lymphocytes in the blood, and, therefore, a fall in the share of the total white corpuscles due to finely granular cells. If this disturbing factor be eliminated," continue these investigators, "and the percentage of the finely granular oxyphile cells be taken of the adult white corpuscle only, this is found to be always very high: in man, 75 to 90 per cent."

Metchnikoff, referring to the phagocytic properties of these cells, writes as follows[54]: "Even outside the organism these amœboid cells readily inglobe a large number of foreign particles with which they may come in contact, and they may often be seen literally crammed with all sorts of granules. Like the amœbæ, they swallow not only inert bodies, such as granules of carmine or other substances that are insoluble in the fluid surrounding the leucocytes, but also a large number of living organisms." This is merely quoted to emphasize the fact that the leucocytes differentiated by Ehrlich from all others by the term "neutrophile" are, irrespective of the form of their nucleus, the wandering cells which Metchnikoff has shown to fulfill the physiological function he has termed "phagocytosis."

THE NEUTROPHILE LEUCOCYTES IN ASSIMILATION.—The property which these cells so strikingly show: *i.e.*, their ability to ingulf or rather ingest substances of all kinds, seems to me to suggest that they are intrusted with another rôle in the body: *i.e.*, *its nutrition.* Macallum[55] observed, in sections of intestines taken from animals first starved, then fed upon a substance containing albuminate of iron, free leucocytes crowded with granules of iron-pigments in the intestine. Some of these cells appeared to pass out through the epithelial cells, while others advanced into the subepithelial elements. Macallum also found them in the venules of the villi, the spleen, etc.

We have just seen the reference of Kanthack and Hardy to the "considerable increase in the number of lymphocytes in the blood, and, therefore, a fall in the share of the total white corpuscles" caused by a meal. Both these two phenomena become normal events instead of a "disturbing factor" if the process of digestion includes the use of a large proportion of adult or fully developed leucocytes to transport various

[54] Metchnikoff: *Loc. cit.*, p. 115.
[55] A. B. Macallum: Journal of Physiology, vol. xvi, 1894.

materials from the intestinal canal to various parts of the organism. It is evident that under these circumstances the immediate neoformation of lymphocytes, and their rapid growth, as is probably their wont, to the state of mature cells, becomes a *sine qua non* of continued existence.

Overlooking the possibility of such a function, and led by his own hypothesis to ascribe to intracellular processes the presence of food-products in the leucocyte, Metchnikoff[56] writes: "The digestion of proteid substances by the leucocytes is well shown by the gradual changes that take place in the muscular fibers which have been inglobed by leucocytes in cases of acute muscular atrophy. The presence of *peptone* in leucocytes, which has been so often proved by Hofmeister, is sufficiently accounted for by this fact of intracellular digestion, and need not, therefore, be referred, as done by this author, to an *absorption* by these cells of the *peptone formed in the alimentary canal.*" I need hardly observe, however, that, added to the foregoing testimony, Hofmeister's view seems sustained.

Indeed, the process to which the peptones owe their presence within the cell is not difficult to trace, if the latter's mechanical functions, as I have construed them, are taken into account. The presence of peptones within the perinuclear vacuole being an accepted fact (since it is recognized by both investigators), the presence therein of substances from which the peptones are elaborated must be accounted for. Metchnikoff traces these to products of degeneration, as suggested by his comparison, and perhaps to waste-products of digestion. Hofmeister's conception differs only from this in implying a closer or more direct relationship between the leucocytes and the intestinal contents of their host. In other words, while Hofmeister associates leucocytes with the process of digestion, Metchnikoff looks upon them only in the light of scavengers. That phagocytes may fulfill both rôles is obviously suggested not only by their own chemico-physiological characteristics, but also by their itinerary in the system. Both Hofmeister and Metchnikoff are right, therefore, each in his own way.

In his review of the absorption of proteids Stewart[57]

[56] Metchnikoff: *Loc. cit.*, p. 124.
[57] Stewart: "Manual of Physiology," 4th ed., 1900.

writes: "Although a certain amount of egg-albumin and other native or slightly altered proteid substances can be absorbed as such by the small, and even by the large, intestine, there can be no doubt that the greater part of the proteids of the food is first changed into proteoses and peptones. But proteoses and peptones are absent from the blood, and, indeed, when injected into the blood they are excreted in the urine. When injected in larger amount they pass also into the lymph, from which they gradually reach the blood again, and are eventually, as before, eliminated by the kidneys. The clear inference is that when absorbed from the alimentary canal they must be changed into one or both of the chief proteids of blood and lymph (serum-albumin and serum-globulin) in their passage through its walls. And it has actually been shown that during digestion of a proteid meal the mucosa of the stomach and intestine contains proteose and peptone, while none is present in the muscular coat or in any other organ. They rapidly disappear from a portion of the mucous membrane kept at a temperature of about 40° C. outside the body; but not if it has been thrown into boiling water immediately after excision, nor even if it has been heated at 60° C. for a few minutes and then kept at 40° C. Now, a temperature of 60° C. does not destroy an unorganized ferment, but kills a *living cell*. The regeneration of the proteose and peptone must, therefore, presumably take place in *cells*, and the only available cells in this locality are those which line the intestine, or the *leucocytes* which wander between them. Accordingly, both have been credited with the power of absorbing and transforming these substances."[58]

If my views concerning the functions of the epithelial cells of the intestines, as submitted in the seventh chapter, are sound, they subserve an entirely different function from that now generally ascribed to them: *i.e.*, that of supplying the intestinal tract with a secretion calculated mainly to asepticize the intestinal contents. On the other hand, I showed that the lymph-follicles, including Peyer's patches, supply leucocytes, formed in the cytogenic area of the follicles (Flemming's central nodule), to the intestinal cavity through the fenestrated

[58] All italics are my own.

membrane overlying each follicle. As the inquiry did not afford evidence to the effect that all these leucocytes served to insure destruction of pathogenic bacteria, I stated that *some* of them carried out this function. Indeed, there was good ground for this limitation, for I had already referred to the iron-laden leucocytes observed by Macallum and had been led later on to allude to those charged with the return of bilirubin to the circulation. That the leucocytes supplied to the intestinal canal by the cytogenic follicular areas include some—and probably a large proportion—whose functions it is to ingest proteids *with* the iron and bilirubin, then re-enter the intestinal wall by way of the villi, is very likely. To the various agencies thus incorporated in the organism can now be added that referred to by Metchnikoff in the sentence: "The presence of peptones in leucocytes which has been so often proved by Hofmeister." While this contributes further evidence to show that my conception of the whole process must be poised upon solid premises, it also suggests that leucocytes ingest *proteids*, and not peptones, from the intestinal canal, because peptones are the terminal products of the digestion of proteids.

If leucocytes ingest proteids, these must accumulate in their perinuclear vacuole and find their way into the nuclear canaliculi. These cells being freshly supplied to the intestinal canal from the follicles, the proportion of blood-plasma in them must be limited when, laden with proteids, they enter the venules of the villi to find their way to the portal vein. Even in this vessel they must again find a dearth of adrenoxidase, for we have seen that this channel is essentially venous. We must not lose sight of the fact, however, that potent additions to its contents are obtainable here: the spleno-pancreatic internal secretion, *i.e.*, trypsin, to which the plasma of arterial blood and dextrose may be superadded when the precincts of the hepatic artery, *i.e.*, the hepatic lobules, are reached.

If these cells do take up proteids and other bodies utilized in nutrition or in the building up of various organic structures, their own canaliculi, *i.e.*, those of the cell-substance, must serve as the eliminatory channels. In other words, proteids ingulfed by the leucocyte must be submitted to a process of digestion in the nucleus and its vacuole, and the products be passed out as granules. *This elevates leucocytes to the rank of glandular organs,* but we must not overlook the fact that glands in general supply their secretion in the form of granules. Referring to the parotid, for instance, Foster speaks of the secretion as "generally in the form of granules" and of the "granules" which in the submaxillary gland "may obscure the nuclei." The granules of the pancreas, of the intestinal epithelial cells, etc., are also familiar examples. Indeed, all these granules only differ from those of leucocytes in being less complicated molecularly and smaller. They seem to me fully to represent a true, cellular secretion.

What is the nature of the neutrophile's secretion, *i.e.,* the composition of its granules? Milroy and Malcolm[59] state that the finely granular amphophile (or neutrophiles) granules "are usually taken to be proteid in nature," and refer to the fact that Sherrington had suggested that they might be "of nucleo-proteid nature": a view which their own researches confirm. Under the action of alcohol kept at boiling-point, neither fine nor coarse oxyphile granules were dissolved; ether also at boiling-point gave similar results. These agents being then used successively, the granules remained practically unaltered: a fact which leads the authors to conclude that the granules *cannot consist* of *fat* or *lecithin.* Weak alkaline solution at 115° to 120° C. almost entirely removed the granules from the finely granular cells, "but the most striking feature was the persistence of two structures, the nuclei and the coarse oxyphile granules." Solutions of sodium carbonate (½ to 1 per cent.), followed by careful washing, almost entirely removed the fine oxyphile granules in from one to sixteen hours, while the coarse ones were left. Oxalic acid (0.4 per cent. in alcohol, then 1¼-per-cent. watery solution) entirely removed the small granules, a few of the coarsely granular oxyphile cells containing pink-stained granules, while others were vacuolated. As a result of these tests (which should be read *in extenso* in the original paper) Milroy and Malcolm write as follows: "The possibility of both types of granules consisting of the same kind of organic matter either *differently bound* or with organic salts atached in such a way as to alter the solubilities is certainly a

[59] Milroy and Malcolm: Journal of Physiology, vol. xxv, 1899.

strong one. That it is not *simply* albumin or globulin appears evident from the comparatively insoluble character of both types of granules, but especially the coarse oxyphile ones. Again, the fact that the fine granules are not only oxyphile, but also basophile, supports the view that they are composed of a *complex proteid substance.*"[60] . . . The concordance of these facts with those previously recorded appears to me conclusive.

Milroy and Malcolm's researches not only seem to me to give neutrophile granules their own identity (though showing a distinct kinship to the larger acidophile granules), but also to emphasize the fact that these minute masses of proteid substance represent the end-result of the intracellular process that occurs during the journey of the leucocytes from the intestinal villus to the general circulation *via* the portal and hepatic vessels.

Is it only in the cells that the reactions which serve to convert proteids into assimilable products occur? The investigations of Milroy and Malcolm will greatly assist us in elucidating this question.

In their first article on the "Metabolism of Nucleins"[61] these investigators say, in the course of a review of the metabolism of the nucleins under physiological conditions: "When nucleins are taken by the mouth, the first change that they undergo in the alimentary tract is a simple solvent one in the stomach, and that only to a very slight degree. They are never split up into their constituents. They are easily broken up, however, by the *pancreatic secretion*[62] into an organic phosphorus-holding acid (not nucleic acid) and albumose or peptone. The important points to notice are that the phosphorus is still in organic combination, and that neither ortho- nor meta- phosphoric acid is so formed. It is probable that the organic phosphorus-holding acid so formed is similar to thymic acid. It forms soluble compounds with albumose and peptone, and is, in all probability, so absorbed. After absorption the bodies derived from the nucleins cause a well-marked *leucocytosis,* and the excretion of phosphoric acid in the urine is

[60] The italics are my own.
[61] Milroy and Malcolm: Journal of Physiology, vol. xxiii, No. 3, July 26, 1898.
[62] All italics are my own.

increased. Whether a hypoleucocytosis *always* precedes the hyperleucocytosis is difficult to say. Almost all the writers on this subject have emphasized the fact that, on giving nucleins by the mouth, the phosphoric acid excretion in the urine is increased; but they have omitted to show that this excretion *cannot be accounted for* by the phosphorus taken in the form of nucleins, there being really more phosphorus excreted by the kidneys than was present in the original nucleins."

Again, as a result of a series of experiments, Milroy and Malcolm are led to the following conclusions among others: "1. The digestion products of nuclein-holding tissues, nuclein and nucleic acid, cause, on being absorbed, a temporary leucocytosis, which is accompanied by a rise in the P_2O_5 excretion above that derivable from the absorbed phosphorus. These alterations are especially well marked after giving nucleic acid. 2. The alloxuric bodies are excreted in excess, after nucleic acid has been given, and in all probability also after large doses of nuclein-holding tissues or nucleins, although in our experiments, owing to the small amount of thymus taken, there was no distinct increase. 3. The uric acid excretion after nucleic acid was only slightly, if at all, increased. We were exceedingly anxious to give larger doses of nucleic acid, but were unable to do so because of certain rather disagreeable symptoms (*severe muscular tremors*) which arose after the *larger* quantity had been given."

The augmented phosphoric acid excretion to which the authors refer, and which they state cannot be accounted for by the phosphorus taken in the form of nucleins, has doubtless suggested to the reader as primary cause the increased functional activity of the adrenal system induced by the phosphorus ingested: an interpretation sustained by the presence of severe muscular tremors, "which arose after the larger quantity had been given." Of course, phosphorus here acts like any other toxic as a stimulant, the anterior pituitary body responding to the effects of organic poisons as well as those foreign to the system as a chemical entity.

Still, this involves the necessity of showing that leucocytes are themselves the seat of the enhanced metabolism and the source of the excess of phosphoric acid to which the muscular

tremors are due, in accord with my previous statements to that effect. Again, if, as I have suggested, the granules represent the leucocytic secretion, an excess of granules must occur under the influence of the stimulation of the adrenal system induced. That such is the case is shown by the following casual remark of Stokes and Wegefarth,[63] who, as stated, based their studies of the free granules derived from leucocytes upon examinations of blood taken from about five hundred persons: "In perfectly fresh specimens the granules were not numerous, but they seemed somewhat increased in patients who had been taking *tonics* or various alcoholic drinks."

This, in turn, involves a query as to the manner in which the anterior pituitary body becomes primarily stimulated when nucleins are taken in excess, for it would seem that locked up in the perinuclear vacuole of the leucocytes their phosphorus could not influence the adrenal system through the blood-stream. This would doubtless hold were the intracellular process to cease at any time, but, as this must begin as soon as the cells enter the hepatic capillaries, after acquiring therein their adequate supply of adrenoxidase, their normal production of granules must start at once. An inordinate proportion of nucleins in the food soon supplies the blood-stream, through the agency of the cells, with an abnormal quantity of these minute phosphorus-laden bodies. These at first give rise to excessive functional activity, including among other signs the "severe muscular tremors" to which Milroy and Malcolm refer, coupled with an excess of P_2O_5 production. Persisted in, however, the excessive (relative) ingestion of nucleins brings on, as do other toxics, adrenal insufficiency, which, by entailing a reduced production of adrenoxidase and trypsin, upon which the physiologically perfect intracellular reactions mainly depend, correspondingly lowers the efficiency of the cleavage-processes. This means, instead of the physiologically perfect granules which, we have seen, Milroy and Malcolm found to be proteid in nature, an accumulation in the blood of proteid toxalbumins.

In their first paper, the above-mentioned investigators draw attention to the two decomposition products considered "as more or less characteristic signs of the decomposition of the

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

nucleins, viz.: the alloxur bases and phosphoric acid." If my conception as outlined in the preceding paragraph is justified, these alloxuric bases are products of *inadequate* metabolism, while phosphoric acid is the product of perfect metabolism. Uric acid having likewise been considered as a product of the complete process, a rise of alloxuric excretion cannot occur along with excessive phosphoric acid production. That my conclusion, based mainly on Horbaczewski's work, was warranted, is shown by what Milroy and Malcolm term "points of special importance" as results of a series of experiments, namely: "1. There is no doubt that the P_2O_5 excretion is increased even when very small doses of thymus are given. 2. Relatively, also, the P_2O_5 is increased in proportion to the nitrogen. 3. With the small amount of thymus taken there was practically no appreciable alteration in the excretion of the alloxuric bodies, either absolutely or relatively to the total nitrogen or total P_2O_5." All this serves to emphasize another feature of the problem: *i.e.*, that *phosphoric acid is the prototype of uric acid as a product of perfect or physiological intracellular metabolism, and that the phagocytic leucocytes which take up nucleo-proteids from the intestinal food-products are the seat of the reactions through which these bodies are converted into assimilable products.*

Although I have only dwelt so far, as regards the intracellular processes with which nucleo-proteids are concerned, with neutrophile leucocytes, these are not alone the seat of reactions which, normally performed, end in the production of uric and phosphoric acids. Indeed, we have seen that all leucocytes contain nuclein in their "nucleus"—a fitting name under the circumstances, and the physio-chemical process reviewed only typifies that which occurs in all varieties of leucocytes. Wherein the neutrophile cells are distinguishable, however, is in their ability as phagocytes to take up nucleo-proteids from the intestine, and to break them up, by means of the trypsin and adrenoxidase subsequently absorbed by them, into peptone and an organic compound containing phosphorus.

How are the various bodies, the presence of which this suggests, utilized? The presence of pancreatic secretion in the intestine, and of the spleno-pancreatic secretion in the portal

vein, would suggest that the leucocytes must be carriers of carbohydrates: an important question when we consider the leading functional rôle which myosinogen plays in muscular contraction. Dextrose, formed from glycogen, itself in turn a product derived from starches, forms part of a chain of events which would, in a measure, have to occur within the cell itself. That such is the case is suggested by the investigations of Zabolotny,[64] who found that phagocytes devoured particles of starch-paste and digested them: features which led this investigator to conclude that "the presence of an amylolytic ferment in the phagocytes cannot be doubted." But Zabolotny likewise states that when leucocytes ingest starch they become iodophile. This, as is well known, has been termed by Ranvier and other physiologists the "*glycogen* reaction."

Foster, referring to this question, says: "In the case of many corpuscles, at all events, we have evidence of the presence of a member of the large group of *carbohydrates,* comprising starches and sugars, viz.: the starch-like body *glycogen.* . . . This glycogen may exist in the living corpuscle as glycogen, but it is very apt, after the death of the corpuscle, to become changed by hydration into some form of sugar, such as maltose or dextrose." Indeed, he furnishes us complementary evidence, alluding to the cellular proteids in the following sentence: "One of these proteids is a body either identical with or closely allied to the proteid called *myosin,* which we shall have to study more fully in connection with muscular tissue." I have shown that myosin is the *post-mortem* product of the action of what remains of oxygen in the plasma upon myosinogen, and that this is the cause of *rigor mortis.* Foster says, in this connection: "And we have reasons for thinking that in the living white corpuscle there does exist a body identical with or allied to *myosinogen,* which we may speak of as being in a fluid condition, and which, on the death of the corpuscle, is converted, by a kind of clotting, into myosin, or into an allied body which, being solid, gives the body of the corpuscle a stiffness and rigidity which it did not possess during life." All this seems to me clearly to suggest that these leucocytes, in the light of my

[64] Zabolotny: Russian Archives of Pathology, April, 1900.

views, supply the muscle-cells of the entire organism with myosinogen.

Still, our analysis alone so far points to the neutrophiles—by far the most numerous leucocytes in the blood-stream—as the ones upon which this great function would devolve. It becomes necessary, therefore, to control this conclusion by showing that excessive muscular exercise, by creating a demand for myosinogen in the cells of all muscles,—skeletal, cardiac, vascular, etc.,—engenders a leucocytosis in which the neutrophiles prevail. The data for this are available in a study of this subject by R. C. Larrabee,[65] who writes as follows: "The paper is based on a study of the blood of four of the contestants in the Boston Athletic Association's Marathon race of 1901. This is a road-race of about twenty-five miles (40 kilometers), held each spring. The severity of the contest will be apparent when it is said that the winner—not included in my four—covered the distance in less than two and one-half hours. This is about ten miles an hour, about as fast as an ordinary man rides his bicycle for pleasure. . . . The blood of these four cases [counted by the author, assisted by Dr. W. H. McBain] before the race showed no abnormalities. The percentage of polymorphonuclear neutrophiles may perhaps run a little high, but this is to be expected in active young men in the best possible physical condition. After the race the blood was taken immediately, within five minutes from the actual finish. In every case a leucocytosis was found, varying from 14,400 to 22,200. The differential count showed that the increase was *mainly* in the polymorphonuclear *neutrophiles*."

That the exciting cause of the leucocytosis was the increase of waste-products which in turn stimulated the adrenal system, hardly needs to be dwelt upon. Vagal influence incited to inordinate activity and controlled the organs charged with the genesis of these particular cells, while the inordinate oxidation processes started by the overactive adrenals in all tissues accounts for the general leucocytosis which the word "mainly" implies.

Myosinogen being a member of the globulin group of proteids, the other members of this group should be represented

[65] R. C. Larrabee: Journal of Medical Research, Jan. 1902.

among the cell's products, particularly fibrinogen found in the blood-plasma in association with serum-globulin and serum-albumin. That such is the case is demonstrable. Stewart[66] alludes to the sources of nucleo-proteid in the following words: "In shed and clotting blood, the only possible sources of nucleo-proteid, so far as we know, are the corpuscles and the blood-plates. The red corpuscles we may at once dismiss, for, although they contain a small amount of nucleo-proteid, not only do they remain intact under ordinary circumstances during coagulation, but there is the strongest evidence, as has already been pointed out, that they do not make any essential contribution to the process. We have left over the leucocytes and the platelets. The latter are said and the former are known to yield *nucleo-proteids* when they are broken up in the laboratory; and it is highly probable that from both, but especially from the white corpuscles, nucleo-proteid is liberated in the first moments after blood is shed, and that this nucleo-proteid *is then changed into fibrin-ferment*."

The relationship between the cellular nucleo-proteids and fibrin which this quotation suggests finds itself sustained by Ranvier,[67] who, alluding to the rôle of granules in the formation of fibrin, says: "Free granulations, which we found in the blood besides the red and white corpuscles, are very numerous; they were termed 'elementary vesicles' by Zimmermann. In a preparation of human blood examined after rouleaux of red corpuscles have formed these granulations may easily be observed, two varieties being distinguishable. The first are spherical, small droplets of fat; the others are angular or variable in shape, and appear at first as if they were fragments of white corpuscles, but differ from the latter in not being altered by water. They are *stained by iodine,* but remain colorless in carmine solutions. We will see that these are also the characteristics of fibrin." After reviewing the phenomena that attend coagulation, and exposure by washing of the fibrinous net-work, he says: "When this preparation is examined and magnified four hundred to five hundred diameters, the fibrinous reticulum can be seen distinctly, and is disposed in a very interesting

[66] Stewart: *Loc. cit.*
[67] Ranvier: *Loc. cit.*, 213.