reader[96] "of the existence" in muscle "of a *proteolytic* enzyme" and finally states that "glycolysis occurs in many tissues, and that the agent or ferment to which this is due, is believed by Cohnheim[97] to be rendered active by the internal secretion of the pancreas." That trypsinogen is common to all cells is evident.

The presence of *nucleo-proteid* has been sufficiently emphasized in the preceding section. I showed therein that all cells, from the lowest unicellular organisms up to the highest members of great cell colonies, contained nucleo-proteid granules. I may add the testimony of Chittenden,[98] who wrote recently: "Nucleo-proteids of various kinds are conspicuous constituents of all cells; they are found in all tissues, in all glandular organs, and their widespread distribution may be taken as evidence of their great physiological importance."

The term "oxidizing substance," we have seen, is synonymous with "oxidase," and, in the higher organisms, with *adrenoxidase*. "It has been positively proved by the researches of Jaquet, Salkowski, Spitzer, Röhmann, Abelous and Biarnès, Bertrand, Bourquelot, DeRey-Pailhade, Medvedew, Pohl, Jacoby, Chadot and Bach, and others," says Hammarsten,[99] "that in the blood and different *tissues* of the animal body, as also in plant cells, substances occur which have the property of causing certain oxidations and are therefore called oxidation ferments or oxidases. Little is known in regard to the nature or the manner of action of these bodies." In the thirteenth chapter[100] I stated that, while Claude Bernard, Pavy and Lépine had observed that blood-plasma could oxidize sugar, Pohl, Spitzer and others had found that intercellular and tissue juices produced a similar effect; that Loew had been led by his researches to conclude that "there does not exist a group of organisms or any organ, or even a single vegetable or animal cell that does not contain some catalase;" and finally that "this general occurrence of catalase in the *organized world* cannot be accidental and must have a certain significance." We have

[96] Halliburton: *Loc. cit.*, p. 31.
[97] Cohnheim: Zeit. f. phys. Chemie, Bd. xxxix, S. 336, 1903.
[98] Chittenden: Boston Med. & Surg. Jour., Aug. 17, 1905.
[99] Hammarsten: *Loc. cit.*, p. 7.
[100] *Cf.* this vol., p. 813.
[101] Jolles: Münch. med. Woch., Nov. 22, S. 2083, 1904.

seen also that recently Jolles[101] found that the catalase which decomposes hydrogen peroxide is associated with the red corpuscles. As I have shown,[102] catalase is a name given to adrenoxidase, or its homologue in animals in which adrenals do not exist and in plants, owing to the catalytic properties of its active principle. Adrenoxidase is thus endowed with two properties, viz., that of a catalytic and that of an oxidizing agent. Loew's generalization, therefore, applies to adrenoxidase or its homologue in lower forms. Its presence in the latter is further emphasized by the fact that sponges protect their bodies, according to Ray Lankester,[103] not only by fringes and palisades of spicules, but "also by excretion of poisonous ferments from the surface of the body which have a strongly oxidizing action."

Not only are the three components of trypsin—the ferment itself in fact—thus shown to be present in all tissue-cells and in all organisms, but tissue-catabolism corresponds in its general characters with the digestion of nucleo-proteids as carried on by trypsin—the hydrolytic triad—in the intestine.

As stated by Barnes,[104] there is a remarkable uniformity in the decomposition of products of all cells. "No matter what the organism from which they are derived," says this plant physiologist, "no matter how simple they are or how complex, when broken up by the process of digestion or by boiling with acids, they yield invariably a series of products which have become in the last few years much better known. These are amino- or amido-acids, such substances as leucin, tyrosin, arginin, glutamin, glycocoll, etc." As this applies to the digestive process in the intestine as well as to artificial digestion, we can conclude that if these decomposition products are also excreted by the tissue cells, these cells are the seat of a digestive process similar to that of the intestinal canal.

That such is the case is shown by the fact that the precursors of urea, which include the amino-acids, can be traced to the tissues. Salkowski,[105] Schultzen and Nencki,[106] and other chemists, have shown that amino-acids are converted into urea during the transit through the body. That the liver is

[102] *Cf.* this vol., p. 822.
[103] Ray Lankester: *Loc. cit.*
[104] Barnes: Science, vol. xxi, No. 529, p. 241, 1905.
[105] Salkowski: Zeit. f. physiol. Chemie, Bd. iv, S. 100, 1879.
[106] Schultzen and Nencki: Zeit. f. Biol., Bd. viii, S. 124, 1872.

not the only organ in which this conversion occurs, as was formerly believed, may be shown in various ways. Experimental removal of this organ by Slosse,[107] Nencki and Pawlow,[108] and others, failed to arrest the formation of urea, while Kauffmann[109] found that when the liver and kidneys were entirely isolated from the circulation (to which, we have seen, the lymph carries waste-products derived from the cells), the blood was found to contain an excess of urea. This was confirmed by the researches of Wurtz, which showed that under the same conditions, "lymph contains more urea than does the blood of the same individual" (Schäfer[110])—considerably more, in fact, since the ratio is 0.009 parts per cent. in the blood to 0.016 parts per cent. in the lymph.

Finally, Halliburton[111] states that "there can be but little doubt that muscular tissue, being our most abundant tissue, is the ultimate source of most of the nitrogenous waste that leaves the body as urea." Indeed, Barnes refers to the ease with which lactic acid (and this applies also to the familiar muscular acid, *i.e.*, sarco-lactic acid) "can be converted into an amido-acid, glycocoll." Chittenden also says:[112] "Muscles, liver, kidneys, lymph-glands, lungs, spleen, etc., all contain proteid-dissolving ferments, and when the tissues are subjected to autodigestion or autolysis, such products as the amido-acids, leucin and tyrosin, tryptophan, glycocoll, hexone bases or diamino-acids and ammonia result from the breaking down of the various proteids of the tissue." He closes the paragraph with the statement that "the general trend of action with these intracellular proteolytic ferments is *hydrolytic cleavage*, much the same as the influence exerted by mineral acids, or by *ordinary digestive enzymes*."

It is evident, therefore, that the tissue cells are the seat of a digestive process similar to that in the intestinal canal, and that it is carried on by the same hydrolytic triad "trypsin."

Two sources of confusion in the current interpretation of tissue metabolism require attention in this connection. The

[107] Slosse: DuBois-Reymond's Archiv f. Physiol., S. 482, 1890.
[108] Nencki and Pawlow: Arch. d. Sc. Med. de St. Petersburg, T. V.
[109] Kauffmann: C. r. de la Soc. de biol., T. xlvi, p. 323, 1894.
[110] Schäfer: *Loc. cit.*, vol. i, p. 182, 1898.
[111] Halliburton: *Loc. cit.*, p. 41, 1904.
[112] Chittenden: Boston Med. & Surg. Jour., Aug. 17, 1905.

first of these is the multiplicity of ferments which are thought necessary to explain tissue function—a feature which, in my opinion, accounts for the growing complexity of the problem.

Chittenden[113] remarks: "There is practically no process of metabolism so intricate or obscure that it cannot well be explained by the action and interaction of intracellular ferments." The confusing feature just referred to appears, however, when he adds: "New ferments are constantly being discovered, new chemical reactions are being traced to the power of *special* ferments. . . . . " This applies also to the oxidizing ferments. "Oxidation is preëminently one of Nature's ways of bringing about alteration and decomposition," says the same author, "and in intermediary metabolism especially, oxidative processes must be quite conspicuous. Yet to-day we have accumulated a mass of evidence tending to show that oxidation in the tissues is due primarily to the presence and action of a *row* of more or less *closely related*, though chemically distinct ferments, known as oxidases.* Physiological oxidation, therefore, as it occurs in metabolism, is likewise a result of intracellular ferment action." This corresponds with the prevailing view, the various ferments bearing characteristic names, aldehydase, guanase, tyrosinase, adenase, indolphenol-oxidase, nuclease, etc., etc., according to the substances upon which they act, the organs in which they are found, the organic substance with which they happen to be combined, etc.

At best, this multiplicity of ferments—both proteolytic and oxidizing—can only be assumed, since as recently (1905) stated by Halliburton:[114] "Ferments are substances which have, to a great extent, eluded the grasp of the chemist. All he can say," adds this physiologist, "is that they are probably proteid-like in nature, and in some cases the proteid material with which they are either identical or *united* is, as in the case of *fibrin ferment*, of the *nucleo-proteid* variety." On the other hand, this is a suggestive statement in view of the interpretation of the composition of ferments in general I have submitted in the preceding chapter: viz., that there is *but one true ferment*—that

* The italics are my own.—S.
[113] Chittenden: *Loc. cit.*
[114] Halliburton: *Loc. cit.*, p. 30.

represented by the adrenal active principle of adrenoxidase (or its homologue in organisms deprived of adrenals); that all "ferments" contain nucleo-proteid; and finally that the specific action of any "ferment" is due, not to a specific ferment, since there is but one *"ferment of ferments,"* but to the zymogen which the triad termed "ferment" happens to contain.

Once fully apprehended, this simplified conception of the composition of ferments will tend to eliminate many of the obstacles met with when any attempt is made to interpret clearly the intrinsic processes of tissue-metabolism—obstacles which have made it impossible, so far, to discern the true nature of this process. Besides supplying a logical explanation of the manner in which oxygen is supplied to the tissues (the process I have submitted in the thirteenth chapter of this work) we would not be constantly confronted with new "ferments," but with combinations of known tangible bodies, whose chemical properties have been thoroughly scrutinized—all endowed with their quality as a "ferment" by the "ferment of ferments."

Examples are not lacking in which these principles are applicable. Thus, we have seen that Pawlow's enterokinase and Bayliss and Starling's secretin are not ferments, but that they contain adrenoxidase. Cohnheim's "muscle ferment" need only be adrenoxidase to cleave sugar when combined with the secretion of the islands of Langerhans if either the latter or the adrenoxidase contain nucleo-proteid. Finally, Cohnheim's erepsin need not be a "ferment," since, as shown by various investigators, it has the same properties as trypsin; it may be, therefore, only the proteolytic triad known under the name of "trypsin." Yet, Cohnheim holds, on good ground, that erepsin is not trypsin, and that it is endowed with other properties. So is the pancreatic juice endowed with properties other than those of trypsin—those it receives from zymogens other than trypsinogen, and which are all, we have seen, taken up by the digestive leucocytes. It may thus happen that erepsin will prove to be an aggregate of all the hydrolytic triads—proteolytic, amylolytic, lipolytic, glycolytic, etc.—which bathe the intestinal mucosa, and which, through the intermediary of the leucocytes, reach the tissue-cells, to carry on therein a function similar to that performed by them in the intestinal canal, *i.e.*, digestion

by hydrolysis, but having as object in the tissue-cells, the breaking down of worn-out elements.

The second misleading feature now suggests itself: the prevailing belief that the tissue-cells themselves are a source of the "intracellular ferments," and that the intrinsic processes of the cell are ascribable to the presence of a large number of such ferments.

This doctrine has also done much to obscure our knowledge of cellular metabolism by suggesting fictitious functions in the tissue-cell, thus defeating any attempt to discover the sequence of events in the interchanges of which it is the seat. Suggestive in this connection are the following lines by Moore in a recently published work:[115] "Much has been made of the fact that intracellular enzymes have been isolated from living cells which are capable of producing actions hitherto only observed in the presence of the cell, and it has been surmised that all, or nearly all, the chemical activity of the cell may be due to the action of a large number of such intracellular enzymes." . . . . . "Without disparaging the importance and value of such work of separation of intracellular enzymes, it may, however, be urged that there is in such a view no explanation of the phasic activity of the cell, no taking into account of the action of the living cell in co-ordinating, so to speak, the myriad activities going on within it whereby the whole process is regulated."

In the light of all the facts submitted so far in this work regarding the rôle of leucocytes in the nutrition of the tissue-cell, the coördination of the various phases of its life-cycle assumes a normal sequence. The leucocyte not only supplies the "nutritive particles," as Herbert Spencer calls them, thus satisfying the constructive or anabolic phase of the process, but also the hydrolytic enzymes necessary to break down the worn-out nutrient material and prepare it for elimination—the catabolic phase of the process.

A question at once imposes itself, however, in this connection. We are dealing now with the tissue-cells of highly differentiated animals in which the pancreas affords an endless sup-

[115] Moore: Hill's "Recent Advances in Physiology and Bio-Chemistry," p. 11, 1906.

ply of zymogens for the elaboration of "ferments," which the leucocytes transfer to the cells. How can we account for the presence of these same enzymes—or aggregate of enzymes characterized as "trypsin"—in an animal devoid of pancreas, down, in fact, to the lowest in the zoölogical scale, the unicellular organisms? Even the latter differs in no way, as to the manner in which its life-cycle is sustained, from the tissue-cell of the highest of vertebrates, man. Although it has to provide for itself, it acquires from the organic materials it engulfs in its protoplasm the three bodies required to build up its "ferments," and which, we have seen, are present in all living structures.

All this clearly points to a common governing principle in all organic life concerning the manner in which a living cell acquires the ferments which carry on its metabolism, viz., combined with the food materials it ingests. It is evident that since, as we have seen, it is the function of the digestive leucocytes to provide the tissue-cells their nutrient materials and the hydrolytic ferments required to break the latter down when they are no longer of use, these ferments cannot be said to originate in the tissue-cells themselves, but in the digestive apparatus from which the leucocytes obtain them.

Since I first advanced these views in 1903, the brilliant labors of Abderhalden[115a] have contributed much confirmatory evidence. "Each separate cell with very few exceptions," writes this physiological chemist," disposes of the same or similar ferments as those secreted by the digestive glands in the intestinal canal." As to the manner in which the ferments are conveyed to the tissue-cells, he says: "Many facts accord with the suggestion that the leucocytes play a part in this connection."

In brief, it seems to me permissible to conclude: (1) *that the tissue-cells do not contain, as now believed, a large number of special ferments differing from those found in the intestine;* (2) *that the tissue-cells of all living organisms contain the three constituents of at least one hydrolytic triad, trypsin, including adrenoxidase, the active principle of which confers upon the latter its properties as a ferment;* (3) *that whereas in*

[115a] Abderhalden: "Defensive Ferments," third edition, 1914.

the intestine and in the digestive leucocytes, trypsin hydrolyses food-proteids prior to their transformation into assimilable granules (anabolism), in the tissue-cell it hydrolyses worn-out proteid granules or chromosomes to convert them into eliminable waste-products (catabolism); (4) *that the trypsin or any other hydrolytic ferment found in tissue-cells does not originate in these cells, but from the digestive apparatus through the intermediary of the digestive leucocytes.*

THE GRANULATIONS OF LEUCOCYTES AND ADRENOXIDASE IN THE FUNCTIONS OF THE NERVE-CELL.

"What the nerve-impulse actually consists in we do not know," says Stewart.[116] Nor have the more recent labors solved the problem.

We have just seen that the chromatin granules, which correspond chemically and tinctorially with the nucleoproteid granulations of leucocytes, are also present in the nerve-cells. Again, in the first volume[117] I pointed out a fact which seemed to me capable of affording a clue to the nature of the nerve-impulse, viz., that the oxidizing substance—the adrenoxidase—circulates in the axis-cylinders of nerves, the cell-bodies and their protoplasmic processes or dendrites, and other nerve-structures, and that the nervous system, as far as the plasma is concerned, is *an extension of the general circulation,* thus constituting what might be termed the intraneural circulation.

That the various nervous structures referred to are longitudinal channels or canaliculi similar to capillaries, and that the blood-plasma circulates in these channels, is sustained by considerable evidence.

Holmgren[118] found that the reticular network of all ganglion-cells was, in reality, a system of lymph-canaliculi and that the nucleus itself received two delicate vessels. This was confirmed by Studnickal, Bethe and others. Donaggio[119] conducted similar researches, using, however, material from various parts of the brain and spinal system. He not only confirmed the findings of his predecessors, but found that the dis-

[116] Stewart: "Manual of Physiology," fourth edition, p. 592, 1900.
[117] *Cf.* vol. i, pp. 532 to 590.
[118] Holmgren: Anatom. Anzeiger, Bd. xvi, Nu. 7, S. 161, 1899.
[119] Donaggio: Rivista sperimentale, Fasc. i, 1900.

tribution and general characters of the minute canaliculi were identical in all types of cells, the only variations being in the caliber of the canaliculi, these minute channels being somewhat larger in some cells than in others. That it is not lymph, however, as these authors believe, that circulates in the ganglionic canaliculi, and that it is the blood-plasma—its vascular homologue—is shown by the earlier (1886) experiments of Adamkiewicz.[120] This observer found that injections into blood-vessels caused the plasma alone, *i.e.*, blood-plasma without blood-corpuscles, to penetrate minute capillaries which coursed in ganglionic cells.

Zoölogy affords many examples in which the blood-plasma circulates in the nerve ganglia. This is best shown in animals whose blood contains no red corpuscles and in which the hæmoglobin is dissolved in the plasma. Thus, Ray Lankester[121] found that in the sea-mouse the chain of nerve ganglia was a bright crimson color, the hue in the supra-œsophageal ganglion being as intense "as a drop of fresh human blood," the color impregnating "the nerve itself." Gamgee[122] also states that "hæmoglobin has been found diffused in the substance of the nervous tissue," and that Hubrecht[123] "found hæmoglobin in the red-colored cerebral ganglia of certain Nemertine worms, which possess no colored blood-corpuscles."

Pathology supplies striking testimony in the same direction. Although the fact that the toxin of tetanus affects mainly the central nervous system has been known a long time, the manner in which it reaches the cellular elements has only been established within the last few years. Marie and Morax, in 1902,[124] found that when this toxin was injected into the tissues it entered the blood. Thence it passed into the motor and vasomotor nerves, beginning with the peripheral nerve endings, and steadily progressed upward by way of the *axis-cylinders,* until the central nervous system, cord, pons, medulla, etc., were saturated. While motor nerves were found to "absorb" toxin more rapidly than others, the sensory and sympathetic nerve endings were also found to take up portions of

[120] Adamkiewicz: Neurol. Centralbl., Bd. xix, S. 2, 1900.
[121] Ray Lankester: Proc. Royal Soc., London, vol. xxi, p. 70, 1872.
[122] Gamgee: Schäfer, *Loc. cit.*, vol. i, p. 187.
[123] Hubrecht: Niederland Arch. f. Zoölogie, Hft. 3, 1876.
[124] Marie and Morax: Ann. de l'Inst. Pasteur, vol. xvi, p. 818, 1902.

the toxin. These observations were confirmed by Meyer and Ransom[125] by independent researches. They observed, moreover, that the symptoms of tetanus occurred early if the region inoculated was near the central nervous system, and that the period of incubation was long when the inoculation was remote from the cord, thus showing that the length of the nerve governed the period of incubation, a fact previously emphasized by Courmont and Doyon.[126] Meyer and Ransom, moreover, found the toxin in the axis-cylinders, and ascertained that when it was injected after these structures had been severed, the upper segment did not contain the poison. The latter travelled centripetally and entered the nerve, not by way of the neural capillaries, but by the bare axis-cylinder endings in the muscle. These and other experiments led these investigators to conclude that the toxin did not reach the central nervous system by the lymphatics, but *solely* by the axis-cylinders. They suggested that there must be in these structures "a current of protoplasm" which carried the toxin to the central cells. That the toxin did not penetrate the axis-cylinders by way of the lymphatics had also been demonstrated by Marie and Morax.[127] These investigators suggested that it was "absorbed" by these structures. With the axis-cylinders as plasma-capillaries, we need no tentative theories to explain this process: It is the blood-plasma that enters these minute channels to which Schäfer[128] refers as "extremely fine tubes filled with fluid" which carries the toxin while coursing through them.

The presence of the oxidizing substance, *i.e.*, adrenoxidase, in these nerve-channels suggests itself in view of the facts that the plasma invariably contains this substance, as we have seen, and that it is the albuminous and main component (94 per cent.) of hæmoglobin, which circulates as just shown in the ganglia and nerves of some animals. But direct evidence to this effect is also available. As stated by Barker,[129] "the conditions in the nerve structures essential to methylene-blue reactions" are, according to Ehrlich (1886), "(1) oxygen saturation, (2) alkalinity." As is well known, injections of methylene-

[125] Meyer and Ransom: Proceedings Royal Soc., vol. lxxii, p. 26, 1904.
[126] Courmont and Doyon: "Le tétanos," Paris, 1899.
[127] Marie and Morax: *Loc. cit.*
[128] *Cf.* vol. i, p. 535.
[129] Barker: N. Y. Med. Jour., May 15, *et seq.*, 1897-98.

blue into animals causes their axis-cylinders, nerve-endings, etc., to become intensely blue, thus proving the presence of considerable oxygen in these structures. Again, since the methylene-blue penetrates the nerves, though injected in the subcutaneous tissues to be absorbed by the blood, it is evident that it is the latter, or rather its oxygen-laden plasma, which carries the stain into the axis-cylinders, nerve-endings, etc.—precisely as is the case with tetanus toxin.

That the methylene-blue actually penetrates into the axis-cylinders was recently demonstrated by Meltzer, of New York.[130] Intravenous injections were followed not only by staining of these structures throughout their entire length, but when a segment of nerve was isolated between two ligatures, it failed to be stained, thus showing that the methylene-blue entered the nerve by way of its extremities, central and peripheral. Although chloride of gold and nitrate of silver solutions penetrated the axis-cylinder from the side, at Ranvier's nodes, staining the axis-cylinders a short distance, the methylene-blue solution circulated from end to end. The concurrence with the circulation of tetanus toxin as to the rôle of the axis-cylinders as channels for the methylene-blue stained plasma is self-evident.

Again, as shown by Apáthy, the structures stained with methylene-blue are also stained by his chloride of gold method. This coincides with Meltzer's observation that the axis-cylinders also take both these stains. Now, Barker writes: "Inside the *ganglion-cells* a recticulum of fine fibrils *derived from the neuro-fibrils* in transit can be stained a beautiful deep-violet color by Apáthy's chloride of gold method." This confirms the observation of Adamkiewicz as to the circulation of blood-plasma in the ganglionic cells. It explains also why Meltzer found that the methylene-blue entered the axis-cylinders by way of the central nerve-cells as well as through peripheral nerve-endings. Indeed, that it is the fluid which circulates in the axis-cylinders that is present in the cellular network of neuro-fibrils is further shown by the familiar fact that the latter is also stained by methylene-blue. The link with Meltzer's observation now appears: "Apáthy, Bethe, Nissl and other histologists have all

[130] Meltzer: Amer. Jour. of Physiol., vol. x, p. xxiv, 1903-4.

found that the neuro-fibrils which reach the cell-body of a neuron by way of its dendrites passed out of it again to *take part in the formation of the axis-cylinder*—thus entering the latter from above, *i.e.*, by way of the central cell."

All this points to another fact, viz., that Apáthy's neuro-fibrils are likewise channels for adrenoxidase-laden blood-plasma, as I suggested in the first volume, for if it is blood-plasma which carries the stains from below, it is the same fluid which carries it from above.

While this affords evidence in favor of the neuro-fibril theory, it does not support Apáthy, Bethe, Nissl and their followers in the belief that this theory overthrows the neuron doctrine now accepted by most neurologists, including Déjerine, Obersteiner and Barker, and histologists such as Kölliker, Ramon y Cajal, van Lenhossek and van Gehuchten, since the neuro-fibrils can no longer be considered as "conductors," as plasma capillaries. Moreover, Ramon y Cajal has shown recently[131] by means of new staining methods, that the ends of the dendrites, *i.e.*, the neuron's protoplasmic extensions, are independent nervous elements with free endings, and that they are varicose, while the neuro-fibrils are smooth. The latter were found to form two close networks, in which the fibrils anastomosed freely, one network extending between the dendrites (Golgi's network), the other sending large fibrils into the cell. In some dendrites large fibrils could be traced to the nucleus, around which they formed a dense perinuclear mass. As these, interpreted from my viewpoint, are all plasma capillaries, the neuron preserves its identity as an independent anatomical structure, just as a kidney remains a kidney though traversed by many blood-vessels, and though its parenchyma contains a multitude of capillaries. The need of these in the formation of the nerve-impulse is shown by the fact that, as stated by Howell,[132] "a nerve placed in an atmosphere free from oxygen loses its irritability, and regains it quickly upon the admission of oxygen." Briefly, we are not dealing, as stated above, with conductors of nerve energy as Apáthy, Bethe and their followers believe, but with *the circulation of the neuron.*

[131] Ramon y Cajal: Archives latines de méd. et de biol., T. i, No. 1, Oct. 20, 1903.
[132] Howell: "T. B. of Physiology," p. 113, 1905.