manner: From an angular granulation, from 11 to 10 μ in diameter, very tenuous fibrils start divergingly, then subdivide, to unite with other fibrils, in order to form a delicate net-work. The preparation is covered with these small net-works, each of which has its central granulation. . . . The granulations which serve as centers for each diminutive fibrinous reticulum have the same microchemical properties as the fibrils."

A normal deduction which seems to me to impose itself in this connection is that fibrin is to the blood what myosin is to the muscle-cells, *i.e.*, a post-mortem product due to arrest of the oxidation process which during life is insured by the adrenoxidase—the supposed "fibrin-ferment." In other words, it not only becomes probable that *peptones, myosinogen, and fibrinogen are products of the same variety of leucocyte, the neutrophile, and therefore chemically similar when liberated from the latter,* but also that *fibrinogen subserves the same purpose in the blood that myosinogen does in muscle:* i.e., *it supplies it with its primary source of functional energy.*

True, the solubility of fibrin differs somewhat from that of myosin, but this is probably due not to a difference in the molecular structure of fibrinogen as against that of myosinogen, but to the influence of the medium in which the granules are dropped by the leucocyte. Indeed, the ashes of fibrin contain a larger proportion of calcium and magnesium phosphate than does myosinogen.

Another conclusion which now seems warranted is that *the neutrophile leucocytes are the agencies which take up proteids in the intestinal canal, and, after submitting them to a process in which various physio-chemical bodies taken up by them in the portal and hepatic systems take part, distribute the products to every part of the organism, including the blood itself.*

Such being the case, the proteids, inclosed in their diminutive carriers, should not be found in the blood of the portal system. Foster writes, in this connection, after referring to the difficulties attending the experimental determination of the path taken by proteids: "Bearing this in mind, we may state that all observers are agreed that peptone is absent from chyle, or at least that its presence cannot be satisfactorily proved. On the other hand, while some observers have succeeded in finding peptone in the portal blood after food, but not during fasting, many have failed to demonstrate the presence of peptone in the blood either of the portal vein or of the vessels at large, *even after a meal containing large quantities of proteids.*" Again: "If an artificial circulation of blood be kept up in the mesenteric arteries supplying a loop of intestine removed from the body, the loop may be kept alive for some considerable time. During this survival a considerable quantity of peptone placed in the cavity of the loop will disappear: *i.e.,* will be absorbed, but *cannot be recovered from the blood* which is being used for the artificial circulation, and which escapes from the veins after traversing the intestinal capillaries. The disappearance is *not due to any action of the blood itself,* for peptone introduced into the blood before it is driven through the mesenteric arteries in the experiment may be recovered from the blood as it escapes from the mesenteric veins. It would seem as if the peptone were changed before it actually gets from the interior of the intestine into the interior of the capillaries."[68] Viewed from my standpoint, *the peptones are hidden in the neutrophile leucocytes which the follicles of the segment continue to produce. These cells, after migrating over the serum-bathed (and thus constantly asepticized) epithelial surface, and ingesting their burden, find their way into the villi's venules and thence into the mesenteric channels.*

If the foregoing analysis and the various deductions submitted are sound, the neutrophile leucocytes must fulfill a rôle in the organism commensurate with their relative proportion in the blood-stream. Indeed, the following conclusion appears to me to have been sustained:—

The neutrophile leucocytes, through the intermediary of their granules, the β granulations of Ehrlich, supply (1) the blood and all tissues (excepting the nervous system) their nutritive elements: i.e., *peptones; and (2) the muscles and the blood, the compounds from which they obtain their mechanical energy when exposed to the action of the adrenoxidase:* i.e., *myosinogen and fibrinogen.*

Ehrlich's Eosinophile Leucocytes.—Metchnikoff does not grant Ehrlich's eosinophiles phagocytic properties, these

[68] All italics are my own.

cells being unable to inglobe foreign bodies. Again, as emphasized by Ehrlich, the granules of these cells are only stainable with acid dyes, the other varieties either taking only alkaline dyes or simultaneously, as does the neutrophile just reviewed, both acid and alkaline dyes, etc. This marked affinity for acids obviously gives the eosinophile an identity of its own, while its non-phagocytic functions as clearly separate it from the finely granular cell just reviewed, which is essentially phagocytic. Ehrlich's eosinophile is usually considered under the heading of "coarsely granular oxyphile cell."

These cells only represent from 2 to 4 per cent. of all the leucocytes in the blood-stream, but this proportion is rapidly increased during disease. Kanthack and Hardy, in the article previously quoted, describe them as follows: "The coarsely granular oxyphile cell, or eosinophile cell, varies in size in different animals, not only absolutely, but relatively to the dimensions of the other classes of cells. In man it is larger than either the hyaline cell, the finely granular oxyphile cell or the finely granular basophile cell. In the rat, rabbit, and guinea-pig, on the other hand, it is smaller than the largest hyaline cells, but larger than the finely granular oxyphile and basophile cells.

"The *nucleus* is typically an elongated body bent to form a horseshoe. In the rat the arms of the horseshoe are carried so far round that in film preparations the ends often overlap, giving to the nucleus the appearance of a circle with a large hole in the center. Sometimes the nucleus is lobed; but we are inclined to regard this appearance as being largely due to the stresses to which the nucleus is subjected when the cell is dying. In the living cell at rest, when it is spherical, the shape of the nucleus, so far as it can be determined by the disposition of the cell-granules, is a simple horseshoe or crescent. A distinct nuclear net-work is present.

"*Cell-granules.*—The cell-granules are relatively large, spherical, or slightly ovoid bodies, and are sharply marked off from the cell-substance by their *very high refractive index,* which is so great that in fluid preparations the granules have a *brilliant, greenish luster.*[69] The cell-substance in which they are imbedded has the appearance of a clear, transparent, structureless jelly. The intensity of the oxyphile reaction of these granules differs in different animals, but is always high. Thus, it is very high in the case of the granules of man, these staining with eosin dissolved in 95 per cent. alcohol. . . . The granules also stain with weak acid dyes, such as Orange *G,* hæmatoxylin, and sodium sulphindigotate. Ehrlich-Biondi's mixture (washed out with 95 per cent. spirit) colors these bodies brown-purple, and the 'neutral' mixture (washed out with water) stains them a very intense purple. Corrosive sublimate increases the oxyphile reaction, as does also heat when applied to the dried film."

[69] All italics other than those of the side headings are my own.

Gulland found Heidenhain's iron-hæmatoxylin extremely valuable to counteract "the bright refraction of the granules" which "blinds the eye to the presence of the threads" (my canaliculi). The granules are stained opaquely in shades of black and gray. He was thus able to ascertain that the granules varied greatly as to size, the smallest granules lying close to the astrosphere and the larger at the periphery, the arrangement pointed out by Heidenhain and shown in Figs. 10, 12, and 16 of Gulland's plate. In the newt's blood, as already stated, "these cells are markedly amœboid, and have the habit of throwing out circular pseudopodia, which are often connected to the main part of the cell only by a very delicate thread." Gulland illustrates this feature in Figs. 3 and 6 of his plate, and states that "it is evident that the threads are often broken through and the spherical portion of the cell-body set free, as the blood contains a large number of them." He also refers to the fact that, "when the eosinophile cells are found degenerated in blood or pus examined in the fresh state, the granules are always in the Brownian movement."

In the study of the granules of neutrophile cells I referred to the chemical analysis of Milroy and Malcolm and to various points of dissimilarity between these cells and the coarse oxyphiles now in question. Considered from the standpoint of the latter, these investigations showed that, while neither alcohol nor ether, nor both of these agents used successively, produced alterations in either variety, the failure of the latter process *excluded* the possibility of their consisting of

fat or *lecithin*. Weak alkaline solutions at about 120° C. caused (a feature referred to by the authors as striking) the removal of practically all the granules of the finely granular cells (the neutrophiles), and "persistence of two structures, the nuclei and the coarse oxyphile granules." Acetic acid in alcoholic solution and oxalic acid caused partial removal of both granules, but "sodium ethylate in alcoholic solution removed the fine oxyphile granules almost completely and only affected the coarse ones to a slight extent."

The authors, while concluding that the granules might also be nucleo-proteid in nature, *i.e.*, similar to those of the neutrophile cells, account for the discrepancies in the results of their analyses by the following argument: "The fact that weak acid solutions dissolve both types of granules at least partially is not against the view that they are nucleo-proteid in nature, because these bodies are more easily soluble in weak acid solutions than almost any other complex proteid. The fact that some granules are undissolved, while others are removed, is probably due to the fact that the former have undergone coagulation, while the latter have been rapidly fixed, although it may be also due to the nature of the salts which are combined with the proteid."

Still, the very high refractive index to which Kanthack and Hardy and Gulland refer is not characteristic of the neutrophile granules, and this seems to me to testify against an absolute functional similarity between them and the granules of the eosinophiles. Indeed, with the plasma as excipient for the adrenoxidase, we can as readily account for the presence of the "brilliant, greenish luster" witnessed by the above authors as we can for the phosphorescence of the photogenic organs of lightning-bugs: *i.e.*, by the simultaneous presence of phosphorus and oxygen. This seems to me to indicate that we are dealing with a nucleo-proteid body, as Milroy and Malcolm contend, but with one richer in phosphorus than that forming the neutrophile granules.

What are the functions of the eosinophile leucocytes in the organism? The high percentage of phosphorus in their granules suggests the possibility of their being lecithin carriers; but we have seen that the investigations of Milroy and Malcolm clearly show that this organic body is absent. L. F. Barker,[70] of Baltimore, noted the presence of iron in the granules of the eosinophile leucocytes,—a point which he thinks may be of some value in determining the significance of the leucocytic granulations,—but we cannot consider them as the cells intrusted with transportation of iron from the intestine, for they are not phagocytic. Indeed, it has now become evident that the neutrophiles are intrusted with this function, for Macallum used albuminate of iron. The intestinal leucocytes of his previously starved animals evidently took this substance up as they would the proteids of their usual food. Barker's observation, however, adds another link to the chain of evidence which unites the eosinophiles to the neutrophiles, for, in addition to being both nucleo-proteid carriers, they now become also iron carriers. By tracing the itinerary of this iron we may, therefore, obtain a clue to the true identity of its cellular host.

The phagocytes seen by Macallum to ingest the albuminate of iron being assimilated to those charged at all times with the duty of selecting proteids from the intestinal foodstuffs, it becomes a question as to where they can part with their iron in order to facilitate its absorption into the hæmoglobin molecule, of which, as is well known, it forms an important constituent. From the intestine the iron is carried to the portal system, thence into the hepatic lobule. It must be here that the phagocytic leucocytes must take part in some process related to the elaboration of hæmoglobin, for we have seen on page 335 that in the spleen the leucocytes are formed *in situ*, pass out into the pulp-channels, take up the iron-pigment (probably that of disorganized red corpuscles), and carry it to the liver. Again, and for reasons which are there given, I was led to conclude (page 339) that bilirubin and iron were used to build up the hæmoglobin in the lobular (hepatic) capillaries. The liver, therefore, seems to receive iron from both directions—intestine and spleen—a normal mechanism when we consider that the liver's blood passes almost directly to the heart, and thence to the lungs.

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[70] L. F. Barker: Johns Hopkins Hospital Bulletin, Oct., 1894.

How do the eosinophile (*non-phagocytic*) leucocytes acquire their iron? We can hardly imagine that when the splenic or intestinal leucocytes reach the hepatic lobule their contents or any part thereof is disgorged to enable another cell to appropriate it. Indeed, there is not the slightest evidence that such a process occurs, although the eosinophile has already been shown to contain not only iron, but also the other main constituents of the neutrophile cell. There exists a physiological process, however, through which the eosinophile can acquire all the attributes of the latter: *i.e.*, by mitosis, a mode of cell-multiplication known to apply to leucocytes and particularly to neutrophiles. Gulland refers to this feature in the following lines: "The cells which one sees dividing or about to divide have generally the appearance of medium-sized hyaline cells, with a relatively large, rounded nucleus and a comparatively small cell-body, in which the mitoma is not easily made out. But there is no doubt that cells with horseshoe-shaped nuclei [the eosinophiles] divide, and that the nuclei may even advance as far as the spirem stage without altering their shape. Cells with more markedly polymorphous nuclei, as, for instance, the *ordinary oxyphile cells*, certainly divide also, but they seem generally to go through a preliminary resting stage in which the polymorphous nucleus returns to the rounded form."

In Gulland's plate, Figs. 3 and 6, which refer to eosinophiles from newt's blood, graphically portray a secondary process through which these cells can subdivide, or rather yield a portion of their substance. In 3, a spherical pseudopod is in the act of being formed; in 6, three similar masses appear, the lowest of which is on the point of being separated by the mother-cell. Referring to the bridges that connect net-works of granules with basophile leucocytes, Gulland remarks: "I have little doubt that when that stage is reached [he associates the phenomenon with a supposed process of degeneration] these bridges are torn across and the granules are actually left behind. This forms an exact parallel to what happens in the *eosinophiles* of the newt's blood."

It thus becomes evident that recognized cytological phenomena sustain the conclusion that *neutrophile leucocytes are the parent-cells of eosinophile leucocytes,* and that *eosinophiles can part with segments of their cell-substance.*

But does the process of neutrophilic mitosis actually occur in the liver? M. Duval,[71] in his study of the hæmatopoietic functions of this organ, refers to the proportion of the red to the white corpuscles in the blood of the portal vein as compared to that in the hepatic vein, and writes: "Researches in this connection give as result: 1 white corpuscle to 746 red in the *portal* vein, and 1 white corpuscle to 170 red in the *subhepatic* veins. This difference can only be due to a production of white corpuscles in the liver or to a destruction of red corpuscles." That red-corpuscle destruction is a function of the spleen is sustained by the presence "in the spleen-pulp," using Foster's words, of red corpuscles "in various stages of disorganization, some of them lying within the substance of large colorless corpuscles, and, as it were, being eaten by them." The presence of blood-pigments in the liver has been thought to indicate that red corpuscles were destroyed in this organ; we have seen, on the contrary, that it is the seat of a reconstructive process of which hæmoglobin is the product. Though the liver may be a seat of destruction for red-cell fragments, the likelihood that any entire corpuscle leaves the capillaries of the hepatic lobules to penetrate the cells is so remote that it can be left out of question. On the other hand, we have seen that these capillaries are the seat of the more important processes connected with the blood. It seems probable, therefore, that the liver, owing in part to the inordinate temperature of its lobular channels (106° F.; 41.9° C.), is also the seat of the mitotic process.

"At a certain period," write Böhm, Davidoff, and Huber,[72] "the embryonic blood consists principally of nucleated red cells, which proliferate in the circulation by indirect division. The colorless blood-cells, the development of which is not yet fully understood, appear later. It is possible that they also are elements of the blood-islands, which do not contain any hæmoglobin. In a later period of embryonic life the liver becomes a blood-forming organ. Recent investigations have shown, how-

[71] M. Duval: "Cours de Physiologie," p. 200.
[72] Böhm, Davidoff, and Huber: *Loc. cit.*, p. 168.

ever, that it does not take a direct part in the formation of the blood, but only serves as an area in which the *blood-corpuscles proliferate* during their slow passage through its vessels. The *blind, sac-like endings of the venous capillaries* seem to be particularly adapted for this purpose, as in them the blood-current stagnates, and it is here that the greater number of blood-cells reveal mitotic figures. The newly formed elements are finally swept away by the blood-stream and enter the general circulation."

Gulland likewise states that the eosinophile cell is derived from the "finely granular acidophile" (the neutrophile), and the latter is itself traced back to the lymphocyte. "The transition-forms between the finely granular and the coarsely granular acidophile cells are seen much more frequently in the bone-marrow than in the blood," says this investigator, "and it seems certain that both from this source and from mitotic division the main source of the eosinophile cells is in the bone-marrow." That there is ample margin for my view that mitosis may occur in the liver is also suggested by the following additional lines: "They must arise *elsewhere,* however, *in abundance,*[73] for Schaffer[74] and I[75] have shown that they are present in the thymus and in lymphatic glands before either bone or bone-marrow is properly formed at all, and Engel[76] has seen them in the chick's blood on the fifth day of incubation. In the transition-forms (see Figs. 2, 8, 11) there is little in the general shape of the cell and nucleus to distinguish them from the preceding stage." All the evidence tends to show, therefore, that *the process of mitosis, through which eosinophile leucocytes are formed from neutrophile leucocytes, is carried on in the capillaries of the hepatic lobules, though it can also occur elsewhere in the organism.*

I have referred to the direct path which leucocytes can follow from the liver to the heart and thence to the lungs. If eosinophiles are formed in the liver, therefore, the lungs should show indications of the presence of these leucocytes. Proof that such is actually the case is obtainable with the aid of pathology: *i.e.,* the significant fact that in several pulmonary diseases eosinophile cells are to be found in the sputum. Teichmüller,[77] for instance, has not only found this to be the case in pulmonary tuberculosis, but considers an increase of these cells favorable from the standpoint of prognosis. In asthma, though a non-ulcerative process is present, eosinophiles are to be found in abundance in the sputum, and Gollasch[78] states that they are connected with the formation of the Charcot-Leyden crystals. Lenhartz[79] states that "it is not improbable that the majority of cells designated as 'alveolar epithelia' are variously altered forms of leucocytes. The protoplasm very frequently shows fine or *coarsely granular* fatty metamorphosis, which is characterized by the *strongly refractive index.*"

[73] The italics are my own.
[74] Schaffer: Centralbl. für die med. Wissen., 1891.
[75] Gulland: Journal of Path. and Bacteriol., 1894.
[76] Engel: Archiv f. mikr. Anat., vol. lxiv, 1894.

The irregularity of the granules, and the manner in which they form fibrin, as described by Ranvier, and the peculiar color of the granules are recalled by the following description of the Charcot-Leyden crystals by Lenhartz: "The Charcot-Leyden crystals are delicate, very sharply pointed octahedra which occur in very variable size. They present a sometimes water-clear, transparent, sometimes a slightly yellowish-green, Rhine-wine color; they occur either isolated or in dense collections which here and there are jumbled together, or in uniform rows, following the mucous shreds." The same author also says: "The crystals were first found in the sputum by Friedreich in croupous bronchitis. On the other hand, Leyden has drawn attention to their frequent occurrence in asthmatic expectoration."

The association with various pulmonary diseases obviously suggests that their presence is pathological, whereas we consider their presence in the lung as normal, and their *elimination* in their recognizable form as an accompaniment of the morbid state. That such is the case is shown by the fact emphasized by Lenhartz that: "The longer the asthmatic subject *is free from* paroxysms,—that is, the more time allowed for the formation of the crystals,—the more densely the spirals are studded with these crystals."

[77] Teichmüller: Lenhartz's "Manual of Clinical Microscopy," translation by H. T. Brooks, 1902.
[78] Gollasch: Fortschritte der Med., vol. 1889.
[79] Lenhartz: *Loc. cit.*

While all these facts sustain my opinion that the lungs show ample evidence of the presence in them of eosinophile cells and of their granules, their identity as offsprings of the neutrophiles should be demonstrable here, as elsewhere, through their chemical properties. Indeed, their identity as daughter-cells of neutrophile leucocytes does not disappear even in the lungs, for both acids and alkalies can dissolve them, while the test common to both neutrophile and eosinophile granules, *i.e.*, insolubility in alcohol, is also applicable here. Lenhartz not only confirms this assertion by saying, in reference to the crystals: "They are readily dissolved in warm water, acids, and alkalies, but are *insoluble*[80] in alcohol"; but we also, it seems to me, can consider, as confirmation of my interpretation of the identity of the granules from which the crystals were derived, his statement that: "fixation of the air-dried preparation for one hour in absolute alcohol and subsequent staining with Chenzinsky's *eosin*-methylene-blue solution also gives very good results."

All these facts further confirm the origin of the eosinophile leucocytes from the liver, for there is no other path that would have brought them to the lungs. They also seem to me to indicate that, *after their formation by mitosis in the liver, eosinophile leucocytes are carried to the pulmonary lobules.*

This question has already engaged the attention of pathologists, including Virchow, Wagner, and Cohnheim. Lenhartz's view is fully sustained by my own investigations, however, when he says: "It is not improbable that the majority of the cells designated as *'alveolar* epithelia' are variously altered forms of leucocytes. The protoplasm very frequently shows *fine* or *coarsely* granular fatty metamorphosis, and is characterized by the strongly *refractive index.*" Again, while Lenhartz expresses his belief that the positive identification of the "alveolar epithelia" is "extremely difficult," he states that he understands thereby "the large oval or round polygonal cells, three to six times as large as a white blood-corpuscle, which are found in almost every sputum. The usually large cell-body is *coarsely granular,* and contains one or several vesicle-like nuclei." The true identity of epithelium of the

[80] These italics are Dr. Lenhartz's.

alveoli and, therefore, of the lobule of which they form part now seems clear, if interpreted in the light of the data I have submitted: The cells to which Lenhartz refers, *i.e., the lobular epithelial cells, are aggregates of the polynuclear neutrophiles and of the daughter-cells of the latter, the eosinophiles.*

We have seen that the neutrophiles start from the intestinal canal; that Macallum and L. F. Barker found leucocytes gorged with iron in this region, and, finally, that *some* bilirubin at least is recovered from the intestine—obviously, now, by leucocytes. We have traced the latter from the intestinal canal, through the portal system, liver, hepatic veins, heart, thence to the alveoli. After giving the formula of hæmoglobin, Foster writes: "It will thus be seen that hæmoglobin contains, in addition to the other elements usually present in *proteid* substances, a certain amount of *iron,* that is to say, the element iron is a distinct part of the hæmoglobin molecule, a fact which of itself renders hæmoglobin remarkable among the chemical substances present in the animal body." Kanthack and Hardy noted, as previously stated, that "in fluid preparations the granules have a brilliant, greenish luster"—a characteristic of fine hæmoglobin crystals. Hæmoglobin is readily soluble in blood-serum, as are the granules, we have seen. Ether coagulates hæmoglobin; it caused, in Milroy and Malcolm's experiments,[81] the granules to lose a part of their refractive power, even when boiling ether was used. The proteid constituents of the granules of the neutrophiles, myosinogen and fibrinogen, belong to the globulin group.

This recalls my statement in the first edition of this work (p. 441), in respect to the manner in which the heart-muscle was nourished: "Paradoxical as the statement may seem, I was led to conclude that the minute granules referred to on page 433"— a general outline of the prevailing views concerning the histology of the myocardium, in which the minute pigment-granules, easily seen therein microscopically, are mentioned—"were actually supplied to the heart through the intermediary of leucocytes. These cells were found to migrate from the liver (also through the hepatic veins) to the inferior vena cava, where

[81] Milroy and Malcolm: *Loc. cit.*, p. 112.