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AUTOLYSIS*

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TISSUE DISINTEGRATION.

THE most constant property of living matter is disintegration. This precedes every other manifestation of life in the simplest and in the most complex animated organism. The development of a fertilized egg begins with disintegration of its original structure. When the egg is placed under conditions which make the initial disintegration impossible, life remains suspended. Disintegration also is the most lasting property of living matter, for when all other functions are extinct this property is still in evidence, and, if conditions are favorable, the disintegration proceeds until all evidence of organization or of structure disappears, giving place to a mixture of organic and inorganic substances.

Disintegration of organic matter present in the cells and tissues is also the primary source of that form of vital energy which controls most functions of the living organism. Contraction of muscle, secretion of glands, peristaltic movements of the gastro-intestinal tract, growth and reproduction of an organism, are possible only so long as the breaking-down process continues. This has been realized by physiologists at all times. However, the mechanism and the exact nature of the chemical reactions associated with animal functions have never been very clear, and even to-day the question remains a topic of considerable controversy.

Lavoisier was the first to emphasize strongly the similarity between the chemical reactions in the living organism and those in the process of combustion. Ever since that time physiologists have adopted the term combustion in order to signify

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the chemical reactions in the organism which result in the production of animal energy. Physiologists speak of burning or non-burning in the human or in the animal body of proteid, fat or sugar, of burning of the body tissues and of body cells. Indeed, the ultimate products of the reactions in the body are very similar to those from the burning of carbonaceous material. Carbon dioxid, water and heat are formed in both instances. However, there has always existed an utter lack of information regarding the agents causing the powerful oxidation of organic material in the animal body. The simplest way out of the difficulty seemed to ascribe the power of combustion to a peculiar property of the living cell.

In recent years there has accumulated a great number of observations tending to show that various functions previously regarded as the result of life, as the result of cell assimilation and disintegration by the animal tissues, are actually occasioned by substances which can be isolated from the living cell. An instance illustrative of this statement is found in the work on alcoholic fermentation. The formation of alcohol from grape sugar by the yeast cell was regarded as a chemical reaction brought about by the activity of the cell. Alcohol thus was considered a catabolic product of cell metabolism. Mme. Manassein, and, more convincingly, Buchner, have demonstrated that one substance from the cell can be obtained which is capable of accomplishing the alcoholic fermentation of sugar in the same manner as the living cell. This startling discovery marks a radical change in our conception of the process of life. For centuries it was thought that the activity of a complex organism was needed to manufacture spirits out of grape sugar, and the foregoing work demonstrated that the cell may be crushed, its life may be extinct, and one substance soluble in water may be extracted which possesses the power to accomplish the work of the entire cell. True, the substance is of a very subtle nature and needs to be handled with great care. It does not resist the action of heat and other strong chemical and physical agents, but while intact it is capable of inducing chemical reactions into which it apparently

does not enter itself, and thus is capable of performing work out of proportion to its own mass. Substances endowed with this power are designated enzymes or ferments. The work of Buchner caused a very intense interest to be directed toward the older and unsystematic work on the enzymotic processes in the cells of the simple and complex organisms.

As already stated, the source of all vital and animal energy lies in tissue disintegration, and the prevailing conception has been that the disintegration was brought about by the power of the living cell to burn its own components. It is the great merit of Salkowski to have shown that a cell or tissue in which all visible signs of life have disappeared still retains the power of self-dissolution, of self-disintegration, of autolysis. True, the phenomenon had not escaped the observation of earlier workers, and in 1871 Hoppe-Seyler wrote:[1] "All organs suffering death within the organism, in the absence of oxygen, undergo softening and dissolution in a manner resembling that of putrefaction. In the course of that process, albuminous matter gives rise to leucin and tyrosin, fat to free acids and soaps. This maceration, identical with the pathologic conception of softening, is accomplished without giving rise to ill odor, and is a process similar to the one resulting from the action of water, acids and digestive enzymes." In 1874 the French chemist, Schutzenberger[2] observed similar changes in yeast which had been allowed to remain for from 12 to 15 hours in water suspension at a temperature of from 35° to 40° C.

However, before going into the details of chemical analyses, attention may be called to the structural, morphologic changes which cells and tissues undergo when they are placed in conditions which do not permit continuation of life. It is well known that animal tissues and organs are readily invaded by micro-organisms, causing putrefaction. The application of

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[1] Tübinger Med. Chem. Untersuchungen, 1871, p. 499.

[2] Compt. rend., vol. lxxviii, and Bull. de Soc. Chemique, vol. xxi, p. 204.

the recent methods of aseptic surgery allows the removal of organs from the animal body and the preserving of them free from all contamination with micro-organisms. Hauser,[3] as well as Rindfleisch and Meissner previous to him, succeeded in preserving tissues for months and years free from infection with any bacteria. In organs kept in an absolutely sterile condition Hauser observed general softening, and microscopically he noted the destruction of the most typical structural part of the cell, the nuclear material, and decay of the mass of the cell, made apparent by the development of changes which are designated by pathologists as fatty degeneration.

Thus the term softening is not merely a figure of speech, but applies to an actual occurrence. This is made evident particularly through the work of Schutzenberger and the workers who followed him. Normal fresh organs on extraction with boiling water give off only a small fraction of their constituents, while those that have undergone the process of softening allow a very considerable part of their substance to pass into the boiling water. Thus, fresh yeast on boiling with hot water leaves a residue consisting of from 20 to 21 per cent. of its original weight, while the residue of yeast kept in water for from 12 to 15 hours does not exceed 13 per cent. However, in the experiment of Hauser, although the tissues were placed in conditions unfavorable for continuation of life, death set in slowly, and the possibility is not excluded that the softening was accomplished by the vital force not yet completely extinct. In the experiments of Schutzenberger also this possibility was not excluded; besides, yeast always contains bacteria and it is difficult to separate the part of the changes wrought by the action of micro-organisms from that induced by the surviving yeast cell.

Salkowski was the first to preserve the material employed in his experiments under conditions which checked all functions but that of dissolution, bacterial growth being impossible. This was achieved by the use of chloroform water instead of the

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[3] Arch. f. exper. Path. u. Pharm., vol. xx, p. 162, 1886.

pure. Salkowski repeated the experiments of Schutzenberger on yeast and arrived at the same conclusions as the first observer. He extended the work to animal tissues, using the liver and muscle. The results are best seen in the following table:

From 1000 grams of liver were extracted by hot water	Autolyzed organ	Control	Difference
Organic substance	45.97 gms.	33.73 gms.	12.24 gms.
Ash	7.95 gms.	7.21 gms.	0.74 gms.
Phosphoric acid	1.957 gms.	1.359 gms.	0.598 gms.
Nitrogen in form of nitrogenous substances	6.239 gms.	3.152 gms.	3.087 gms.

In the main experiment the finely-divided organ was mixed with three times its weight of chloroform water and allowed to stand. At given intervals analyses were made. In the control experiment the organ was heated and then further treated in the same manner as in the principal experiment. The table clearly shows that, on standing, substances soluble in hot water have developed in the organ. Very similar changes occur in tissues subjected to the influence of digestive enzymes, either in the digestive tract or outside of the body. Because of this analogy Salkowski introduced the term "self-digestion" in order to designate the process occurring in tissues allowed to stand under antiseptic conditions. For reasons which will be made clear in the course of the discussion, the process later was named by Hofmeister "autolysis."

Thus the researches of Salkowski have established the fact that tissues, placed in conditions which do not allow contamination with living matter, undergo changes resembling those occurring during the course of digestion: but they offered no information regarding the rôle played by the process in the economy of the organism, in those transformations of matter which create and maintain life. It was still undecided whether or not the capacity of self-digestion was a universal property of all tissues. The probability was not excluded that the autolysis of an organ was brought about by the action of enzymes absorbed from the gastro-intestinal tract and transported to the various organs. The researches following those of Salkowski

endeavored to give an answer to these questions. The solution of the first problem was comparatively an easy matter. It was necessary only to repeat his experiments on various other organs. This was accomplished most successfully by the efforts of Hedin and Rowland.[4] It may be noted here that the last two investigators employed in their experiments not the entire tissue nor the tissue extracts, but the plasma of the organs. In this manner they made certain that no cellular elements were playing any part in their experiments, and that the reactions were caused by a soluble substance present in the plasma. Previously Schwiening,[5] a pupil of Salkowski, had established the same fact by employing filtered tissue extracts. The work was further extended by Martin Jacoby[6] and by Stookey and myself.[7] As a result of all the work it may be regarded as established that the power of self-digestion is shared equally by all organs. The solution of the second problem, namely, of the origin of the autolysing power, required more ingenuity and perhaps more work. Attempts were made to obtain the desired information in various ways. If the digesting power present in the organs be due to a substance derived from the pancreas, the autolysis of organs must be influenced by the same factors and in the same manner as pancreatic digestion; further, if that assumption be correct, one would expect to find among the products of autolysis those substances which arise on tryptic digestion.

The chemical composition of animal organs is very complex, but the pancreatic gland is capable of disintegrating all the principal tissue constituents, although it resorts to a different mechanism, perhaps to a different substance, for the digestion of the individual substances. The principal components of tissues are albuminous material, carbohydrates and fats. In the course of self-digestion all these components are disintegrated, and it is a matter of convenience to discuss separately

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[4] Zeitsch. f. phys. Chem., vol. xxxii, 1902.

[5] Virchow's Archiv., cxxxvi, 1894.

[6] Zeitsch. f. phys. Chem., vol. xxx, 1900.

[7] Jour. Med. Research, vol. x, 1903.

the change which each of the components undergoes in the course of autolysis. Of all enzymotic processes, that resulting from the breaking up of the proteid molecule has been studied in the greatest detail. For this reason the study of the proteolytic action of organs was employed for the investigations into the origin of the autolytic power of tissues.

Two proteolytic enzymes of distinct individuality have always been known—pepsin, elaborated by the glandular apparatus of the stomach, and trypsin, formed in the pancreatic gland. The principal point of distinction between the two substances is that one requires for its action the presence of acid, while the other is most active in the presence of alkali. Further, it has generally been accepted that pepsin is incapable of producing the same degree of cleavage as trypsin. The formation of crystalline products of amino-acids has been noted only on tryptic digestion. Most typical for the cleavage by the ferment of the pancreatic gland is considered the appearance of a substance giving a peculiar color test with bromin, named tryptophan. In the course of digestion by either of the two enzymes, albumoses and peptones are formed.

Biondi, a student of Salkowski, has noted that the proteolytic action of the liver is facilitated by the presence of acids. This difference in intensity of digestion under the two different conditions is made very conspicuous by the following table:

Out of 1000 grams of liver passed into solution	Experiment 1 with 0.28% HCl	Experiment 2 without HCl
Organic substances	100.10 gms.	59.0 gms.
Ash	26.90 gms.	11.12 gms.
N. in nitrogenous substances...	11.76 gms.	7. gms.
Albumose	Trace.	Trace.
Pepton	None.	None.

The conditions influencing the intensity of autolysis were studied in greater detail by Hedin and Rowland,[4] whose investigations were made on tissue plasma obtained by Buchner's method. It was established by these writers that the self-digestion of the majority of organs is facilitated by the presence

of 0.25 per cent. of acetic acid and is depressed by the presence of alkalies, by calcium carbonate and magnesium oxid. The only deviation from this, according to Hedin and Rowland, is in muscle tissue, where the intensity of digestion is not affected by the presence of alkali or acid. On the other hand, cardiac muscle is subject to the general rule of autolysis. The autolysis of nerve tissue, and of the testes also, is facilitated by the presence of acid, as was demonstrated by Stookey and myself.

These observations are important, for the reason that they make very improbable the supposition that self-digestion of tissues is caused by trypsin deposited in the organs by the blood supply. On the other hand, Salkowski, in his early work on autolysis, has noted the appearance of leucin and tyrosin, and in this respect the proteolytic action of animal tissues resembles tryptic digestion. Contradictory to this seemed the observations of Biondi.[8] This author could not detect tryptophan in the experiments in which the absence of bacterial growth was made certain. Another peculiarity of the autolytic cleavage noted by Biondi is the comparatively insignificant formation of albumose and of peptone. Jacoby also, in his very exhaustive study on autolysis, invites special attention to the foregoing difference between tryptic and autolytic digestion. On the other hand, Jacoby demonstrated tryptophan among the products of self-digestion of tissues. Thus the chemical process of autolysis bears some resemblance to either form of digestion, peptic and tryptic, and yet is different from each of them. This alone makes it very probable that animal tissues do not borrow their power of disintegration from either gastric or pancreatic gland, and that self-digestion is one of the general properties of living or, rather, surviving organs.

Additional evidence in support of these assumptions was brought forward by Matthes.[9] It is well known that urine of normal individuals contains a proteolytic enzyme resembling

[8] Virchow's Archiv., vol. cxliv, 1906.

[9] Archiv. f. exp. Path. u. Pharm., vol. li, 1904.

pepsin. Matthes demonstrated that after the removal of the stomach of dogs the enzyme ceases to be eliminated by the urine. It was natural on the basis of this experiment to view the stomach as the source of the urinary pepsin. The same method of investigation was applied by Matthes to the study of the origin of the self-digesting power of organs and tissues. Dogs were deprived of their pancreas and allowed to recover from the operation. The organs were then examined for their proteolytic power. No difference could be detected between the organs of the normal and those of the operated animal. Thus, all evidence seemed unanimously to support the view that self-digestion is a constant property of surviving tissue.

However, for the interpretation of the rôle of this function in the economy of the living organism, it still remained to be established whether or not the process of self-disintegration takes place also in life. Jacoby [9] was the first to give experimental trial of the question. For this purpose he performed on dogs the following operations: The hepatic artery and the portal vein were ligated and, after several hours, the liver was extirpated and analyzed for amino-acids. Leucin and tyrosin were found to be present. Further, he obtained the same results on ligating a part of the liver. These substances were also obtained by Jacoby from organs extirpated aseptically and kept under conditions in which contamination was impossible. It may be remarked that all these methods are open to some objections. More convincing seems to me the analysis of the developing organism. It has been known for some time, through the work of Schulze and his pupils, that in the course of germination and growth of plants, substances appear which arise also on proteolytic digestion of the seeds. I have made a similar observation on the developing egg of fish and of fowl.[10] In the course of development of the egg one can notice the breakdown of the albuminous matter and the appearance of products of the nature of nitrogenous acids.

Zeitsch. f. phys. Chem., vol. xxxv, 1902.

So, at the present time, there is sufficient evidence for the assumption that disintegration or self-digestion is a constant occurrence in living as well as in surviving tissues. However, there is still a lack of information regarding the rôle of this function in the mechanism of life. In the animal tissue, organ or cell, one has to distinguish two different parts, one representing the organized mechanism controlling its function, the other consisting of various organic substances stored up or deposited in the organs, as a supply of fuel material. Blood plasma and lymph, which envelop every part of the organ, are not integral parts of its tissue. They only furnish the material which the organ may or may not use. White of an egg and the greatest part of its yolk are only building material for the developing organism.

In physiology there are two views regarding the production of animal energy. One is that a substance can not be utilized by a living cell unless it has been assimilated and transferred into organized cell substance. Liebig was the author of this theory and Pflüger most vigorously defended it. On the other hand, Carl Voit claimed that in higher organisms the principal supply of fuel material is furnished to the organs by the blood. The albuminous matter carried to the organs was named by Voit "circulating proteid." Opinions on the subject are still divided and it is possible that in a way both views are correct.

Since there was some foundation for the view that the process of autolysis is the one which controls tissue disintegration, it seemed important to make clear whether or not the mechanism is capable of breaking down albuminous matter derived from other sources than that of its own body substance. The first observation in this direction was made by Theobald Smith, who noted that fresh tissues removed from the organism under aseptic conditions were capable of digesting gelatin. On the other hand, Martin Jacoby [11] noted that during the process of liver autolysis, of the proteids only the globulins suffered a

[11] Zeitsch. f. phys. Chem., vol. xxx, 1900, also vol. xxxiii, 1903.

disintegration; and in a later work he observed that the self-digesting liver was completely incapable of digesting lung tissue. Thus, on the basis of this work, one would be led to the view that the process of autolysis is incapable of causing the digestion of circulating proteid, and that the two processes are totally independent of each other. However, Hedin [4] has shown that the spleen possesses the power to digest not only its own proteid material, but also the proteids of the blood. Thus the question still remains an open one.

PRODUCTS OF TISSUE DISINTEGRATION.

The work thus far reviewed possessed primarily theoretical interest only. It aimed to elucidate the mechanism controlling the disintegration of tissue components in the living and in the surviving organs. Nevertheless a detailed knowledge of the products of tissue autolysis is of importance from the standpoint of practical medicine. In the human organism, as well as in that of many animals, all substances which are consumed as food and nourishment, no matter how greatly they differ in their chemical composition, are finally broken down into a few very simple bodies, which are rejected by the organism through the kidneys, bile and other excretory mechanisms. Urea and carbonic acid are the two substances into which nearly all food-stuff is transformed. In a complex organism the metamorphosis is a gradual process. Before a nitrogenous substance is transformed into urea it undergoes numerous degradations. Before sugar is oxidized to carbonic acid it suffers numerous changes. Further, it is not improbable that in a very complex organism individual organs are concerned only in one definite phase of the transformation, leaving the other organs to continue and to complete the work. In his recent address on the subject, Professor v. Noorden [12] pointed out that the information regarding the nature of intermediate products of metabolism, as well as the seat of their formation, is lacking. Attention of investigators has turned to the study of the products of autoly-

[12] This volume, page 18.

sis of various organs in the hope of filling in the gap in our knowledge of the mechanism of nutrition and of self-preservation of the organism.

However, the study of the substances arising in the course of autolysis was preceded by very active work on the normal composition of tissues and tissue components. Indeed, it was to be expected that within the body, tissue constituents would break down into their component parts. Recent years are marked by astonishing progress in the knowledge of the chemical nature of tissues. It was owing to this progress that the study of autolysis was made a comparatively easy matter. As already stated, the principal tissue components are albuminous matter, sugars and fat. The changes which each one of these components undergoes in the course of self-digestion have been the subject of special investigation.

Under the term proteid is generally understood the substance which represents the most important and most characteristic part of living matter. It is colloidal in nature and is composed of various nitrogenous acids. On heating proteid with strong acids or alkalies, the original substance disappears, giving rise to the nitrogenous acids. Of those already known are the following:

Glycocoll.	Lysin.
Alanin.	Arginin.
Aminovalerianic acid.	Histidin.
Leucin.	Prolin.
Glutamic acid.	Tryptophan.
Phenylalanin.	Cystein.
Tyrosin.	

Of the proteids, one group attracts special attention. Its members are present in greatest quantity in the nuclei of all cells, and it has been assumed that the function of the nucleus is closely associated with the presence of these substances. They are named nucleins, nucleoproteids, nucleoalbumins, etc. They are more complex than ordinary proteids, containing in their molecule, besides the usual constituents, a body termed nucleic acid. This acid is composed of substances to which a considerable rôle in the pathogenesis of disease has been attributed.

Its components are as follows: Phosphoric acid, carbohydrate, thymin, uracil, cytosin, adenin, guanin, hypoxanthin.

Normally, components of simple and complex proteids occur as such in tissues in very insignificant quantities. But it is found that in the course of self-digestion an organ may undergc such deep changes that nothing remains of its original structure, in its place the following substances appearing:

	Pancreas	Liver	Spleen	Kidney	Testes
Glycocoll	—	—	—	—	—
Alanin	+	+	+	+	+
Aminobutyric acid	+	+	+	+	+
Aminovalerianic acid	?	?	+	?	+
Leucin	+	+	+	+	+
Glutamic acid	+	+	+	+	+
Aspartic acid	+	+	+	+	+
Pyrrolidin carbonic acid	?	?	+	+	+
Tyrosin	+	+	+	+	+
Phenylalanin	+	+	+	+	+

A glance at the table shows clearly that the action of the autolytic process in organs is as powerful as that of strong acids combined with high temperature. Nearly all the products which are obtained on prolonged boiling of proteids with strong mineral acids arise also in the course of autolysis. However, there are noted some differences in the two processes. If it be allowed to name substances appearing on cleavage with mineral acid as primary cleavage products, the distinction may be made that on autolysis the primary products undergo further transformation. It is a matter of convenience to discuss the points of difference according to the three principal groups of substances in which they occur, namely: 1. The nitrogenous acids containing only one nitrogen in their molecule, monoamino-acids. 2. Acids with more than one nitrogen in the molecule (The substances of this group arising from proteid cleavage were named by Kossel hexon bases. They generally possess basic properties). 3. Substances resulting from the nuclear degradation, nuclein derivatives or nuclein bases. The most

appropriate method for investigation was: first, to study the products obtainable on boiling organs with strong acids; second, to study those arising on autolysis of the same organs and, finally, to analyze the substances appearing on boiling with strong acids of organs previously subjected to self-digestion.

On acid cleavage all the amino-acids are obtained which are known to appear on the breaking down of proteid material. Among the end-products of self-digestion of the pancreas, Emerson [13] discovered oxyphenylethylamin, which is not known to be present in the proteid molecule, and which may be regarded as a secondary product derived from tyrosin. Further, on autolysis of various organs the formation of glycocoll was not observed, and prolin could be demonstrated only in a few experiments. It should be remarked that the present methods of analysis of amino-acids are not fully satisfactory, and too much weight should not be attached to the results thus far obtained. However, the results of the analysis of the amino-acids obtained from the fresh and from the self-digested glands seem to indicate that in the course of the latter process some destruction of the substances takes place. This may be seen from a table showing the results of experiments not yet published, although completed:

	Fresh Spleen 5 pounds	Autolyzed Spleen 5 pounds
Glycocoll	0.700	0.700
Alanin	8.6	1.7
Aminobutyric and aminovalerianic acids	5.25	5.00
Leucin	14.75	12.0
Aspartic acid	2.24	0.8
Glutamic acid	3.12	1.25
Phenylalanin	1.15	1.33
Prolin	Present.	Present (inactive).

The knowledge of the further phases of amino-acid metamorphosis is rather meager. Stolte [14] has shown that amino-acids exposed to the action of tissue extracts give rise to ammonia, and Magnus-Levy [15] has demonstrated the formation of fatty

[13] Hofmeister's Beiträge, vol. i, 1901.

[14] Hofmeister's Beiträge, vol. v, 1904.

[15] Hofmeister's Beiträge, vol. v, 1904.

acids in the course of autolysis. Diamino-acids and other basic substances of the proteid molecule suffer a similar disintegration. Thus, on prolonged autolysis of the pancreatic gland or of the gastric mucosa, the formation of diamins from diamino-acids, a process analogous to the transformation of tyrosin into oxyphenylethylamin, was observed by Lawrow,[16] Langstein,[16] and by myself.[17] It has also been noted that a very considerable part of the diamino-acids suffers a more complete disintegration. Thus five pounds of fresh spleen yields on hydrolysis 3.2 gm. of arginin and 2 gm. of lysin and the same quantity of digested glands only 1.5 gm. of arginin and 1.2 gm. of lysin. The mechanism controlling this degradation was explained by the brilliant discovery of Kossel and Dakin.[18] These authors have demonstrated in various organs the presence of a special enzyme whose function it is to decompose arginin into urea and diaminovalerianic acid. The same enzyme was found by Shiga in the yeast cell.

Before concluding the review of the products resulting from proteid cleavage in the process of autolysis mention has to be made of the formation of plasteins. From the foregoing discussion it is apparent that great activity has been displayed in the study of degradation of the proteid molecule within the body and in the test tube, and an attempt has been made to explain the bearing the work had on our understanding of various physiologic functions. However, there was one great problem which inspired many workers in their labors and which remains unsolved by them. This was to discover a process by which proteid, the chief tissue component, could be constructed out of its simple components, and also to discover the mechanism which the organism employs to build up tissue proteid out of those fragments which are formed in the digestive tract. It has been stated before that the first phase in

[16] Zeitsch. f. phys. Chem., vol. xxxiii, 1901.

[16] Ibid., vol. ii, 1902.

[17] Amer. Jour. of Physiol., vol. xii, 1904.

[18] Zeitsch. f. phys. Chem., vols. xli and xlii, 1904.

proteid digestion consists in converting native proteid, which is insoluble in boiling water, into products which are soluble both in hot and cold water. These products are termed albumose and peptone. Danilewsky and his pupil, Okunew,[19] were the first to make the observation that when the soluble substances are exposed to the action of rennet ferment a substance arises which is insoluble in water. This substance was termed plastein. Kurajeff, another student of Danilewsky, has shown that the plasteins are formed by the action of autolytic enzymes also. It has been shown in recent years that enzymes possess a double function. They break up complex into more simple substances and again rebuild the original substances from the fragments. Hill made this observation on the enzymes digesting starch, and Kastle and Loevenhart[20] on that splitting fat. The first ferment is capable of converting sugar into starch and again starch into sugar; the second possesses the power of converting fat into fatty acids, and the acids into fat.

Attempts were made to ascribe a similar faculty to enzymes digesting proteids, and the plasteins were regarded by many investigators as reconstructed native proteid. The most emphatic supporter of this theory was Herzog.[21] He based his assumption on a very ingenious experiment. The viscosity of a dilute solution of native proteid decreases in the course of digestion. On the other hand, the viscosity of a fairly concentrated solution of albumose exposed to the action of digestive enzymes increases. This, according to Herzog, is to be explained by the reconstruction of proteid. However, if the digestive action of the enzyme is disturbed by the presence of an antiferment, the reconstruction fails. Very recently Kurajeff and his pupil, Grossman,[22] exposed digested plasteins to the action of autolytic enzymes and noted the formation of coagulable proteid, and they seem inclined to believe that this indicates a reversible

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[19] Maly's Jahresb., vol. xxv.

[20] Amer. Chem. Jour., vol. xxiv, 1901.

[21] Zeitsch. f. phys. Chem., vol. xxxix, 1903.

[22] Hofmeister's Beiträge, vol. vi, 1905.

action of the autolytic enzymes. Unfortunately the experiments of various writers on the subject contain many contradictions. While the primary albumoses are considered the mother substance of plasteins by some writers, Kurajeff noted the formation of plastein only from the secondary ones. Further, Bayer,[23] in Hofmeister's laboratory, found that plastein is formed from crystalline cleavage products.

In our own experiments, Stookey and I failed to find any evidence of reconstruction of the lower albumoses into coagulable proteid. By the action of rennet ferment on albumose it was possible to convert the solution into a solid jelly, which was very difficult to fractionate for purely mechanical reasons. If, for the experiment, a solution of albumose of moderate concentration was employed, it turned into a very thick syrup. In the original solution and the one treated with rennet the various albumoses were estimated. The time of treatment with rennet varied from 12 to 48 hours. In no instance was there observed any diminution in the quantity of the lower albumose or of the peptone. Thus there was no evidence of reversion of peptone into coagulable proteid, and it is extremely improbable that enzymotic synthesis of proteid can be made possible before the substances at present known as proteolytic enzymes are divided into their components. Cleavage of proteids by trypsin, pepsin or other proteolytic enzymes is a very complex process. Reversibility of action thus far is known to be a property of enzymes affecting a hydrolytic cleavage. In the course of proteid disintegration in the tissues and in the digestive tract, the primary products so rapidly suffer further metamorphosis that reversion on a large scale is scarcely imaginable. There must be other synthetical processes to which the organism resorts for the purpose of tissue construction.

It has already been stated that special attention has been directed to the study of the products of autolysis of nuclear material. Through the work of Fischer, it was established that the components of nucleic acid were closely related one to

[23] Hofmeister's Beiträge, vol. iv, 1904.

another and all of them, in turn, related to uric acid. In addition, the pathogenesis of many diseased conditions, and particularly that of gout, is closely associated with uric acid. Normally, fresh organs contain the components of nucleic acid in a free state only in a very insignificant quantity. Schutzenberger, Salomon and Lehmann (the last working under Kossel) observed that yeast suspended in water and allowed to stand at body temperature gave rise to free nuclein bases, the fresh cells not containing the substances in a free state. Salkowski and his pupils, Schwiening and Biondi, made it certain that the appearance of the substances was due to an autolytic process. More recent investigations have made it clear that through autolytic action purin bases, as soon as they become detached from the complex molecule of nuclein, suffer complete decomposition. Statements to that effect were made by earlier observers, more recently by Kutscher. The observation of Dakin [24] is in harmony with this statement, although he could detect in autolyzed kidneys only one purin base, hypoxanthin. The correctness of the statements was demonstrated by my own investigations. Quantitative analysis of all purin bases present in the fresh and in autolyzed organs has made clear the destruction of nuclein bases in the course of self-digestion. Very recently also some information has been gained regarding the chemical process of their disintegration.

In fresh tissues adenin and guanin occur in large amounts, the other two purin bases in very small amounts. Jones [25] and I,[17] independently of each other, have shown that by autolysis, these first two bases are changed into hypoxanthin and xanthin, respectively. The following shows the contents of purin bases in five pounds of fresh and autolyzed spleen:

	Fresh Spleen	Self-digested Spleen
Adenin	1.85 gm.	0.0 gm.
Guanin	1.10 gm.	0.0 gm.
Hypoxanthin	0.30 gm.	1.2 gm.
Xanthin	0.40 gm.	0.150 gm.

[24] Jour. of Physiol., vol. xxx, 1903.
[25] Zeitsch. f. phys. Chem., xli, 1904.

Schittenhelm arrived at similar conclusions and demonstrated further that under certain conditions the bases are transformed into uric acid. The other constituents of nucleic acid, the pyrimidin bases, undergo analogous changes.

The mechanism of this transformation was elucidated by the work of Jones.[25] This author accepts the presence in the tissues of two specific enzymes, one capable of acting on guanin and the other on adenin, and further of an oxidizing enzyme, the function of which it is to complete the transformation of nuclear material. The deductions of Schenck[26] are similar to those of Jones, and Schittenhelm[20] has corroborated in a general way the same conclusions. However, he does not support the assumption of the existence of more than one enzyme which can transform the amino-purins into the corresponding oxy-derivatives. According to Schittenhelm, the entire nuclear destruction is accomplished by three enzymes, one breaking up the nucleic acid into its components, the second splitting off the nitrogen from the nitrogenous constituents, and the third completing the oxidation of the purin derivative. It must be admitted that more detailed information concerning the intermediary products of nuclein metabolism is still wanting.

With this I wish to conclude the review of the products arising on autolysis of surviving organs. Reference should be made to the products of autolysis of sugar and fat, but thus far the investigations in that direction are few in number and the results obtained from them not very significant. This also concludes the review of autolytic action in normal organs. It remains to discuss these results in connection with the original problems which led to all these numerous investigations.

DISCUSSION OF RESULTS.

It has been stated already that the principal object of the work was: 1. To elucidate the nature and the mechanism of those chemical reactions which make the functions of the body possible. 2. To interpret the rôle of individual organs in the

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[26] Zeitsch. f. phys. Chem., vol. xliii, 1904.

animal metabolism. 3. To study the intermediate products of metabolism, since there is a general agreement that by the accumulation in the organism of these substances many diseased conditions are occasioned.

The foregoing review leads one to the conclusion that the knowledge of intermediate metabolism has been furthered considerably. On the other hand, a comparative study of the products of disintegration of various organs fails to bring out marked differences among them, although, during life at least, some organs are known to be the seat of special chemical reactions. This leads one to the assumption that in the animal body the process of self-digestion does not control all chemical reactions occurring in organs, perhaps even not all the processes of disintegration. A most conspicuous instance illustrating this statement is found in the work in which Eck's fistula was employed. By this name is designated a fistula between the vena cava and the portal vein. The aim of the fistula is to exclude the liver from the portal circulation. The organism of the dog possesses a very intense power of burning uric acid; the acid is present in the urine of this animal only in traces, even after injection of two to three grams of the substance. Dr. Sweet and I have demonstrated that animals kept for weeks on a diet free from all precursors of uric acid excrete considerable quantities of it in the urine as soon as an Eck fistula is performed on them. The output is especially increased after the administration of the substance itself.

Evidently under these conditions the organism fails to disintegrate uric acid, although the process of self-digestion is not depressed in the tissues. Thus the mechanism of "burning" uric acid in the living organism is not known yet. Proteid combustion also in the normal living organism apparently is different from the proteid disintegration in the course of autolysis. The great mass of products in the process of self-digestion remain in the stage of nitrogenous acids. A small part of them lose their nitrogen and a still smaller part give rise to carbon dioxid. In the living organism the splitting off of nitrogen from proteid material is a very rapid process, and

the transformation of all carbonaceous material to carbon dioxid also occurs with much greater rapidity than it possibly could take place in the course of self-digestion. But it has been stated that disintegration by the process of autolysis does occur during life. This and the foregoing are not contradictory to each other. Every tissue consists of cells of different age, of different states of nutrition and of different resistance. Work on hemolysis has brought out most clearly that individual blood cells vary in their vulnerability. Cells in a state of defective nutrition succumb to the process of self-digestion. In the course of that process enzymes are liberated which are capable of digesting extraneous material also.

It is difficult to demonstrate the correctness of this view on a complex organism, but it is made very clear from observations on the yeast cell. It is the function of that organism to convert grape sugar into alcohol. So long as conditions for this function are favorable there is little evidence of the process of self-digestion in a colony of yeast cells. But as soon as conditions are so altered as to make the normal life and the alcoholic fermentation impossible, a very active proteid-splitting enzyme is developed by the yeast cell which causes digestion of the cell proteid and of other proteid material. In the living organism the two forms of metabolism undoubtedly coexist. One is the result of the function of the organs, the other of their disintegration. The supply of energy required for the maintenance of life is furnished possibly by the first process. It has already been stated that by means of autolysis proteid is converted principally into amino-acids. By this conversion proteid could not furnish the organism with its full calorific requirement. On the other hand, autolytic enzymes may act on cell proteid and on the surrounding proteid in a manner similar to that of the enzymes of digestive glands, namely, rendering them a more suitable material for rapid combustion.

It is marvelous that, notwithstanding the presence of destructive agents in all tissues, organs succeed in guarding their integrity. A most clever investigation of recent years throws light on the mechanism by which this is accomplished. The

integrity of the gastric wall, the function of which is to elaborate digestive enzymes, has been the cause of much speculation. Weinland [27] demonstrated that this was due to the presence of an antiferment in the digestive glands. In the blood also were found antitryptic substances by Hahn,[28] Landsteiner,[29] Glaessner [30] and Cathcart.[31] The same property was noted in tissue extracts by Dr. Stookey and myself.[7] Furthermore, we have demonstrated that tissue extracts exercise an action antagonistic to that of autolytic enzymes. However, in health the two tendencies are so regulated that the tissue disintegration is sufficient to permit the organs to perform their function, while excessive wear is avoided. But as soon as the normal nutrition of the organism is disturbed the autolytic power of tissues increases. The mere fasting of an animal suffices to occasion in the tissues an exaggerated tendency for self-destruction. This was demonstrated by the experiments of Lane-Claypore and Schryver. It has been known for years that in cases of starvation animal organs lose in weight and that the loss varies in different organs. It is not improbable that products formed by disintegration of some organs serve to support the integrity of other more important organs. More marked is the high destructive power of tissues in diseases of a grave nature. Thus, in diseases of the respiratory system and of the heart, an intense self-digesting tendency of the tissues was noted by Schlesinger. In infectious diseases a similar observation was made by Flexner.[32] The work of this author preceded that of Schlesinger, and is very important for the reason that it furnished an interpretation for some old observations of pathologists.

Flexner demonstrated an unusually high rate of self-digestion in organs removed from individuals who succumbed to typhoid

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[27] Zeitsch. f. Biol., vol. xliv, 1903.
[28] Münch. med. Wochft., 1903.
[29] Cent. f. Bacteriol., vol. xxvii, 1900.
[30] Hofmeister's Beiträge, vol. iv, 1903.
[31] Jour. of Physiol., vol. xxxi, 1904.
[32] Univ. of Penn. Bull., July, 1903.

fever and other infectious diseases. The observation on typhoid is of special interest, since the exaggerated autolysis in the course of the disease can not be ascribed to the action of the micro-organism, for it is known that the proteolytic power of that germ is very slight. That this high rate of self-digestion is not merely a post-mortem phenomenon may be concluded from the old clinical observation, that products of proteid digestion are eliminated by the kidneys in the course of infectious diseases. The occurrence of peptonuria in these pathologic forms is not infrequent. Thus, in the light of the new investigation, this symptom acquires a special significance. So long as the nutrition is in a sufficiently good condition to prevent wasting of the tissues of the body, peptone is not present in the urine.

However, the softening and the wasting of tissues is most striking when these are under the influence of protoplasmic poisons. For this reason the study of phosphorus poisoning has attracted great attention. Martin Jacoby [6] first pointed out the high rate of autolysis of the organs removed from animals killed by phosphorus poisoning.

In this condition self-digestion takes place not only in surviving organs, but also during life. This may be concluded from the work of Abderhalden [21] and his co-workers, who recently have demonstrated the presence of crystalline components of the proteid molecule in the urine of animals poisoned with phosphorus.

The importance attached to the study of phosphorus poisoning is largely due to the resemblance which the clinical symptoms of this condition bear to that of a spontaneous pathologic form known as yellow atrophy of the liver. While the morphologic changes in the liver in the two conditions are not absolutely identical, still they present many points of similarity. The most striking are the disintegration of cellular elements and the so-called fatty degeneration of the organ. Through the work of many investigators, and particularly through that of Wakeman and Waldvogel, it has become evident that the changes which the liver undergoes in these pathologic conditions are identical with those which the organ suffers in the course

of autolysis. Indeed, the fact that the liver of persons who have succumbed to yellow atrophy contains products of proteid digestion was demonstrated by Salkowski more than twenty years ago, and was recently corroborated by Alonzo Taylor.[33] However, in neither of the two forms are the changes limited to one organ. Jacoby has demonstrated that the blood in phosphorus poisoning presents a striking loss of coagulability. Still more striking is the power it possesses of liquefying coagulated blood. This peculiarity was interpreted as being due to the presence of a proteolytic enzyme in the blood. In the course of yellow atrophy, products of a proteid cleavage have been found in the blood by Frerichs. This has been corroborated by many investigators, and very recently Neuberg and Richter[34] have shown that leucin, tyrosin and lysin may be present in the blood in quantities which clearly show that their origin could not be limited to the liver alone.

Thus, in the foregoing forms all tissues are apparently affected in the same manner. Marked autolysis in them may be considered a symptom of decline in general health and nutrition. However, there are conditions in which self-digestion is located in one organ only; thus the atrophy of the thymus, the involution of a puerperal uterus, are accomplished by a process of autolysis. The softening of tumors is brought about by the same mechanism. This was made clear through the work of Petry,[15] who demonstrated that freshly removed tumors contain products of proteid cleavage. The same author further demonstrated that the rate of self-digestion of the new growth is higher than that of a normal tissue. An attempt was also made to study the toxicity of the products resulting from this process. However, neither from a chemical nor a pathologic point of view could a difference between the end-products of autolysis of tumors and those of normal tissues be detected. Indeed, the intensity of self-digestion is high in all organs composed of cellular elements endowed with rapid growth.

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[33] Jour. of Med. Research, vol. viii, 1902.

[34] Deutsch. med. Wochft., No. 16, 1904.

The occurrence of local autolysis is not, as a rule, productive of a lowering in general health. On the contrary, it tends to restore normal conditions when these have been disturbed.

There are other conditions in which the process of autolysis is of aid to the organism in the efforts to maintain its integrity. It has been stated already that in the course of infectious diseases tissues possess a high power of autolysis. Investigations of Blum,[14] Conradi [35] and Levaditi [36] have shown that autolysis may be one of the means to which the organism resorts in order to elaborate protective substances. Substances of two distinct groups are formed in the organism as the result of infection. Those of one group aim to destroy the micro-organism and are designated bactericidal; the purpose of the other is to neutralize the toxin elaborated by the micro-organism—these are commonly named antitoxin. Normal tissues in course of autolysis may give rise to substances of either group.

Blum has shown that the products of autolysis of lymph glands possess the power to neutralize tetanus and diphtheria toxins and cobra venom. The mechanism of this action is not based merely on the physical properties of the autolyzed gland, for it is possible to save animals from death by injecting the products of autolysis subsequent to the injection of toxin.

Further, Conradi has tested the bactericidal power of the products of self-digestion of various organs. This author noted that the last, added to a suspension of bacteria in broth, prevented their growth. The intensity of bactericidal power varied in different organs, as presented in the following table:

Muscle	Strong
Lymphatic gland	Strong
Liver	Strong
Spleen	Strong
Thymus	Marked
Suprarenal	Marked
Bone marrow	Slight
Pancreas	None
Thyroid	None

[35] Hofmeister's Beiträge, vol. i, 1902; vol. v, 1904.
[36] Ann. de l'Inst. Pasteur, vol. xvii, p. 186.

Glancing over the table, one is struck by the fact that organs rich in leucocytes are most efficient in elaborating protective substances against infection. And one is naturally led to the analysis of the rôle played by the white blood cells in the effort of the animal body to maintain its integrity. A review of all the information gained concerning the action of leucocytes on the tissues shows that this is similar to the action of the digestive tissue enzymes. A tissue invaded by the white blood cells is subject to the action not only of the local enzymes, but of those of the blood cells as well. Digestion caused by the last, strictly speaking, cannot be regarded as self-digestion. However, the two processes present so much similarity that they are classified by most writers under the same head of autolysis.

The formation of proteid-digestion products through the action of leucocytes was first noted by a Russian writer, Eichwald, in 1864. The observation has been corroborated by many scientists and clinicians. Peptone has been demonstrated in pus and in the urine in all conditions associated with abscess formation. But the work that has attracted most attention and stimulated most research is that of Friedrich Müller. This author has demonstrated that in croupous pneumonia the resolution of the exudate is accomplished by a process of autolysis. The products arising in the course of resolution are identical with those occurring in proteid digestion. The mechanism of this process has been interpreted by Flexner, who, studying the intensity of autolysis in different stages of croupous pneumonia, observed that the intensity was very high in the stage of gray hepatization and very low in the stage of red hepatization, and explains this by the abundance of leucocytes in the exudates in the first condition and their scarcity in the other. Flexner also noted that in unresolved pneumonia the autolytic power is very imperfect. In this condition, as is well known, the exudate is very poor in leucocytes.

Flexner is inclined to interpret the failure of absorption of the exudate in unresolved pneumonia as being due to the disproportion between the leucocytes and the other constituents. Indeed, the correctness of the assumption that the absorption of

an exudate is accomplished by the action of leucocytes was established by the work of Opie,[37] who has noted that a fresh exudate does not possess the same degree of autolytic power as an old one. He has also noted that the lack of self-digestion in the first is due in some degree to the presence in the serum of a substance checking the digesting action of the blood cell. The rôle of the leucocyte in the absorption of inflammatory exudates had been previously described by Ascoli and Mareschi [38] and by Umber,[39] but the details of the mechanism were not fully understood until the appearance of the work of Opie. The discovery of the substance capable of keeping in check the destructive work of the blood cell is of great value for the understanding of the process. And it is now established that in the white cell the organism finds its most active factor for repairing the damaged and inflamed tissue.

There still remains to be discussed one point in the mechanism of leucocytic action. Regarding other tissue enzymes it has been stated that the destruction of the cell always precedes their liberation. Many writers have surmised that this applies also to the white blood cell. And yet this has not been fully established. The observation that in leukemia autolysis is noted during life seems to contradict the foregoing assumption. However, it is possible that the dead cell exercises at least a greater digestive action than the living. Hedin and Opie have demonstrated that from the white cell two enzymes can be obtained. One resembles the enzyme elaborated by the pancreatic gland, and the other the autolytic enzymes. It is conceivable that the first is secreted during the life of the cell, the last liberated only after its death. It is possible also that, once set free, the autolytic enzyme increases the power of the other enzyme. An analogous action of the spleen on the pancreas has been assumed by many writers and was proven by

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[37] Jour. of Exp. Medicine, vol. vii, 1905.

[38] Maly's Jahresb., vol. xxxii, 1902.

[39] Münch. med. Wochft., No. 49, 1902.

Stookey and myself. Thus waste and repair are controlled to a large extent by the same factors.

Autolysis, then, is the process by which dead tissue is not only prevented from becoming a burden to the organism, but, in addition, becomes converted into useful fuel material. By the aid of this process diseased tissues, pathologic formations and new growths are removed and replaced by normal healthy tissues. The organism resorts to this process also for elaboration of protective substances against bacteria and bacterial toxins. It still remains to be demonstrated whether or not in the course of autolysis enzymes are formed which are concerned in producing that energy which makes all animal functions possible.