That they are *living* bodies is another conclusion imposed by their correspondence with spermatozoa, which are themselves regarded as living cells. Landois,[166] for example, writes that "the spermatozoa of the frog may be frozen four times successively without injury; they endure a heat of 43.75° C. and continue to *live* for seventy days in the testicle transplanted to the abdominal cavity of another frog." Now, we have seen that the head of the spermatozoön contains chromatin and also that all cells show among the granules in their protoplasm some that correspond in their staining properties and chemical composition with those of leucocyte granulations.

If, however, these granulations are living structures, the cellular chromatic granules should also be endowed with life. Henle (1841), and subsequently Béchamp and Estor, Maggi and Altmann held that "*microsomes* are actually organic units or bioblasts capable of assimilation, growth and division, and hence to be regarded as elementary units of structure standing between the cell and the ultimate molecules of living matter.[167] Altmann's conception,[168] the most comprehensive, was that a cell was a colony of such elementary granules or "bioblasts" capable of leading an independent existence. Bacteria, for instance, were considered as "bioblasts;" so were waste-products, oil-droplets, and many other heterogeneous substances. Such a widespread application of the theory caused it naturally to be received with great skepticism, and practically to be set aside when bacteria were found to be cells. As shown below, however, there is good ground for the view that the microsomes of a cell are living entities. Indeed, it is possible also that leucocyte granulations may prove to be cells in keeping with spermatozoa. Minchin, for example, refers to the granules derived from the trophocytes of sponges as "excessively minute cells" having "a nucleus and a clear cytoplasm." Müller[169] also alludes to markedly active granules found in lymph "enclosed in an albuminoid covering."

Many other investigators, as previously stated, have based comprehensive theories on the presence in all cells of living

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[166] Landois: "T. B. of Human Physiology," Amer. edition, p. 945, 1905.
[167] Wilson: *Loc. cit.*, p. 21.
[168] Altmann: Archiv f. Anat. u. Physiol., Physiol. Abth., S. 524, 1889.
[169] Müller: cited by Berdal, "Histologie normale," Paris, 1894.

or viable units. The "physiological units" of Herbert Spencer, to which this great philosopher ascribed the phenomena of regeneration, development and heredity, Weismann's "biophores," Beale's "bioblasts," Verworn's "biogens," and the various hypothetical units of cellular organization and function introduced in the writings of Darwin, de Vries, Haeckel, Foster and other authorities, certainly point to the need of such elementary bodies to account for vital phenomena. Indeed, Verworn refers[170] to his "biogens" as "the real bearers of life," hence to living units.

Again, Wilson, after reviewing some of the foregoing evidence, and referring to cells in general, writes: "Many of the granules, especially the larger and more obvious of them, are unquestionably inert bodies, such as reserve food-matters, suspended in the meshwork. Others are the nodes of the network or optical sections of the threads. But there is some reason to believe that, apart from these appearances, discrete *living* particles may form a constant and essential feature of the protoplasmic thread. These particles, now generally known as *microsomes* (Hanstein, '82), are embedded in threads of the network." The latter does not alone embody the living constituents of the cell, for he says, elsewhere,[171] after reviewing the labors of Van Beneden, Heidenhain, Reinke and Schloter: "When all these facts are placed in connection, we find it difficult to escape the conclusion that no definite line can be drawn between the *cytoplasmic microsomes* at one extreme and the *chromatin* granules at the other. And inasmuch as the latter are certainly capable of growth and division, we cannot deny the possibility that the former may have like powers."

This conclusion harmonizes with my own reached from a different direction, both as to the functional relationship of the nutritional leucocyte with the tissue-cell, and as to the kinship of its granulations with the biochemical structure of the spermatozoön and its rôle in reproduction. Just as the germ-cell is a living unit, so is the chromatin granule or microsome a living unit, and the leucocyte granulation, being naught else than a chromatin granule when transferred to the tissue-cell, is likewise, therefore, a living unit. So close a kinship between the spermatozoön and the leucocyte granulation appears anomalous; but we must not overlook the fact that, interpreted from my standpoint, the granulation perpetuates, as nutritional substance, what the spermatozoön initiates, *i.e.*, the life process.

[170] Verworn: *Loc. cit.*, p. 484.
[171] Wilson: *Loc. cit.*, p. 223.

This evidence appears to me to warrant the following conclusions: (1) *that the granulations of leucocytes are living units which perpetuate in the organism what the spermatozoa initiate in the ova, viz., the vital process, the cellular development;* (2) *that when leucocytes migrate from the blood to the tissue-cells in the lymph spaces, the granulations they secrete therein penetrate into the cells as spermatozoa penetrate into the ova;* and (3) *that the granulations of nutritional leucocytes become the nucleo-proteid chromatin granules or microsomes of tissue-cells.*

THE ACTIVE PRINCIPLE OF ADRENOXIDASE AS THE DYNAMIC ELEMENT OF LIFE.

The leucocytes, as we have seen, supply granulations to the tissue-cells and constitute their chromatin granules. An important discrimination becomes necessary in this connection, however. Wilson, referring to the investigations of Heidenhain, confirmed by Reinke and Schloter, states that "the nuclear network contains granules of two kinds, differing in their staining capacity. The first are *basi*chromatin granules, which stain with true *nuclear* dyes (basic anilines) and are identical with the 'chromatin granules' of other authors. The second are the *oxy*chromatin granules of the *linin* network, which stain with *plasma* stains (acid anilines, etc.), and are closely similar to those of the cytoreticulum" or network. The first are evidently the nucleo-proteid granules derived from leucocytes, since Heidenhain found, in accord with other physiological chemists, that "basichromatin is a substance rich in phosphorus (*i.e.*, nucleic acid)." The identity of oxychromatin is as self-evident, but its importance in the vital process is so great that I will submit, along with the data which indicate the rôle of oxychromatin, testimony to the effect that this substance is adrenoxidase.

Huxley's definition of life: "A universal *disintegration* and waste of oxidation, and its concomitant *reintegration* by the

intussusception of new matter," depicts the sequence of events in cellular metabolism. This conception involves first of all the breaking down of worn, though still living substance, and its replacement by materials derived from the exterior, but evidently capable only of *acquiring* life. As Spencer says: "No separate molecule of proteid possesses vitality."

The manner in which the first part of the process, the disintegration of living matter, is brought about, is suggested in the following lines by Chittenden: "Chemical study has shown that nucleo-proteids, by simple *hydrolysis* with mineral acids in a flask, can be broken down in some form of proteid, phosphoric acid and one or more purin bases, such as adenin, guanin, xanthin and hypoxanthin . . . . Too much stress cannot be laid upon the easy convertibility of the free purin bases, adenin, guanin, hypoxanthin and xanthin into uric acid *by virtue of the action of the intracellular enzymes* present in so many of the organs and tissues." This undoubtedly applies to chromatin disintegration, for, as stated by Halliburton,[172] Hoppe-Seyler found that the true nucleins, those found in the cell nuclei, yield "proteid xanthin and alloxuric bases" and "phosphoric acid," and that the nuclei "richest in nucleic acid occur in the chromatic fibers of the nucleus."

In the light of these facts it is evident that "disintegration and waste by *oxidation*," as expressed by Huxley, no longer represents the process through which the *living portion* of the cell is destroyed, according to the more advanced teachings of physiological chemistry. Indeed, as we have seen in the preceding chapters,[173] catabolism is not attended by cellular combustion, but by *hydrolytic cleavage.*

Again, Gautier,[174] who pointed out as far back as 1881, that cellular metabolism occurred without the direct participation of oxygen, as in the case of anaerobic bacteria, also attributed the disintegration of the living substance to hydrolytic cleavage. He writes, however, referring to the cell: "It is at the expense of *its products* that the phenomena that furnish the animal the major part of its heat and energy occur." As interpreted from my standpoint, it is not at the expense of its

[172] Halliburton: Schäfer's "T. B. of Physiol.," vol. i, p. 66, 1898.
[173] *Cf.* this vol., chapters xiii and xiv.
[174] Gautier: "La cellule vivante," Paris, 1881.

products that this bulk of heat energy is liberated. Indeed, the prevailing doctrine does not enlighten us as to the manner in which this energy is produced. "If heat is indispensable to living beings," says Morat, of Lyons,[175] "if the animal has framed, as it were, all its evolutional development so as to regulate it at a fixed and invariable rate; if it spends combustible materials in such quantities to uphold it to a certain level when there is no danger of losing it; and if it wastes and rids itself of it so actively when threatened with an excess of it, heat must be endowed with some rôle of the very first order and absolutely general in its bearing upon the *elementary reactions which sustain life,* while giving the latter its maximal activity and value. It is this rôle that we cannot discern, not even properly define. We are reduced to the realization that such a degree of heat is useful; that another is bearable and that another is harmful, but we cannot give the reasons for this."

Still, Morat touches the keynote of the true process—in the light of my views—when he adds: "It seems, however, that we can connect this factor with the conception, still so vague, of *fermentation.* Fermentation is the paramount chemical process of the living being; fermentation characterizes life; *Life is a fermentation!*"* It is not to hydrolytic cleavage, due to the action of trypsin, however, that Professor Morat refers. After defining the properties of catalytic agents, he writes: "The ferment is thus endowed with the most general characteristic of living beings: it modifies its environment in a specific way without disappearing itself, that is to say, while preserving itself." The final purpose of this catalytic ferment in the vital process is then brought forth in the sentence: "In doing so, it awakens thermogenic reactions."

Yet, how does a catalytic ferment awaken these heat-producing reactions? Obscurity recurs in this connection. The author ascribes the intracellular process to the fact that "*some* agent is capable of converting some such commonplace energy as heat into modalities of energy commensurate with the reaction to be obtained." This is the characteristic effect of the ferment, but the main feature of the problem is left unsolved.

* The italics are Prof. Morat's own.
[175] Morat: Morat and Doyon, "Traité de Physiologie," Paris, 1899.

Indeed, Morat states that "this enlightens us in no way upon the intimate mechanism of its action."

The identity of the agent that is capable of carrying on these functions suggests itself in the light of all the evidence I have submitted, namely, adrenoxidase. We have seen: (1) that its active principle, that of adrenal secretion, is not only a ferment, but the "ferment of ferments" and, therefore, a component of all cells; (2) that it acts as a catalytic agent or "oxygen transmitter" in all animals provided with adrenals, and that its analogue in animals which are not known to possess such organs, and in plants likewise, acts as a catalytic, and finally, (3) that it acts as a thermogenic agent when its loose oxygen is brought into contact with the phosphorus of nucleo-proteid granules.

Adrenoxidase thus meets all the conditions expressed by the word "fermentation" as Morat—voicing the trend of modern research—interprets it. It follows, therefore, that since, as he says, "Life is a fermentation," *adrenoxidase is the dynamic agent in the vital process.*

What function does adrenoxidase fulfill in the cell?

Pfeffer[176] writes: "Various post-mortem *oxidations* may occur after death, as for example when the *sap* of *Monotropa, Vicia faba,* etc., turns brown. These appear to be produced by the action of certain substances to which the provisional name of *oxydases* may be given." Gautier,[177] alluding to this sap, states that "it is always *acid,* while the protoplasmic portion is always slightly alkaline." He also says that it contains, besides acids, various "products of the cell," including, among other substances, pigments, extractives, sugars, fats, and alkaloids, which "the protoplasmic pockets," *i.e.,* the vacuoles, "could be *seen* in certain instances" to eliminate. Now, the animal cell is similarly supplied with excretory channels. Böhm, Davidoff and Huber state,[178] for example, that among the various structures that protoplasm contains "the vacuoles deserve special mention. They are more or less sharply defined cavities filled with fluid," add these histologists, "and vary considerably in number and size. The fluids that they contain

[176] Pfeffer: "Physiology of Plants," vol. i, p. 545, 1900.
[177] Gautier: *Loc. cit.*, p. 17.
[178] Böhm, Davidoff and Huber: *Loc. cit.*, p. 61, 1905.

differ somewhat, but are always secreted by the protoplasm, and are, as a rule, finally emptied out of the cell." It is plain that the products thus eliminated are wastes—the identical wastes or decomposition products which, we have seen, it is one of the functions of the lymph to sweep away. In fact, the fluid secreted by the cell evidently corresponds with lymph, since no fluid other than this is derived from cellular elements. Again, the presence of oxidase in the lymph of plants affords additional proof that we are dealing with lymph, since we know that our tissue-cells are bathed in blood-plasma, which invariably, we have seen, contains adrenoxidase, derived from the adjoining capillaries.

An important feature of the question in point asserts itself in this connection: "The plasma of the blood," writes Howell,[179] "makes its way through the thin walls of the capillaries, and is thus brought into *immediate contact* with the tissues, to which it brings . . . . *oxygen* of the blood and from which it removes the waste products of metabolism." In other words, according to the present (1905) teachings, it is the oxygen of the plasma that is supplied to the tissue-cells, and the plasma itself, which has become lymph, merely "bathes" the exterior of the cells, sweeping off the wastes it eliminates. As stated by Landois,[180] however, "It can be conceived that, through contraction and diminution in size of their cell bodies . . . . they [the cells] might exert suction upon the blood-plasma transuded. If the cells, themselves, then take up the transuded fluid, the conception is permissible, further, that by subsequent contraction they express this fluid in a certain direction, namely, from secretory space to secretory space, toward the lymph-capillaries."

This suggestion harmonizes perfectly with the facts embodied in the preceding paragraph. Indeed, the data I have submitted in the foregoing pages indicate (1) that it is not around the tissue-cells that the blood-plasma passes, but *through* these cells, and (2) that they do not absorb oxygen from the plasma, but that the intrinsic cellular process involves the ac-

[179] Howell: *Loc. cit.*, p. 427.
[180] Landois: *Loc. cit.*, p. 362.

tive participation of the *adrenoxidase* which passes through the cells with the plasma.

This accounts for the fact that oxidase is present in the fluid eliminated by way of the vacuoles, the cellular emunctories, and for the observation of Abelous and Biarnès[181] that tissues are even richer in oxidase than the blood itself. Lillie[182] found, moreover, that animal cells were endowed with oxidizing properties and that lymphocytes and leucocytes contained oxidase. Again, if, as I have pointed out, axis-cylinders and neuro-fibrils contain this substance—adrenoxidase—and that it is on this account that they take methylene-blue, the ground-substance or cytoplasm of cells should also take this stain. Gulland,[183] in an illustrated study of the granular leucocytes, shows several methylene-blue stained cells, in which the cytoplasm, along with other structures to be described presently, took the stain. It is evident, therefore, that the cells absorb the adrenoxidase-laden plasma as it enters the lymph-spaces, and that they eliminate it—probably by contracting periodically—with all wastes, by way of their vacuoles.

The framework of the cell, the *cytoreticulum*, was called by Leydig its "spongioplasm," so greatly did it recall that of the sponge. Referring to this structure, Wilson[184] states that "it is composed of irregular rows of distinct granules which stain intensely blue with hæmatoxylin, while the substance in which they are imbedded, left unstained by hæmatoxylin, is colored by red acid aniline dyes, such as Congo red or acid fuchsin." Elsewhere, in describing a diagram[185] of a cell, he says that "its basis consists of a thread-work (mitome or reticulum) composed of minute granules (microsomes) and traversing a transparent ground-substance." Again,[186] recalling the researches of Van Beneden and his own, he writes: "It is certain that the microsomes are not merely nodes of the network, or optical sections of the thread, as the earlier authors maintained."

What is the nature of these microsomes?

[181] Abelous and Biarnès: *Loc. cit.*
[182] Lillie: Amer. Jour. of Physiol., vol. vii, p. 412, 1902.
[183] Gulland: Jour. of Physiol., vol. xix, p. 27, 1895-96.
[184] Wilson: *Loc. cit.*, p. 213.
[185] Wilson: *Loc. cit.*, p. 14.
[186] Wilson: *Loc. cit.*, p. 213.

The network of the nucleus contains, as previously stated, basichromatin granules and oxychromatin granules. Again, Wilson refers to the latter as "closely similar to those of the cytoreticulum" or cellular network in their staining properties. As basichromatin granules were found by Heidenhain and others to be rich in phosphorus, those of nuclear network are doubtless composed of nuclein or nucleo-proteid. The nuclear network which corresponds tinctorially with the network of the ground-substance or cytoreticulum, therefore, is that composed of oxychromatin granules. Having now seen that the plasma passes through the cells and that oxidase is present in the fluid eliminated by the cell, there is good ground for the belief that the oxychromatin granules are in reality composed of adrenoxidase. That such is the case is shown by the fact that both substances correspond in their staining properties. Thus, in the leucocytes stained with methylene-blue by Gulland, the cytoreticulum took the stain as well as the cytoplasm, but more intensely. This shows that adrenoxidase is present in both structures though in a more condensed form in the network. Again, Wilson states that the *oxy*chromatin network stains with "plasma stains (acid anilines, etc.)," and refers to the fact that the Biondi-Ehrlich mixture of acid fuchsin and methyl-green stains the cytoplasm of leucocytes red. Now, Heidenhain showed that the oxychromatin granules are also stained red by this mixture. Thus, the cytoplasm and the cytoreticulum correspond in their reaction to this stain as they did to methylene-blue. Gulland's plates show the same correspondence with the Biondi-Ehrlich mixture. This is further sustained by the fact that the nucleo-proteid chromatin is stained green by this mixture. Thus Wilson says that the green substance is the "chromatin of Flemming," the basichromatin, which, as stated above, is rich in phosphorus. It is evident, therefore, that *just as the substance in the axis-cylinders of nerves is adrenoxidase* (a fact controlled by the circulation of tetanotoxin-laden plasma in them), *so is the cytoreticulum or spongioplasm of cells composed of adrenoxidase.*

The far-reaching meaning of this conclusion asserts itself in view of the fact that it is a living portion of the cell. "Since I saw the reticulum in *continuous movement* during the life of

a protoplasmic lump," says Heitzmann,[187] referring to observations made by him in 1873, on the leucocyte of the newt, "my conclusion was that the reticulum is made up of the living or contractile matter proper; whereas the meshes contained a liquid, destitute as such of properties of life, filling the meshes of the sponge-like structure, and permitting the contraction of the solid portion—*i.e.*, the living matter." More recently, Wilson also wrote,[188] alluding to the "sponge-like network," "At the present time it seems probable that the more solid portion is the more active and is perhaps to be identified as the *living substance proper*, the ground-substance [the cytoplasm] being passive." The rôle I attribute to adrenoxidase, *viz.*, that it is "the dynamic agent in the vital process," harmonizes clearly with this view, especially when we consider that it is probably in its original form that it occurs in the network.

That the "granules" described are formed only after death of the cell, and owing to the methods of fixation employed, is probable, however. We have seen that in its original state—as the albuminous portion of oxyhæmoglobin—adrenoxidase leaves the red corpuscles in the form of droplets, the so-called "blood-platelets" or "hæmatoblasts." Indeed, Löwit[189] found blood-platelets "within capillary blood-vessels just removed from animals, and in which the blood was still fluid," though none could be discovered in the circulating blood. These vessels being the very ones that supply plasma to the lymph spaces, the adrenoxidase droplets reach the cells almost at once after traversing the capillary walls.*

In the preceding section, I pointed out that the granulations of leucocytes were living substance and that they entered the cells as spermatozoa penetrate ova. We have now seen that adrenoxidase is the dynamic agent in the vital process. It is

* The semifluid nature of adrenoxidase suggests that the network or cyticulum is made up of delicate capillaries, or minute tubes or canals such as axis-cylinders are thought to be by Schäfer and others. In the first volume[190] I submitted reasons which had led me to conclude, referring to leucocytes, that "the intracellular and intranuclear network of fibers in mature leucocytes are canaliculi for blood-plasma and for the substances contained in this fluid," and to compare them to neuro-fibrils. As the conclusions of the first volume were intended as suggestions, while the second volume contains only what appears to me fully sustained by available evidence, I will adhere for the time being to the prevailing view that the network is made up only of granular threads, *i.e.*, strings of granules in close apposition.—S.

[187] Heitzmann: Jour. of the N. Y. Micros. Soc., July, p. 66, 1893.
[188] Wilson: *Loc. cit.*, p. 17.
[189] Löwit: Archiv f. exp. Path. u. Pharm., Bd. xxiv, S. 188, 1887.
[190] *Cf.* vol. i, p. 668.

therefore owing to the presence of adrenoxidase in the leucocytes[191] that the nucleo-proteid granulations become living entities. We also find adrenoxidase forming a delicate and close network throughout the cell—a network recognized as the living portion of the cell.

On the whole, the evidence submitted appears to me to warrant the following conclusions: (1) *that adrenoxidase, owing to its ability to act simultaneously: as a catalytic, as oxidizing body, and as the ferment of all ferments, is a dynamic agent in the vital process;* (2) *that the nucleo-proteid granules are evolved as living units by the digestive leucocytes because these cells contain adrenoxidase;* (3) *that the blood-plasma does not, as now believed, on penetrating the lymph-spaces circumvent the tissue-cells and yield its oxygen to them;* (4) *that the blood-plasma on entering the lymph-spaces at once enters the cells and thus supplies them directly with adrenoxidase;* (5) *that the "oxychromatin" network of the nucleus and of the cytoplasm or ground-substance, are both composed of adrenoxidase;* and (6) *that these networks are the living portion of the cell.*

What is the functional relationship between adrenoxidase forming the cellular networks and the nucleo-proteid granules of the cytoplasm?

THE ACTIVE PRINCIPLE OF ADRENOXIDASE AS THE DYNAMIC ELEMENT OF LIFE (continued).

An important constituent of the cell has so far received but little attention. I have repeatedly referred to the function of the nucleo-proteid, but only to that of its phosphorus-laden constituent, the nuclein. Its proteid moiety plays, none the less, a prominent rôle in the vital process; indeed, when the granulations of leucocytes were referred to as living entities in a preceding section, a more detailed analysis of the question—inappropriate at the time—would have pointed to this moiety as a living substance. Thus, Verworn[192] says that "it is the proteids whose presence constitutes the general essential condition and *focus* of life." This estimate is fully sustained when the vital process is interpreted from my standpoint, since the

[191] *Cf.* this vol., p. 894.
[192] Verworn: *Loc. cit.*, p. 480.