in reality not ferments at all, but compounds of several substances having marked affinity for one another, and formed adventitiously when removed from their normal environment, the living body. As will be shown in the next chapter, one of these substances, *proteid, is a passive constituent of these so-called ferments,* while the three others, the zymogen, the nuclein and the adrenoxidase, are active, the active principle of adrenoxidase remaining, as stated above, the only true ferment.

THE TRIADS.—The compounds now termed "ferments" represent, nevertheless, aggregates of bodies which jointly take part in all fermentative processes, and it is necessary, in order to interpret satisfactorily the functions in which they take part, to treat them as autonomous entities. Especially is this desirable in view of the fact that as such they retain the specific properties now attributed to them as "ferments." In succeeding chapters, however, they will not be referred to as ferments, but as "triads," each being composed of a zymogen, nuclein and adrenoxidase. To indicate the specific action of each triad, the familiar terms "proteolytic," "amylolytic," "lipolytic" and "glycolytic" will be employed as qualifying adjectives, according to whether the food-stuff hydrolyzed is proteid, starch, fat or sugar. Thus the triad which acts on proteids will be referred to as the "proteolytic triad;" that acting on sugars as the "glycolytic triad," etc.

CHAPTER XV.

THE ADRENAL ACTIVE PRINCIPLE AS THE DYNAMIC ELEMENT OF LIFE AND THE GRANULATIONS OF LEUCOCYTES AS THE LIVING SUBSTANCE.

THE LEUCOCYTES AS TISSUE BUILDERS.

Beddard, referring to the end-products of intestinal digestion in Leonard Hill's recently published (1906) treatise, writes:[1] "Our knowledge of the actual path taken by different substances is extremely meagre. We know that the products of fat digestion pass into the epithelial cells, but we know nothing of the path taken by the products of proteid and carbohydrate digestion."

In the first volume[2] I pointed out that the granulations of the leucocytes played a far more important rôle in physiological functions than was credited to them in text-books, and that in all tissues these granulations were a source of energy when brought into contact with the oxidizing substance, *i.e.*, adrenoxidase. The final conclusion submitted, after showing that the leucocytes took up the products of intestinal digestion in the alimentary canal to convert them into granulations which they deposited in the tissues, was that these cells supplied "the entire organism with the agencies which combine with the oxidizing substance to insure the continuation of life and the efficiency of all organic functions." Additional researches have only served to strengthen this conclusion.

A bar to progress in our knowledge of the function of these minute bodies is the prevailing view that "elementary granules [the granulations of leucocytes] are minute particles of proteid matter, probably arising from the *disintegration* of white corpuscles or of the blood-platelets." That leucocytes do not physiologically yield their contents by becoming decom-

[1] Beddard: Hill's "Recent Advances in Physiology and Bio-Chemistry," p. 643, 1906.
[2] *Cf.* vol. i, pp. 677 to 728 in the first three editions.

posed or ruptured in the intestinal cavity or in the blood, has been repeatedly emphasized. Dastre,[3] after a careful review of the labors of other investigators and personal experiments, lays stress upon "the erroneous character of the classic doctrine," having found, in accord with the views of Buchner, that leucocytes were not the fragile cells they were generally believed to be. Microscopical observations showed that, as previously observed by Hayem, Ranvier and Stassano, coagulation of blood, to which these cells are known to contribute, occurred without any destruction of leucocytes, a process thought necessary, according to the prevailing doctrine, for the escape of the cell contents. Such being the case, he was led to ascribe the elimination of fibrin-ferment by these cells to osmosis. Arthus[4] ascertained experimentally, however, that they actively *secreted* this ferment. "In the protection of the organism against hæmorrhages," says this observer, "leucocytes play a secretory, active, physiological, rôle. They are not the passive agents which, as has been erroneously supposed, generate fibrin-ferment by cadaveric destruction." Indeed, I suggested in the first volume[5] that the networks in these cells are canaliculi which traverse them in all directions, and that their digestive vacuole, their nucleus, and their canalicular system (the mitoma), point to them as highly-organized, though diminutive, secreting organs. "The various ferments they contain," says Carles, in a recent work,[6] "make of each cell a minute laboratory."

Considerable evidence to the effect that leucocytes are secretory organs is afforded by the researches upon the identity and functions of the leucocytic granules, which Hankin, fourteen years ago, was first to regard as secretory products. As we have seen,[7] Kanthack, Hardy and Keng have taken much the same view. No less an authority on the subject than Ehrlich also considers granulations in the light of a secretion, and holds that certain basophile granules constitute a preliminary step (*Vorstufe*) in the elaboration of typical eosinophile granu-

[3] Dastre: C.-r. de la Soc. de Biol., vol. lv, Nov. 14, 1903.
[4] Arthus: *Ibid.*
[5] *Cf.* vol. i, pp. 677 to 728, in the first three editions.
[6] Carles: "Du rôle des leucocytes dans l'absorption et l'élimination," Paris, 1904.
[7] *Cf.* vol. i, p. 679 *et seq.*, in the first three editions.

lations. That they are developed with relative suddenness from non-granular mononuclears, and that they are constantly being developed, also suggests, according to Levaditi,[8] that they are secretory products. Stokes and Wegefarth[9] observed, in blood taken from some 500 patients, that at 35° C. the leucocytes became actively amœboid, their rapid motions resembling the swarming of bees around a bee-hive, and that after this the number of free granules in the plasma was increased. Sangree[10] saw eosinophile granulations leave the cell and wander away oscillating very actively. Bail[11] also saw granulations leave the periphery of the cell. Gulland[12] noted that leucocytes show a marked tendency to leave their granules behind them. Finally, Leo Loeb[13] observed that if a cell was drawn out with a needle, in order to produce the elimination of a widely diffused mass of granules, many granules could be "seen to move very actively."

What is the origin of these granulations? Are we dealing with a *sui generis* product of the leucocytic protoplasm, or with the end-products of materials ingested by the cell? If, as shown above, we have abundant proof that leucocytes secrete their granules, we have in phagocytosis as evident a demonstration that they ingest assimilable materials; and if to this we add the known facts that they contain proteolytic ferments, and that they are able to digest what they ingest, we cannot but conclude that their granulations are elaborated out of the materials they appropriate while carrying on their function as scavengers.

This, in turn, suggests another question: May the food-materials of the alimentary canal not serve as their main source of supply for this purpose? Not only do the leucocytes ingest food-stuffs in the intestinal canal, as shown below, but also the "ferments," *i.e.*, the various hydrolytic triads which carry on the digestive process in the intestine, to continue this process in their digestive vacuole and elaborate granulations as end-products.

[8] Levaditi: "Le leucocyte et ses granulations," Paris, 1902.
[9] Stokes and Wegefarth: Bull. Johns Hopkins Hosp., Dec., 1897.
[10] Sangree: Phila. Med. Jour., Mar. 12, 1898.
[11] Bail: Berl. klin. Woch., Oct. 11, S. 887, 1897.
[12] Gulland: Jour. of Physiol., vol. xix, p. 385, 1896.
[13] Leo Loeb: Univ. of Penna. Med. Bull., Apr., 1905.

According to present teachings, the products of intestinal digestion pass into the general circulation in liquid form. Moore,[14] for instance, says: "The new materials formed by the action of the *intestinal epithelial cells* on the absorbed products of digestion pass out of these cells into the lymphoid tissue of the villus underlying them. The modified carbohydrates and proteids pass in solution into the lymph which bathes the tissue, and in *soluble form* are absorbed by the capillary vessels of the villus, thus passing directly into the portal circulation, while the fats leave the epithelial cells as fat globules, and are carried as such past the capillary network of the villus, to enter the lacteal situated in the axis of the villus." Yet, as stated by other physiologists, Brubaker,[15] for example, "the mechanism by which the [epithelial] cells effect this passage of the food is but imperfectly understood." Indeed, in a recent monograph L. Mendel,[16] reviewing the advances in our knowledge of the chemical processes of digestion, remarked that "beyond the intestinal wall, in the blood and lymph stream, the cleavage products seem, for the most part, to be *missing*." This fact, in itself, affords strong evidence in favor of my view that proteids are supplied to the tissues in the form of leucocytic granules, since this conception involves the ingestion by intestinal leucocytes of the cleavage-products from the alimentary canal.

It is but normal, under these conditions, that these products should be absent from the blood and lymph streams. Indeed, Howell[17] states that: "The form in which proteid is absorbed remains . . . . a mystery."

Twenty-five years ago Hofmeister[18] compared the leucocyte to the red blood-corpuscle, the white cell carrying *peptones* from the intestinal wall to the tissues, while the red cell carried oxygen to the latter. Laboring, however, under the impression that peptones reached the tissues as such and in *fluid* form, being liberated by the leucocytes in some unexplained way, he was unable to defend his theory successfully, and it is

[14] Moore: Schäfer's "T. B. of Physoil.," vol. i, p. 432, 1898.
[15] Brubaker: "Human Physiology," eleventh edition, p. 115, 1902.
[16] L. Mendel: Med. News, May 20, 1905.
[17] Howell: "T. B. of Physiol.," p. 716, 1905.
[18] Hofmeister: Zeit. f. physiol. Chem., Bd. iv, S. 253, 268, 1880; Bd. v, S. 127, 1881; Bd. vi, S. 69, 1882.

now but rarely referred to. A view similar to Hofmeister's was recently (1905) advanced by Pavy.[19]

Hofmeister's view was opposed mainly by Neumeister[20] and Heidenhain,[21] but on very weak grounds. Moore,[22] for instance, reviewing the evidence against Hofmeister's contention, says: "In the first place proteid is not absorbed to any appreciable extent by the lymphatics." But this only shows that it is not their function to do so; granular leucocytes, which are *known* to contain *peptones*, being present in the intestine, thus become the normal absorbents. The second reason adduced is that "albumoses are not changed, as Hofmeister himself has shown, in the blood, which contains plenty of leucocytes." This only proves that it is the function of the leucocytes to absorb albumoses in the intestinal canal but nowhere else, and that it is within these cells that the albumoses are changed. The third reason is that "Heidenhain has shown that the amount of leucocytes in the wall of the intestine (and the amount of active mitosis in these) is too small to render them adequate for the purpose." This is an invalid conclusion unless Heidenhain can give the actual number required *during* the digestive process, and one in fact contradicted by more recent observations, as we shall see. The fourth reason is that Shore[23] has shown that "after slow injection of a small amount of peptone (.049 gramme) into a lymphatic of the hind-limb in a dog, this can be detected again in the course of twenty minutes in the chyle flowing from a fistula of the thoracic duct, showing that it has traversed the lymphatic system unchanged." This merely affords proof that the lymph—and, I may add, the blood—are not media in which the *digestive* leucocytes ingest peptones, and that the only organs in which they do so physiologically are those of the alimentary canal, viz., the stomach and intestinal canal.

Hofmeister based his conception that these cells carried peptones from the intestine to the tissues upon (1) the presence of an enormous number and active proliferation of leucocytes in the intestinal wall during proteid absorption; (2) the

[19] Pavy: Transvaal Med. Jour., Oct. 1, 1905.
[20] Neumeister: Zeit. f. Biol., Bd. vi, S. 277, 1888.
[21] Heidenhain: Arch. f. d. ges. Physiol., Bd. xliii, Suppl., S. 69, 1888.
[22] Moore: *Loc. cit.*, vol. i, p. 441.
[23] Shore: Jour. of Physiol., vol. xi, p. 553, 1890.

increase of leucocytes in the blood-stream, which follows a proteid meal; and (3) the marked decrease of leucocytes in the intestinal canal during fasting. On the other hand, he found *no peptone in the blood-plasma*, and it is now a recognized fact that neither peptones nor albumoses appear as such in the blood. Indeed, when injected into the latter, it is not assimilated and is excreted in the urine (Bunge). Hofmeister also found peptones in blood-clots where leucocytes had accumulated, and a large amount of peptones in the spleen where these cells were correspondingly numerous. Moreover, Pohl[24] observed an increase of leucocytes in the intestine during digestion when the meal was rich in proteids, but not during the digestion of carbohydrates, fats, salts and water. He found that this increase during the digestion of proteids was due to cells which migrated from the intestinal wall. He also noted a very large increase of leucocytes in the intestinal veins during the digestion of a meal rich in proteids, over and above the normal number of cells in the corresponding arteries.

Furthermore, the cells are traceable beyond the intestinal walls. Goodall, Gulland and Noël Paton[25] found experimentally that there occurs "during digestion a slight preliminary fall in the total number of leucocytes in the blood-stream" (which points to a possible concentration of the appropriate leucocytes towards the intestinal structures); that "a fairly regular rise in the total number of leucocytes then follows and the maximum in about four hours after food"; that the increase "is due (*a*) to a lymphocytosis which is very constant as regards its incidence and degree, (*b*) in the majority of cases to a polymorphonuclear leucocytosis." Cabot[26] also states that the increase of leucocytes during digestion is a polymorphonuclear leucocytosis. As polymorphonuclears are all granulation-forming cells, it is evident that such cells migrate to the intestine. In the first volume, I attributed to neutrophile leucocytes the rôle of taking up food-products from the intestine and showed that as lymphocytes they could originate from the lymphatic glands of the intestine. This is

[24] Pohl: Arch. f. exp. Path. u. Pharm., Bd. xxv, S. 31, 1888.
[25] Goodall, Gulland and Noël Paton: Jour. of Physiol., vol. xxx, p. 1, 1903.
[26] Cabot: "Clinical Examinations of the Blood," 1901.

sustained by Gulland's[27] researches, which have shown that "lymphocytes are the precursors of all forms," and Escherich and Schurg's[28] conclusion that lymphocytes are polynuclear leucocytes with neutrophile granulations. Neutrophiles, as is well known, are by far the most numerous of the leucocytes found in the blood.

Do leucocytes ingest materials in the intestinal canal?

The investigations of Macallum[29] were briefly reviewed in the first volume. This observer having administered albuminate of iron to animals, found that this metal was "deposited *in* leucocytes." These iron-laden cells were found "between the epithelial cells of the tips of the villi," and even more loaded ones were observed to "form a cap, as it were, for the extreme end of the lacteal vessel." He remarks in this connection that "the iron of these cells originates from the food in great part, for if the animal be kept without food for a week, the tips of the villi give but a feeble reaction." Animals that had fasted about four days were fed with commercial peptonate of iron, 100 to 200 grains being given daily for three days. The intestinal mucosa down to the distal end of the small intestine became black when placed in an ammonium sulphide solution, thus showing that the iron had been taken up throughout the entire length of the small intestine. The leucocytes were again found on the tips of the villi, but "others were massed below and in great numbers, and a large number had wandered *between* the epithelial cells in such a way as, in many of the villi, to displace and distort the cells." In an illustration he shows three cells, "in two of which the inner ends appear loaded with iron;" these were fixed "*in the act of transferring*" this metal "to the underlying tissue." This evidently applies to food-stuffs as well, for Asher and Erdely[30] found that while proteids, fats or carbohydrates were being fed to rats, the number of leucocytes in the villi was increased.

Macallum's observations were confirmed experimentally by Cloetta.[31] They not only support those of Hofmeister and

[27] Gulland: Jour. of Physiol., vol. xix, p. 385, 1895-96.
[28] Escherich and Schurg: cited by Einhorn: Post-Graduate, July, 1906.
[29] Macallum: Jour of Physiol., vol. xvi, p. 268, 1894.
[30] Asher and Erdely: Centralbl. f. Physiol., Bd. xvi, S. 705, 1903.
[31] Cloetta: Arch. f. exp. Path. u. Pharm., Bd. xl, S. 29, 1897.

Pohl, but are themselves indirectly sustained by Metchnikoff,[32] who found that however introduced into the body—by way of the skin, the peritoneum, the blood, etc.—soluble preparations of iron were taken up by leucocytes. Kobert[33] also observed that a soluble saccharate of iron was at once absorbed by these cells.

Macallum noted, moreover, that "in all the preparations, the epithelial cells themselves were comparatively free from the iron compound," and that in sections of the small intestine of lake lizards, fed on iron albuminate, the leucocytes were "loaded with an excess of iron, so much so that the ammonium sulphide gave them the appearance of huge collections of greenish-black granules." That the epithelial cells only play a secondary rôle in the process of absorption is also shown by the fact that Hochhaus and Quincke[34] found in a series of micro-chemical experiments that the iron albuminate was precipitated in the form of granules among the epithelial cells of the duodenum, and that leucocytes then passed these granules to the center of the villi. In Macallum's illustration the actual transfer is clearly depicted. Macallum then found the iron-laden leucocytes beyond the villi, *i.e.*, in their venules, in the capillaries of the liver, the spleen, etc., a fact which shows that these cells are capable of taking up a substance in the intestine and of transferring it to the tissues.

This process is also exemplified by the absorption of fats. Thus, in the first volume (1903), I stated that "while leucocytes which ingest proteids from the intestinal food-stuffs pass between the epithelial cells and enter the venules, the leucocytes which ingest fats only carry the latter from the inner limits of the epithelial cells to the interior of the lacteal, and deposit them therein," and that other leucocytes took them up and carried them to the tissues. Schäfer (1885) had previously regarded leucocytes as the agents in fat absorption, while Reuter (1902) found, as stated by Ferguson,[34a] fat droplets "in the tissue spaces as well as in the *lymphatic corpuscles* of the diffuse lymphoid tissue." As the latter author remarks,

[32] Metchnikoff: Annales de l'Inst. Pasteur, vol. viii, p. 706, 1894.
[33] Kobert: Arb. d. pharmakol. Inst. z. Dorpat, S. 123, 1893 u. 1894.
[34] Hochhaus and Quincke: Arch. f. exp. Path. u. Pharm., Bd. xxxvii, S. 159, 1896.
[34a] Ferguson: "Normal Histology and Microscopical Anat.," p. 298, 1905.

this fact "would seem to indicate that other agencies aid in the transit of the fat from the epithelium to the lacteal than are accounted for by the purely mechanical theory of Schäfer." In truth, Schäfer's view differs from mine only in that he observed the first part of the process, *i.e.*, that "leucocytes by their amœboid activity, include the emulsified droplets in the intestinal lumen and convey them into the substance of the villi;" while mine is supplemented by the second phase of the process, that previously witnessed by Reuter, the "lymphatic corpuscles" being the leucocytes which I had traced to the thoracic duct and thence to the tissues.

In a recently-published text-book of physiology (1905), Noël Paton writes: "During the digestion of proteids the number of leucocytes is enormously increased, sometimes to more than double their previous number, and in all probability *it is they which carry the products of digestion from the intestine.*" This physiologist also says, however: "By breaking down in the blood-stream they probably set free the proteids for use in the tissues." We have just seen that the leucocytes need not break down, while, as I pointed out two years before Paton's work was published, they convert *the products of proteid digestion into granulations* and carry the latter to the tissue cells. I wrote at the time that leucocytes thus "supply the entire organism with the agencies which combine with the oxidizing substance [now adrenoxidase] to insure the continuation of life and the efficiency of all organic functions."

If now we gauge the importance of these facts by the multiplicity of the substances which leucocytes are able to ingest and digest, their ability to take up peptones as well as iron in the intestine asserts itself. J. Carles[35] (1904) refers to the rôle of these cells in the body as "immense." "Nothing escapes these scavengers of the economy," says this author, "muscular fibers, red corpuscles, weakened leucocytes are themselves devoured and destroyed." He also writes: "Leucocytes, in fact, thanks to the ferments they contain, are also endowed with a most active *transforming* activity. A given toxic substance modified by them becomes capable of acting as their own food and likewise as *food for the entire organism.*" Ehrlich considers

[35] Carles: *Loc. cit.*, pp. 7 and 9, 1904.

the granulations of his *mastzellen* as nutritive reserves which these, and perhaps other leucocytes, transfer to the connective tissue spaces, where, according to his view, they are supposed to accumulate.

Evidently Hofmeister is no longer alone to speak of the leucocytes as the nutrient cells of the organism. But here it is necessary to recall the misleading feature of his conception: the belief that the leucocytes supplied *liquid peptones* to the tissues. I was first to point out, in the first volume of this work,[36] which appeared in January, 1903, that *peptones* engulfed by leucocytes in the intestine were *converted* into products suitable for assimilation by the tissue cells, this conclusion being based on the foundation upon which Carles also poises his statement, viz., the wonderful "transforming activity" of which leucocytes are capable in the course of their function as phagocytes. I then submitted[37] the conclusion that "the granules in leucocytes are the products of an *intracellular metabolic process* and represent a true secretion."

As I have previously stated, the intestinal digestive process is continued in the leucocytes. The end-product of the intracellular digestion is then built up, and gradually transferred to the periphery of the cell, as explained in the first volume. That the intracellular digestive process is similar to that in the intestine is emphasized by the fact that, as shown below, leucocytes contain the constituents of the pancreatic juice, *i.e.*, the various zymogens and the two components of enterokinase, including adrenoxidase.

The presence of *adrenoxidase* is demonstrable in various ways. In the first volume[38] I stated—Ehrlich having found that the essential conditions to methylene-blue staining were "oxygen saturation and alkalinity" (Barker)—that the fact that leucocytes took this stain showed they contained the oxidizing substance and blood salts. I sustained this conclusion by showing that other stains endowed with reducing properties were taken up by leucocytes. That these cells contain the oxidizing substance is conclusively shown, moreover, by the

[36] *Cf.* vol. i, p. 668 *et seq.*, in the first three editions.
[37] *Cf.* vol. i, p. 682, in the first three editions.
[38] *Cf.* vol. i, p. 540, in the first three editions.

fact that Portier[39] found that leucocytes contained "oxidase" as shown by the guaiac test, an observation confirmed by Brandenburg.[40] M. Labbé[41] states that "the coagulating ferment of the blood, fibrin-ferment, is secreted by leucocytes," and he refers to Hewson,[42] Brücke[43] and Glénard,[44] as having demonstrated this fact experimentally. We have seen that fibrin-ferment as shown by Arthus gives the guaiac-blue test. Labbé also states that the researches of Portier, Salkowski, Abelous and Biarnès and Brandenburg have shown the existence of oxidizing ferment, oxidase, in the white corpuscles.

The presence of *nucleo-proteid* in leucocytes, whether as such, or in the form of its antecedents—the phosphorus-laden side-chain which, by combining with a proteid nucleus, forms this body (Kossel)—is also evident. Thus, as stated by Schäfer,[45] "in plasma obtained by subsidence of the corpuscles, there is most nucleo-proteid in the lower layers which contain most leucocytes; and least in the upper which contain very few." Again, we have seen that nucleo-proteid, as shown by Stassano and Billou,[46] was present in enterokinase. So closely is this reproduced in leucocytes that Delezenne,[47] after a series of experiments, concluded that the "cytase" of leucocytes and enterokinase were identical.

The presence of *trypsin* and other ferments in leucocytes is now recognized as a fundamental feature of phagocytosis. Metchnikoff's "cytase" is regarded by him and by Bordet and others, as a trypsin; Kanthack and Hardy also attribute the proteolytic activity of leucocytes to soluble ferments. The more recent writers refer increasingly to the presence in leucocytes of such a ferment. Thus, Bulloch states[48] that "thousands of facts point to the conclusion that our leucocyte-forming tissues are our great defensive organs against parasitic invasions," and Beattie,[49] referring to leucocytes, says, "the cells act as phagocytes. They englobe, and by a special secretion or

[39] Portier: Thèse de Paris, 1897.
[40] Brandenburg: Münch. med. Woch., Feb. 6, S. 183, 1900.
[41] Labbé: "Le Sang," p. 42, 1902.
[42] Hewson: Sydenham Edition, London, 1846.
[43] Brücke: Arch. f. path. Anat., Bd. xii, S. 81, 172, 1857.
[44] Glénard: Bull. de la Soc. Chim., T. xxiv., 1875.
[45] Schäfer: *Loc. cit.*, vol. i, p. 165.
[46] Stassano and Billou: *Loc. cit.*
[47] Delezenne: C. r. de la Soc. de biol., pp. 283, 591, 893, 1902.
[48] Bulloch: Brit. Med. Jour., Sept. 10, 1904.
[49] Beattie: *Ibid.*, p. 586.