . . .

"Can these granules be actually seen to leave the leucocyte? It is certainly not easy to be sure, even after continuous observation for an hour or more, that one has actually seen one of these granules leave an amœboid leucocyte. We think, however, that we have observed this phenomenon upon several occasions, both in fresh specimens of blood exposed to 35° C. and in blood to which 1 per cent. of alcohol has been added." Farther on in their text they say: "Many fine granules can be seen in the clear plasma and around the neutrophile, and it would seem that occasionally a granule leaves the active leucocyte and becomes free in the surrounding fluid."

Bail's observation, however, that the granules actually leave the periphery of the cell has been sustained by other observers. Gulland refers to this feature of the problem in the following words: "It has often been remarked that the large cells show a great tendency to leave their granules behind them; thus, one might come on a group of granules while the nearest cell was far away. Ballowitz was, I think, the first to declare that all or most of these groups of granules were attached to the cell by fine protoplasmic bridges. It is not always easy to show this." Gulland then says, referring to a figure in one of his plates (not shown in that reproduced herein), in which the granules are evidently disunited from the cell: "In the cell shown in Fig. 31, which was so isolated that there could be no doubt that all the granules represented belonged to it, no trace could be made out of threads extending from granule to granule. They are probably stretched too much to allow them to be visible."

The absolute separation of the granules from the cell witnessed by Bail finds its complementary confirmation in the observation of E. B. Sangree,[46] who, after patient watching,—sometimes several hours at a time,—states that he saw "three granules escape from an eosinophile cell, and wander away until lost under rouleaux of red corpuscles, after having reached a distance of some six diameters from the parent-cell." . . . "While inside the cell," says this pathologist, "these granules had participated in the constant, though rather sedate, movements of the granule mass,—but owing, doubtless, to the difference in specific gravity of the containing medium,—instantly upon emerging from the parent-cell they underwent the wildest possible gyrations. The first to come out were two attached pole to pole, and these rolled frantically over each other, pushed this way and pulled that, all the time oscillating widely and rapidly, yet constantly and definitely traveling farther and farther from the cell, until finally lost to view. The single granule behaved in an exactly similar way. I noticed, too, that before becoming lost to view the motion of these granules had become considerably less marked and approximated more that ordinarily seen in these bodies." If these facts are considered as a normal sequence to the evidence adduced that the cellular

[46] E. B. Sangree: Philadelphia Medical Journal, March 12, 1898.

net-work of fibers represents the secretory system of the leucocyte, it seems permissible to conclude that:—

The granules in leucocytes are the products of an intracellular metabolic process and represent a true secretion.

THE PHYSIOLOGICAL CHEMISTRY OF LEUCOCYTES.—A feature which clearly points to the autonomy of the nucleus and of the net-work of canaliculi in all leucocytes is the uniformity with which they all stain with similar dyes. The nuclear canaliculi and granules and the canaliculi of the cell-substance all take the aniline dyes, methylene-blue and methyl-green, for example: evidence that in *all* leucocytes the structures mentioned must find in the adrenoxidase a source of energy as do other organs.

Beginning with the nucleus, with what chemical body contained in this part of the cell could the adrenoxidase initiate and sustain a reaction? It is, of course, not the composition of the nuclear *granules* that this question involves, but that of what might be termed the nuclear ground-substance. Foster refers to this substance in the following words: "There is present, in somewhat considerable quantity, a substance of a peculiar nature, which, since it is confined to the nuclei of the corpuscles, and further seems to be present in all nuclei, has been called *nuclein*. This nuclein, which, though a complex nitrogenous body, is very different in composition and nature from proteids; is remarkable, on the one hand, for being a very stable, inert body, and, on the other, for containing a large quantity (according to some observers, *nearly 10 per cent.*) of *phosphorus*, which appears to enter more closely into the structure of the molecule than it does in the case of proteids." We evidently have, in the nuclein of the nuclear ground-substance, a body which, as does lecithin in the myelin of nerves, myosinogen in muscles, etc., enters into active combination with the oxidizing substance, *i.e.*, adrenoxidase, and the resulting reaction must necessarily yield functional energy, as elsewhere in the organism.

The character of the reaction which the simultaneous presence of nuclein and the oxidizing substance within the precincts of the nucleus sustain is clearly suggested by the kind of dyes taken by the canaliculi (both of the nucleus and of the cell-substance) and the perinuclear vacuole. E. T. Williams,[47] in a study of the chemical properties of leucocytes, refers to this feature of the problem in the following words: "The nuclei of all three classes stain best with alkaline dyes, as methylene-blue, methyl-green, or dahlia. They are, therefore, acid." Farther on, he says: "We have seen that all nuclei are acid. They owe this property, without doubt, to the nuclein which they contain. Nuclein is acid. When boiled *with alkalies* it yields phosphoric acid. Phosphoric acid, it may be remarked, is the only mineral acid which does not coagulate albumin. It is the presence of this acid undoubtedly which makes nuclein acid. According to the experiments of Kossel, quoted by Vaughan and Novy,[48] nuclein, when boiled with acids, yields certain organic, albuminoid bases, as adenin, sarcin, xanthin, spermin, and others." . . . "We must conceive, therefore, of nuclein as some sort of a phospho-albumin whose composition has not been precisely determined." The source of the various chemical bodies involved in these processes is shown in the following lines of Professor Foster's: "The ash of the white corpuscles is characterized by containing a relatively large quantity of potassium and of phosphates, and by being relatively poor in chlorides and in sodium. But, in this respect, the corpuscle is merely an example of what seems to be a general rule (to which, however, there may be exceptions), that, while the elements of the tissues themselves are rich in potassium and phosphates, the blood-plasma on which they live abounds in chlorides and sodium salts."

The chemical process involved may easily be traced with the foregoing factors as main elements: The blood-plasma (if the views already submitted are sound) evidently reaches the nucleus through the intercanalicular substance of the cell-body; this is shown by the fact that this substance likewise—though to a less marked degree—stains with methylene-blue. Under ordinary circumstances, according to microscopical evidence, the perinuclear vacuole is practically collapsed: *i.e.*, its nuclear wall is more or less close to that of the cell-body. This is well shown in Gulland's plate, by Figs. 10 and 12. The nucleus

[47] E. T. Williams: Boston Medical and Surgical Journal, Sept. 5, 1901.
[48] Vaughan and Novy: "Ptomaines and Leucomaines," 1891.

thus bathes in blood-plasma, and its canaliculi become filled with the latter along with the vacuole. The nuclein of the nucleus under these circumstances itself bathes in the plasma, being thus exposed to the action of the latter's oxidizing substance or adrenoxidase.

Still, this suggests the presence of a stream of plasma flowing through the nucleus itself, with the canaliculi as emunctories. The contraction and retraction of the canaliculi—or reticulum—to which Gulland and others refer represent the only mechanical device in the cell by means of which the vacuole can be drained.

These minute vessels probably serve as continuous channels for the stream of plasma, which contains, besides the adrenoxidase, the alkaline salts necessary to the intracellular process. The plasma's adrenoxidase and the nuclein's phosphorus, thus brought into contact, liberate considerable heat, and the alkaline salts in the plasma then take part in the reaction to which Williams refers, and which involves, we have seen, the formation of phosphoric acid and other agencies to which I will presently allude.

We must not lose sight of the fact, however, that nuclein is derived from nucleo-proteids, and that during the oxidation process waste-products are formed: we have in the "adenin, sarcin, xanthin, spermin," etc., to which Williams refers, a series of catabolic products. This awakens an important pathological feature. We have seen that, when nucleo-proteids undergo cleavage in the organism, the process involved must be brought to a finish: *i.e.*, to the stage of phosphoric acid formation. The penalty, if completion does not attend the series of reactions, is the presence in the blood-stream of the above-mentioned purin bases, which are now considered, we have seen, as the source of the so-called "gouty diathesis." Slight insufficiency of the adrenal system, therefore, by reducing the adrenoxidase and thyroiodase in the blood, must inhibit the intracellular reactions that I have just outlined, thus giving rise to this disorder. Or the injudicious use of rich foods, by surcharging the proportion of nucleo-proteids taken up by the cells, may lead to the same result though the normal proportion of adrenoxidase and thyroiodase—the latter, we have seen, playing an important rôle by sensitizing the phosphorus to oxidation —be present in the plasma.

Another phenomenon which appears to me elucidated by the presence of the adrenoxidase of the plasma is the manner in which worn-out leucocytes are destroyed. As frequently observed by histologists, each of the varieties may be seen at a given time to become "oxyphile," or oxygen-loving, and to undergo disintegration, a preparatory step to proteolysis here. Even the eosinophile leucocytes, which, according to Metchnikoff,[49] are unable "to inglobe foreign bodies, and therefore cannot act as phagocytes," are destroyed by proteolysis. The affinity of these cells for acid dyes might account for their oxidation, however, and suggest a limit; but such a limit does not exist, for basophile cells also yield to the same agency. Indeed, Gulland, referring to a figure in his colored plate which gives a vivid illustration of a cell undergoing disintegration, describes it as follows: "Degenerated basophile cell from the mesentery of newt. Methylene-blue." In other words, an eosinophile is always acidophile, while a basophile is only acidophile when it is dead or about to die. We have seen that methylene-blue stains oxygen-laden media; hence, the adrenoxidase is evidently an active factor (as amboceptor) in the destructive process.

It seems to me that we can conclude from the above data regarding the physiological chemistry of leucocytes, or white blood-corpuscles, that:—

1. The granules which constitute the secretion of all varieties of leucocytes are the products of a continuous reaction in the nucleus, in which the nuclein of its nucleus, the materials ingested by the cell, and the plasmatic adrenoxidase, thyroiodase, and alkaline salts take part.

2. When a leucocyte becomes functionally incompetent it is destroyed by proteolysis in the blood-plasma.

Classification of Leucocytes.—I have proceeded as far as I could with our analysis of the leucocytes as a unit, and it now becomes necessary to ascertain, if possible, the functions of the various types which histologists, headed by Ehrlich, have established with the aid of staining methods.

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[49] Metchnikoff: *Loc. cit.*, p. 115.

Kanthack and Hardy[50] not only give a clear, though succinct, outline of the various varieties of cells, but they emphasize features of the problem which are of special interest to us. After briefly reviewing the more prominent contributions to our knowledge of the subject since Wharton Jones's memoirs, published in 1846, including the investigations of Rindfleisch (1863) and Max Schultze (1865), they write as follows:—

"After Max Schultze, no further advance was made or, indeed, was possible in the histological analysis of the sporadic mesoblast, until Ehrlich, in 1878, furnished a rational basis for the use of staining reagents by his far-reaching discovery that the elective affinity of certain constituents of tissues for particular stains could be referred to two factors: the *chemical* nature of the staining substance employed and—a point too often neglected by workers who have followed his methods—the nature of the medium in which the stain is dissolved.[51] Ehrlich drew particular attention to the granules, the possession of which characterizes various forms of wandering cells. These he divided into five classes, differing either in their special affinity for bases, acid, or neutral dyes, or in size. The α or eosinophile granulation colors *only* with acid dyes; the β granulation colors with both acid and basic dyes (amphophile); the γ granulation colors *only* with basic dyes, and the individual granules are large; the δ granulation colors *only* with basic dyes, but the individual granules are small; and the ε granulation colors *only* in neutral dyes.

"The nomenclature of the granules was extended to the cells bearing them. Thus, the various forms of white cells found by Ehrlich in blood were: I. A small cell free from granules, to which the name lymphocyte was given, from the fact that it appears to be developed in lymphoid tissue. This is the small, non-amœboid form of Max Schultze. II. A cell characterized by possessing fine granules and one or several nuclei. This is by far the most numerous form of white blood-corpuscles in mammalia, and was found by Ehrlich to be neutrophile in man, and amphophile in rabbits and guinea-pigs.

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[50] Kanthack and Hardy: *Loc. cit.*, p. 82.
[51] All the italics are my own.

III. The eosinophile cell, or coarsely granular cell of Wharton Jones and Max Schultze. It occurs only in small numbers in the blood of mammalia, but is abundant in the blood of lower vertebrates. IV. A basophile cell with fine basophile granules (δ granulation).

"The mononuclear amœboid cells of Max Schultze are apparently grouped with the neutrophile cells by Ehrlich. In addition to these forms Ehrlich describes a basophile cell with coarse granules (γ granulation), occurring mainly in connective tissues and also in the blood of frogs, but not in the blood of mammals. These he calls 'Mastzellen.' . . .

"From what we have said so far it will be seen that the group of finely granular blood-corpuscles described by Max Schultze includes the amphophile and neutrophile and the finely granular basophile cells of Ehrlich. Since Ehrlich's work no contribution to our knowledge of the morphology of the wandering cells has been made except on points of detail. Mention must, however, be made of the group of cells recognized by Metchnikoff[52] in his treatise on inflammation. The term 'leucocyte,' originally applied by the French school of physiologists, is used to designate wandering cells, and the following varieties are recognized: (I) lymphocytes; (II) mononuclear leucocytes with abundant protoplasm and a round nucleus; (III) polynuclear leucocytes, or 'leucocytes neutrophiles'; (IV) eosinophile leucocytes."

My purpose being to ascertain the physiological functions of the various types, Ehrlich's four classes, by affording definite microchemical limits, will probably prove more useful than the simplified groupings that other histologists have introduced, and which, by reducing the number of divisions, have tended to efface landmarks that can serve as clues for research. I will preserve, therefore, Ehrlich's classification, and try to ascertain whether the various types of cell do not differ physiologically from one another as they do histologically.

Lymphocytes and Hyaline Cells.—The first cell of the Ehrlich series, the lymphocyte, seems fully entitled to the position accorded it by histologists in general: that of a leucocyte in process of development. The cellular substance is

[52] Metchnikoff: *Loc. cit.*

devoid of canaliculi (or mitoma) and of granules, although the nucleus itself is supplied with both, and is evidently functionally active. Lymphocytes are considerably smaller (6 to 6.5 μ) than leucocytes, and represents less than one-fourth of the total number of these cells. They are devoid of amœboid motion. Hyaline cells have been classed in the same category, the cell-body being likewise free from granules, as shown in Gulland's plate, Fig. 1. Both may become active, however, before complete maturity is reached.

Neutrophile Leucocytes.—These are extremely important members of the leucocyte family, for they represent fully three-fourths of the white cells of the blood, and constitute Metchnikoff's main group of phagocytes. They are termed "neutrophile" by Ehrlich because their granulations stain with both acid and basic dyes. Their reaction to acid dyes is very much less intense, however, than is the case with purely acidophile cells, according to Kanthack and Hardy. Their granules are small as compared to those of other acidophiles. Though termed "polynuclear" leucocytes by Metchnikoff, the masses thought to represent as many nuclei are united by thin bridges, thus constituting a single nucleus. Especially is this likely, since the only other type of cell deemed phagocytic by Metchnikoff is a mononuclear cell. Gulland contends that no shape of nucleus is invariably associated with granules of a special kind. It seems evident, therefore, that the phagocytic cells are only distinguishable by their affinity for alkaline dyes and a slight affinity for acid dyes, and by the concurrence of these histological properties with small granules.

Kanthack and Hardy, who refer to this leucocyte as a "finely granular oxyphile cell," speak of it as follows: "It has a very limited and precise distribution, for, under normal conditions, it is entirely absent from extravascular spaces, and occurs *only in the blood*,[53] where it is by far the most numerous corpuscle, forming 20 to 70 per cent. of the total number of white corpuscles. The fluctuation in this percentage is probably due, in the main, to the great periodic variations in the number of lymphocytes present in the blood. Thus, the effect of a meal is to cause a considerable increase in the number of

[53] All italics are my own.