THE ENZYMES IN PHAGOCYTMIC CELLS OF INFLAMMATORY EXUDATES.

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Those who have insisted upon the predominant importance of phagocytosis in the mechanism of natural and acquired immunity have not deserved Buchner's reproach that multiplied observation of this phenomenon can afford no explanation of its significance. Innumerable experiments have been devised to determine the conditions under which cells of various kinds ingest micro-organisms and other substances and the fate of the ingested material. The conditions under which phagocytosis occurs are now fairly well understood, but less is known concerning intracellular digestion.

Metschnikoff has shown that a close relation exists between phagocytosis in higher animals and the intracellular digestion of food by amœbae, planarians, actinîdae, and other animals of relatively simple structure. Mouton has studied the proteolytic enzyme which can be extracted with glycerine from the bodies of amœbae and has found that it liquefies gelatin and digests feebly coagulated egg albumen; it acts both in a weakly acid and in a weakly alkaline medium. From the phagocytic cells which digest the food of actinîdae, or sea anemones, Krukenberg and Mesnil have obtained a similar enzyme which the latter has named actinodiastase. By analogy with the intracellular digestion of these invertebrates Metschnikoff has attempted to explain the process which occurs within phagocytic cells of vertebrates.

Common to all mammalian species are two types of cell capable of ingesting solid particles. One type is the polynuclear leucocyte

1 L'immunité dans des maladies infectieuses, Paris, 1901.
2 Annal. de l'Inst. Pasteur, 1902, xvi, 457.
4 Annal. de l'Inst. Pasteur, 1901, xv, 352.
(polymorphonuclear) with fine granulation, the neutrophile leucocyte of human blood, which leaves the blood vessels and forms the chief cellular element of most inflammatory exudates. A second type of phagocytic cell is larger than the polynuclear leucocyte and has usually a single large nucleus which is round or somewhat irregular in outline; its protoplasm does not contain granules exhibiting a specific reaction to dyes. The polynuclear leucocyte with specific granulation is readily recognizable either within or without the blood vessels and histological and experimental studies have shown that it is derived from cells with similar specific granulation which divide by karyokinetic division in the bone marrow. The characters of the large mononuclear phagocyte are less distinctive and the identity of cells of this type occurring in different situations under different conditions has been much disputed. Such large mononuclear phagocytes, which Metschnikoff has called macrophages to distinguish them from the smaller polynuclear cells or microphages, are always found in inflammatory exudates which have accumulated in serous cavities; during the late stages of the inflammatory process they are actively engaged ingesting and destroying red blood corpuscles, polynuclear leucocytes, and other cellular elements. The large mononuclear phagocytes of the spleen, lymphatic glands, and other organs which during typhoid fever ingest red blood corpuscles and lymphocytes are doubtless similar in character.

The two types of phagocytic cells react differently to different substances. Polynuclear leucocytes it is well known are attracted by almost all kinds of bacteria and are especially concerned in the inflammatory reaction which follows the invasion of these micro-organisms. When for example Staphylococcus pyogenes aureus, Bacillus pyocyaneus, or Bacillus coli is injected into the peritoneal cavity of the guinea-pig in quantity insufficient to cause its death they are quickly ingested by polynuclear leucocytes; a few bacteria are taken up by mononuclear phagocytes. When, however, cellular elements such as red blood corpuscles or spermatozoa are injected into the peritoneal cavity of this animal they are as Metschnikoff \(^5\) has shown attacked and

\(^5\) Annal. de l’Inst. Pasteur, 1899, xiii, 737.
ingested by large mononuclear phagocytes or macrophages. Phagocytosis of certain parasites belonging to the animal kingdom, such as the malarial parasites and trypanosomes (Laveran and Mesnil⁶), is accomplished in great part at least by the same cells. The spirocheta of relapsing fever (Sawtschenko and Melkich⁷), and the similar parasite of geese (Metschnikoff⁸), organisms of which the zoological position is as yet undefined, suffer the same fate when injected into an insusceptible animal. It is noteworthy that certain bacteria, the bacillus of tuberculosis and the bacillus of leprosy (Iwanow⁹), are subject to phagocytosis by mononuclear cells, attracting in less degree polynuclear phagocytes.

It has long been known that intracellular digestion by amoebae and other protozoa occurs in the presence of acid. Granules of litmus ingested by some of these organisms assume a red color and alizarin-sulphate and other indicators of reaction show that the contents of the digestive vacuoles is acid. Similar observations have shown that the phagocytic cells which form the digestive cavity of certain low animal species such as sponges, planaria, and actinians, and by intracellular digestion prepare food for digestion, secrete acid into digestive vacuoles surrounding particles of food. Various substances ingested by such phagocytic cells assume in the presence of neutral red a brownish red color which according to Metschnikoff indicates the presence of acid. When phagocytic cells of the higher animals are allowed to ingest granules of litmus or tournesol-blue no change occurs; alizarin-sulphate is unaffected, for the reason, as Metschnikoff suggests, that it has a toxic action upon the phagocytic cell. When, however, a drop of exudate from a guinea-pig which has received in the peritoneal cavity nucleated red blood corpuscles from a bird is treated with a weak solution of neutral red, the partially digested corpuscles, or nuclei of corpuscles, which have been engulfed by macrophages assume the brownish red color which

* Annal. de l'Inst. Pasteur, 1901, xv, 673.
* Ibid., 1901, xv, 497.
* L'immunité dans les maladies infectieuses, p. 169.
* Annal. de l'Inst. Pasteur, 1902, xvi, 705.
Metschnikoff attributes to a feebly acid reaction. J. Plato,\textsuperscript{10} who first studied the effect of neutral red upon material ingested by phagocytes, found that bacteria are stained only after they are taken up by leucocytes and lose their color when the cell dies; he attributes the changes to oxidation and reduction of the dye, which with loss of oxygen becomes colorless. Himmel\textsuperscript{11} has confirmed the observations of Plato; the reaction indicates, he believes, that intracellular digestion of bacteria occurs in the presence of a feebly acid reaction. Bacillus tuberculosis fails to exhibit this reaction even when ingested by the phagocytes of an insusceptible animal though other related bacteria which resist decolorization with acid assume a red color under the same conditions. Metschnikoff believes that intracellular digestion of bacteria usually occurs in the presence of a feebly acid reaction but may, as with the tubercle bacillus, occur in a weakly alkaline medium. The experiments which will be described do not support this opinion.

It is unnecessary to review the arguments with which Buchner, Metschnikoff, and others have maintained that the alexine or complement or cytase of the serum is contained in the leucocytes from which it is set free, according to Buchner by a process of secretion, or according to Metschnikoff by disintegration of cells (phagolysis), nor to consider the views of those who have opposed these theories. Buchner has suggested that the alexine is of the nature of an enzyme and not improbably is identical with the proteolytic ferment which is present within the leucocytes. Metschnikoff has claimed that the disintegration of red blood corpuscles which occurs within the macrophage is analogous to that which is produced by proteolytic enzymes within the digestive vacuoles of ameobae and phagocytic cells of low invertebrate species. The enzyme or cytase which accomplishes intracellular digestion of bacteria he believes identical with the body discovered in the serum by Nuttall and named by Buchner alexine but later more accurately defined by Ehrlich and designated complement. The observations which Metschnikoff has

\textsuperscript{10} Arch. fär mikros. Anat., 1900, lvi, 868.
\textsuperscript{11} Annal. de l’Inst. Pasteur, 1902, xvi, 663.
brought forward in support of his belief that the macrophages contain macrocytase or haemolytic complement while the poly-nuclear leucocytes or microphages contain a second enzyme-like body, microcytase or bactericidal complement, will not be cited. Analogy with intracellular digestion among lower forms of animal life make it not improbable according to Metschnikoff that these bodies have the nature of proteolytic enzymes.

The occurrence of proteolytic enzymes in the cells of purulent exudates has been demonstrated by Fr. Müller, whose observations are recorded by Kossel. A glycerine extract of purulent phthisical sputum was found to digest fibrin and coagulated albumen in a weakly alkaline medium. Other purulent sputa have the same power but purely mucous sputa do not exhibit it. Leber has demonstrated the presence of proteolytic enzymes in purulent exudates and Achalme has shown that they may liquefy gelatin and dissolve fibrin, coagulated egg albumen, and casein. By means of the Kjeldahl method, determining before and after digestion the amount of nitrogen contained in substances precipitable by tannic acid, Ascoli and Mareschi have shown that the sediment from a sterile exudate obtained by the injection of aleuronat undergoes autolysis under toluol. Autolysis does not occur when the sediment has been subjected to a temperature of 60° C. during three hours.

Umber, employing the method of Kjeldahl, found that the peritoneal fluid from two patients suffering with abdominal tumor underwent autolysis, but Schütz found by the same method no measurable destruction of proteids though the exudates which he used contained in two instances a considerable number of leucocytes. Zak studied pleural and peritoneal exudates from twelve patients. He found no autolysis in half of the cases and

13 Ueber die Entstehung der Entzündung, Leipzig, 1891.
15 Eleventh Italian Congress for Internal Medicine, Pisa, October 27-31, 1901.
16 Münchener med. Woch., 1902, xlix, 1169.
17 Zent. f. innere Med., 1902, xxiii, 1161.
18 Wiener klin. Woch., 1905, xviii, 376.
could demonstrate no relation between the abundance of cells and the degree of autolysis. A somewhat similar observation has been made by Hahn, who has found that an artificially produced pleural exudate does not cause liquefaction of gelatin unless several days have elapsed since the injection of the inflammatory irritant.

Experiments which I have described in a previous publication have explained the discrepant observations just cited. The serum of the inflammatory exudate and of the blood inhibits the activity of the proteolytic enzymes contained in the leucocytes. When the leucocytes of the exudate are separated from the serum they undergo autolysis and are capable of digesting actively foreign proteid. In the late stages of inflammation produced by the injection of aleuronat into the pleural cavity of dogs there is some diminution of the anti-enzymotic action of the serum of the exudate.

Methods.—The methods employed have been described in the article to which reference has just been made. Exudates rich in leucocytes have been obtained by injecting a suspension of aleuronat in water containing starch (aleuronat, 5 grm.; starch, 1.5 grm., water, 100 c.c.) into the pleural cavities of dogs. Since the serum of the exudate inhibits the activity of the proteolytic enzymes contained in the leucocytes, it has been necessary to separate carefully cells from serum by repeated centrifugation with normal salt solution (0.85 per cent.). In order to compare the activity of enzymes contained in leucocytes of different exudates the washed cells were measured in a graduated centrifugal tube after centrifugation and suspended in a definite quantity of salt solution—equal to nine (1:10), nineteen (1:20), or twenty-nine (1:30) times the volume of the cells which were obtained. A measured quantity of the suspension of leucocytes was allowed to act upon heated blood serum, which being readily obtained formed a convenient means for testing proteolytic enzymes. Blood serum of the dog or of the ox mixed with an equal volume of salt solution does not coagulate, though it becomes

opaque, when heated to 75°C. for one half hour, and may be accurately measured in a pipette. At this temperature the proteolytic enzymes of the serum described by Delezenne and Pozerski and by Hedin, as well as its anti-enzymotic action, are wholly destroyed.

In all of the experiments to five cubic centimeters of heated serum (ten cubic centimeters of the diluted serum) were added in a small sterilized flask five cubic centimeters of the suspension of cells to be tested. Normal salt solution in sufficient quantity to bring the entire volume to twenty-five cubic centimeters was added, acid or alkali being substituted for salt solution in sufficient quantity to make any desired strength. After adding one cubic centimeter of toluol the flask was securely closed with a rubber stopper and kept at 37°C. in most instances during five days.

The same quantities of cells and of serum used for digestion were coagulated by heating to boiling and the nitrogen in uncoagulable form determined by the Kjeldahl method. The difference between the nitrogen of uncoagulable substances present in a flask kept at 37°C. and that of the control coagulated immediately represents the coagulable proteid converted by digestion into uncoagulable form. For the sake of convenience nitrogen determined by the Kjeldahl method will be represented by cubic centimeters of 1/10 N sulphuric acid, since for comparison of results it is unnecessary to convert these figures into the corresponding quantities of nitrogen.

In the earlier experiments much difficulty was experienced in accurately coagulating the undigested proteid, complete coagulation being dependent upon a reaction which varies with the quantity of proteid. The method finally adopted was as follows: To each flask containing twenty-five cubic centimeters of fluid was added an equal volume of a sixteen per cent. solution of magnesium sulphate (20 grm. to 100 c.c. of water). After heating to boiling, acetic acid in sufficient quantity to react with litmus was added. After boiling, a weak solution of sodium hydroxide

21 Compt. rend. de la Soc. de Biol., 1903, lv, 327, 690, 693.
22 Jour. of Physiol., 1904, xxx, 195.
was added in such excess that on boiling again the coagulated proteids formed a flocculent coagulum which after cooling subsided and left the fluid perfectly clear. Since an excess of sodium hydroxide combines with magnesium sulphate to form insoluble magnesium hydroxide, the reaction of the solution is thus made approximately neutral.

In a publication previously cited I have shown that the enzymes contained in the leucocytes of an inflammatory exudate are capable of digesting proteid both in an alkaline and in an acid medium, though their activity is greater in the former. Hedin and Rowland 23 had shown that autolysis of parenchymatous organs, such as the spleen, kidneys, liver, heart, and lymphatic glands, occurs more actively in an acid than in an alkaline medium. From the spleen Hedin 24 has succeeded in isolating two enzymes, one of which acts with the aid of acid, the other in the presence of alkali. I have shown 25 that the bone marrow resembles the cells of an inflammatory exudate in its action on proteids, digesting far more actively in an alkaline than in an acid medium, and differs from other organs such as the liver, spleen, kidney, and lymphatic glands of which the proteolytic action is stronger in an acid medium. Since histologists with few exceptions are agreed that the polymuclear leucocytes with fine granulation are derived from the bone marrow, proteolysis produced in an alkaline medium by these cells may be referred to an enzyme formed in the bone marrow. The purpose of the following experiments has been to determine if leucocytes of the inflammatory exudate contain one enzyme which acts both in an acid and in an alkaline medium, or two enzymes one of which, like pepsin, acts in the presence of acid while the other, resembling trypsin, acts in the presence of alkali.

Preliminary Experiments.—Since the exudates obtained by the injection of aleuronat into the pleural cavities of the dog frequently, contain red blood corpuscles which are present in considerable number especially when the injection has been

24 Jour. of Physiol., 1904, xxx, 155.
accompanied by injury to the lung, it was considered necessary to test the action of red blood corpuscles on heated serum and to exclude the possibility that they contain enzymes capable of causing proteolysis either in an acid or in an alkaline medium. The following experiment shows that the proteolytic action of these cells is insignificant.

**EXPERIMENT I.**—Mixtures containing 5 c.c. heated serum and 5 c.c. of a suspension of red blood corpuscles washed three times by centrifugation and suspended in salt solution (in dilution of 1:20) were kept at 37°C for five days.

<table>
<thead>
<tr>
<th></th>
<th>With 0.2% acetic acid</th>
<th>Without addition</th>
<th>With 0.2% sodium carbonate</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.05 c.c.</td>
<td>2.3 c.c.</td>
<td>2.45 c.c.</td>
<td>2.15 c.c.</td>
<td></td>
</tr>
</tbody>
</table>

In the experiments which I have recorded in previous articles the ability of the enzymes of the exuded leucocytes to digest proteids in a medium made acid by acetic acid was tested. In order to exclude the possibility that proteolysis occurs only with acetic acid, other acids, namely hydrochloric and sulphuric, were used. By adding two and a half, five, or ten cubic centimeters of the \( \frac{1}{4} \) N acids to each of a number of flasks containing five cubic centimeters of a suspension of leucocytes together with five cubic centimeters of heated serum and bringing the total volume of each flask to twenty-five cubic centimeters with normal salt solution, the strength of the acid present was made respectively \( \frac{1}{4} \) N, \( \frac{1}{2} \) N, and \( \frac{3}{4} \) N. For the sake of comparison flasks containing sodium carbonate in the same strength were prepared.

**EXPERIMENT II.**—Leucocytes from an exudate removed from the pleural cavities of a dog five days after the injection of aleronat were suspended in nineteen times their volume (1:20) of salt solution. The following figures represent in cubic centimeters of \( \frac{1}{4} \) N sulphuric acid the amounts of nitrogen in substances uncoagulable by heat present in 5 c.c. suspension of cells and 5 c.c. heated serum after digestion five days at 37°C in the media which have been mentioned:

<table>
<thead>
<tr>
<th></th>
<th>Acetic Acid</th>
<th>Sulphuric Acid</th>
<th>Hydrochloric Acid</th>
<th>Sodium Carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{1}{4} ) N</td>
<td>21.15 c.c.</td>
<td>5.25 c.c.</td>
<td>4.4 c.c.</td>
<td>20.35 c.c.</td>
</tr>
<tr>
<td>( \frac{1}{2} ) N</td>
<td>18.95 &quot;</td>
<td>18.2 &quot;</td>
<td>13.95 &quot;</td>
<td>28.05 &quot;</td>
</tr>
<tr>
<td>( \frac{3}{4} ) N</td>
<td>—</td>
<td>20.7 &quot;</td>
<td>17.9 &quot;</td>
<td>29.05 &quot;</td>
</tr>
</tbody>
</table>

Digestion without addition of acid or alkali 24.55 c.c.
Control 4.0 "
It is noteworthy that the maximum digestion occurs in a slightly alkaline medium. When no addition has been made the normal alkalinity of the heated serum though diluted with four times its volume of fluid gives slight alkalinity to the medium. The efficiency of the three acids in favoring proteolysis varies considerably; sulphuric and hydrochloric acids in the same strength (\(\frac{1}{3}\) N) in which acetic acid favors digestion almost wholly prevent it, while in much weaker solution they cause active digestion. If, as it is not improbable, the maximum efficiency of hydrochloric acid is represented by a strength less than \(\frac{1}{3}\) N the relative activity of the three acids follows the same order as their dissociation.

Preservation of proteolytic enzymes of leucocytes by glycerine.—In the experiments just described as well as in those recorded in previous publications the enzymotic action of cells has been tested immediately after removal from the animal. For certain experiments preservation of the enzymes was desirable and several methods of preserving them were attempted. Suspensions of cells in fifty per cent. glycerine preserved their ability to digest proteid in an acid as well as in an alkaline medium; proteolysis produced by the suspension in glycerine was only slightly less than that caused by the same cells diluted to the same extent with salt solution and used in the fresh state.

Experiment III.—Leucocytes from a dog killed five days after the injection of aleuronat into the pleural cavity were suspended in nine times their volume of salt solution (1:10). To a part of this suspension was added an equal volume of salt solution and the ability of the diluted suspension (1:20) to digest heated serum tested in the presence of 0.2% acetic acid, in 0.2% sodium carbonate, and with an unchanged reaction. To the remainder of the original suspension was added an equal volume of glycerine, making the dilution of the suspension 1:20. At the end of a week and again at the end of a month the action of 5 c.c. of the suspension was tested under conditions similar to those previously employed. In all instances digestion occurred during five days at 37°C.

<table>
<thead>
<tr>
<th></th>
<th>Suspension of fresh cells</th>
<th>Suspension in glycerine after 1 wk.</th>
<th>Suspension in glycerine after 1 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>With 0.2% acetic acid</td>
<td>20.9 c.c.</td>
<td>17.5 c.c.</td>
<td>18.8 c.c.</td>
</tr>
<tr>
<td>Neutral</td>
<td>26.0 &quot;</td>
<td>&quot;</td>
<td>26.55 &quot;</td>
</tr>
<tr>
<td>With 0.2% sodium carbonate</td>
<td>28.65 &quot;</td>
<td>26.95 &quot;</td>
<td>29.8 &quot;</td>
</tr>
<tr>
<td>Control</td>
<td>2.9 &quot;</td>
<td>4.0 &quot;</td>
<td>6.8 &quot;</td>
</tr>
</tbody>
</table>
At first sight the figures suggest that the activity of the enzyme has at the end of a month increased rather than diminished. It may be noted, however, that there is a corresponding increase in the figure representing the control, doubtless as the result of slow autolysis occurring in the glycerine extract kept at room temperature. When the figure representing the control is subtracted from that obtained at the end of digestion it is found that in an acid medium digestion is less active when the glycerine extract is used (13.5 c.c.) than with the fresh suspension (18 c.c.); activity of the enzyme has diminished slightly at the end of a month (12 c.c.). In an alkaline medium the glycerine extract is somewhat less active (22.95 c.c.) than the fresh suspension (25.75 c.c.) and has remained unchanged during a month (23 c.c.).

Preservation of Proteolytic Enzyme of Leucocytes by Drying after Treatment with Alcohol and Ether.—The attempt was made to determine if leucocytes dried and reduced to a powder retain their enzymotic action. The cells, obtained from an exudate by centrifugalization, after repeated washing with many times their volume of salt solution were treated with about fifteen times their volume of absolute alcohol; part of the alcohol was poured off after the cells had settled and replaced with fresh absolute alcohol. After standing about an hour the alcohol was removed by filtration and replaced with ether. After a short time the ether was removed by filtration and compression between pads of filter paper. The cells, allowed to dry in the air, were reduced in a mortar to a fine powder, which on account of the presence of a small amount of red blood corpuscles had a dull red color.

**Experiment IV.**—Leucocytes were obtained from a dog eight days after the injection of aleuronat into the left pleural cavity and dried by the method described. The dried powder (20 mgr.) was allowed to act upon heated serum (5 c.c.) in acid, neutral, and alkaline media for five days at 37° C.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Volume (c.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With 0.2% acetic acid</td>
<td>7.55</td>
</tr>
<tr>
<td>Without addition</td>
<td>22.8</td>
</tr>
<tr>
<td>With 0.5% sodium carbonate</td>
<td>18.8</td>
</tr>
<tr>
<td>Control</td>
<td>5.8</td>
</tr>
</tbody>
</table>
EXPERIMENT V.—Powdered leucocytes (20 mgr.) obtained from a dog two days after the injection of aleuronat acted on heated serum eight days at 37° C.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>With 0.2% acetic acid</td>
<td>5.5 c.c.</td>
</tr>
<tr>
<td>Without addition</td>
<td>19.05 c.c.</td>
</tr>
<tr>
<td>With 0.2% sodium carbonate</td>
<td>12.55 c.c.</td>
</tr>
<tr>
<td>Control</td>
<td>2.45</td>
</tr>
</tbody>
</table>

EXPERIMENT VI.—The proteolytic activity of the dried enzyme from an exudate obtained twenty-four hours after the injection of aleuronat was tested four and a half months later with the following result:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>With 0.2% acetic acid</td>
<td>6.3 c.c.</td>
</tr>
<tr>
<td>Without addition</td>
<td>22.85 c.c.</td>
</tr>
<tr>
<td>With 0.2% sodium carbonate</td>
<td>21.1</td>
</tr>
<tr>
<td>Control</td>
<td>5.35</td>
</tr>
</tbody>
</table>

The powder prepared from leucocytes of a sterile exudate digests proteid actively in an almost neutral and in an alkaline medium but has almost completely lost the power of the fresh cells to digest in an acid medium. The most probable explanation of this fact is that the leucocytes of the exudate contain two ferments, one of which digests in a neutral or slightly alkaline medium, while the other acts in an acid medium; the former is uninjured during the process of preparing the dry powder, while the latter is destroyed. The enzyme which acts in an alkaline medium has preserved its activity unchanged more than four months in the dry condition.

For comparison with results to be described later an experiment showing the effect of various quantities of the enzyme upon a measured quantity of proteid is recorded.

EXPERIMENT VII.—Various quantities of the powder used in Experiment VI were allowed to act during five days at 37° C. upon 5 c.c. heated serum without addition of acid or alkali.

<table>
<thead>
<tr>
<th>Quantity of Powder</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mgr. powder</td>
<td>19.05 c.c.</td>
</tr>
<tr>
<td>20 mgr. powder</td>
<td>22.8 c.c.</td>
</tr>
<tr>
<td>50 mgr. powder</td>
<td>26.05 c.c.</td>
</tr>
<tr>
<td>Control</td>
<td>5.8</td>
</tr>
</tbody>
</table>

The figures only very roughly indicate the velocity of the reaction produced by various quantities of ferment but show that a considerable increase in the amount of the latter causes a moderate increase in the amount of proteolysis at the end of a given time. E. Schütz,²⁶ studying by polarimetric estimation

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the amount of pepton formed from egg-white by the action of pepsin, reached the conclusion that the rapidity of digestion is proportional to the square root of the quantity of enzyme. Using Mett's tubes, in which a measured column of coagulated albumen is subjected to digestion, Borissow 27 and Samojloff 28 have obtained similar results. J. Schütz 29 has found that digestion may be shown to follow this rule by estimating by the Kjeldahl method the nitrogen of coagulable proteid converted into uncoagulable form. That the same rule is applicable to tryptic digestion has been maintained by Pawlow 30 and by Vernon 31. For the present purpose the mathematical accuracy of these statements is not significant; it is sufficient to point out that only a slight increase in the degree of proteolysis may be caused by large increase in the amount of enzyme.

Effect of Temperature on the Proteolytic Enzymes of Leucocytes.—Further evidence that the leucocytes of the inflammatory exudate contain two enzymes is furnished by the different effect of various temperatures upon proteolysis when tested in an acid and when tested in an alkaline medium. For this purpose suspensions of fresh cells were used; after five cubic centimeters of the suspension of cells in salt solution had been subjected to various temperatures for one half hour, five cubic centimeters of heated serum were added together with acid or alkali in quantity to bring the entire amount of fluid (25 c.c.) to a strength of 0.2 per cent.

Experiment VIII.—Leucocytes from an exudate removed from the pleural cavity of a dog twenty-three hours after the injection of aleuronat were suspended in nineteen times their volume of salt solution (1:20). The result of digestion caused by this suspension of cells heated to various temperatures was as follows:

<table>
<thead>
<tr>
<th>Leucocytes</th>
<th>In 0.5% acetic acid</th>
<th>In 0.5% sodium carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>unheated</td>
<td>13.65 c.c.</td>
<td>24.05 c.c.</td>
</tr>
<tr>
<td></td>
<td>13.5 &quot;</td>
<td>26.0 &quot;</td>
</tr>
<tr>
<td>heated at 50° C.</td>
<td>11.2 &quot;</td>
<td>27.4 &quot;</td>
</tr>
<tr>
<td></td>
<td>8.7 &quot;</td>
<td>27.45 &quot;</td>
</tr>
<tr>
<td>65° C.</td>
<td>9.05 &quot;</td>
<td>25.65 &quot;</td>
</tr>
<tr>
<td></td>
<td>4.0 &quot;</td>
<td>4.75 &quot;</td>
</tr>
<tr>
<td>100° C.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

27 Inaug. Diss., St. Petersburg, quoted by Pawlow.
28 Pflüger's Archiv, 1901, lxxv, 86.
31 Jour. of Physiol., 1901, xxvi, 405.
EXPERIMENT IX.—Leucocytes obtained twenty-four hours after the injection of aleuronat were subjected to various temperatures as before.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Leucocytes unheated</th>
<th>Leucocytes heated</th>
</tr>
</thead>
<tbody>
<tr>
<td>55 ° C.</td>
<td>16.6 c.c.</td>
<td>14. &quot;</td>
</tr>
<tr>
<td>60 ° C.</td>
<td>12.45 &quot;</td>
<td>12.1 &quot;</td>
</tr>
<tr>
<td>65 ° C.</td>
<td>8.65 &quot;</td>
<td>4.55 &quot;</td>
</tr>
<tr>
<td>70 ° C.</td>
<td>3.65 &quot;</td>
<td>5.2 &quot;</td>
</tr>
<tr>
<td>75 ° C.</td>
<td>3.65 &quot;</td>
<td>3.65 &quot;</td>
</tr>
<tr>
<td>80 ° C.</td>
<td>3.65 &quot;</td>
<td>3.65 &quot;</td>
</tr>
<tr>
<td>100 ° C.</td>
<td>2.65 &quot;</td>
<td>3.65 &quot;</td>
</tr>
</tbody>
</table>

Temperatures between 55 ° and 70 ° C. diminish the enzymotic activity of the fresh leucocytes exhibited in an acid medium but do not materially diminish proteolysis in an alkaline medium. In an alkaline medium, indeed, the activity of proteolysis is slightly increased when the cells have been subjected to low temperatures. A somewhat similar observation has been made by Biernacki, who found that heating trypsin in an alkaline solution in the presence of certain salts or of albumoses at 45 ° C. during from five to ten minutes increased its proteolytic activity so that digestion proceeded more rapidly than was usual.

Though the power to digest in an acid and in an alkaline medium is differently affected by temperatures below 70 ° C., proteolysis in both media is prevented by higher temperatures.

EXPERIMENT X.—Leucocytes removed from the pleural cavity of a dog five days after injection of aleuronat were suspended in nineteen times their volume of salt solution (1:20). Power to digest heated serum was tested in acid and in alkaline media after heating to temperatures between 70 ° and 100 ° C.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Leucocytes unheated</th>
<th>Leucocytes heated</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 ° C.</td>
<td>16.35 c.c.</td>
<td>4.75 &quot;</td>
</tr>
<tr>
<td>75 ° C.</td>
<td>3.05 &quot;</td>
<td>3.6 &quot;</td>
</tr>
<tr>
<td>80 ° C.</td>
<td>3.65 &quot;</td>
<td>3.75 &quot;</td>
</tr>
<tr>
<td>100 ° C.</td>
<td>2.65 &quot;</td>
<td>3.65 &quot;</td>
</tr>
</tbody>
</table>

Although in this experiment the effect of heating at 70 ° C. is greater than in the two preceding experiments, it is evident that enzymotic activity of fresh cells is wholly destroyed at a temperature between 70 ° and 75 ° C. This statement applies only to the conditions of the experiment, since Biernacki has shown that reaction, and the presence of certain salts or of albumoses affect the

ability of trypsin and pepsin to withstand various temperatures.

Changes in the Proteolytic Enzymes of Cells of Inflammatory Exudates Occurring during the Progress of Inflammation.—When aleuronat is injected into the pleural cavity of the dog, as Buchner first showed, an active inflammatory reaction ensues and leucocytes together with a large quantity of serum accumulate in the cavity. At the end of from fifteen to twenty hours after injection of from five to ten cubic centimeters of a suspension of aleuronat there is a considerable quantity, in some cases more than one hundred cubic centimeters, of thin grayish-pink or red fluid containing usually not more than five per cent. of cells (measured after centrifugalization) among which red blood corpuscles are often numerous. Aleuronat is no longer free in the fluid but is firmly adherent to the pleural surfaces, accumulating along the anterior mediastinum just above the diaphragm. The lymphatic glands in the mediastinum, particularly those behind the upper part of the sternum, enlarge rapidly. After the injection of ten cubic centimeters of the suspension of aleuronat into each pleural cavity the latter usually contain at the end of from three to five days or even longer a considerable amount of fluid which is now much more turbid and richer in cells. Resolution of the exudate occurs, the aleuronat is absorbed, and except for the presence of fibrous adhesions the cavities return to their normal condition.

At the end of from sixteen to twenty-four hours after the injection polymuclear leucocytes with fine granulation form the greater number of the cells which are present. Differential counts show that from eighty to ninety per cent. of the leucocytes are of this type. The remainder of the cells are of the mononuclear type but few lymphocytes are present. The greater number of these mononuclear cells are somewhat larger than polymuclear leucocytes and have a large vesicular nucleus, which is usually somewhat irregular in outline, and fairly abundant protoplasm. They have the characters of the large mononuclear leucocytes of the blood. Since they are capable of active phagocytosis Metschnikoff has designated them macrophages; Kanthack and Hardy 33

33 Jour. of Physiol., 1894, xvii, 81.
and other English writers have called them hyaline cells; Mar-
chand\textsuperscript{34} describes them as leucocytoid cells.

During the later stages of the inflammatory process polynuclear
leucocytes with fine granulation are very numerous but their
percentage is somewhat diminished since the large mononuclear
cells have increased in number. Leucocytes with eosinophile or
basophile granulation are present in insignificant number. At the
end of three or five days large mononuclear cells form from
twenty-five to thirty-five per cent. of the cells; the large propor-
tion noted by Tarassévitch,\textsuperscript{35} namely fifty to sixty per cent.
two or three days after the injection of aleuronat into the pleural
cavities of rabbits, was not found. The percentage counts of large
mononuclear cells give little indication of the changes which
they undergo, since as the exudate increases in age they increase
in volume so that at the end of three or more days there are many
cells with a diameter four or five times that of a polynuclear
leucocyte. At this time very active phagocytosis is in progress
and mononuclear cells with vacuolated protoplasm may contain
red blood corpuscles or polynuclear leucocytes in various stages
of disintegration; one large phagocyte may contain six or more
of such cells. Mononuclear cells at this time are often clumped
together to form masses which may contain from ten to fifty cells.
The inflammatory reaction which follows the injection of aleu-
ronat does not differ materially from that produced by the in-
jection of various bacteria (described by Durham\textsuperscript{36}) or by the
injection of carmine, starch, or lycopodium powder (described by
Marchand\textsuperscript{37}) into guinea-pigs and other animals.

Since the cellular character of the exudate changes during the
course of inflammation opportunity is afforded to study the re-
lation between the proteolytic enzymes which are present and
the types of phagocytic cells which have been described. For this
purpose the power of a measured quantity of cells to digest a
measured quantity of proteid has been tested in acid, in neutral,
The Enzymes in Phagocytic Cells of Inflammatory Exudates

or in alkaline media. In the records which follow the amounts of digestion represented in cubic centimeters of 1/10 N sulphuric acid have been obtained by subtracting the figure representing the control, coagulated immediately, from that representing the amount of nitrogen in substances uncoagulable by heat present after digestion for five days at 37°C. Differential counts of the leucocytes contained in the exudate have been made. Since cells with eosinophile or basophile granules have been rarely encountered, by polynuclear leucocytes are designated cells with fine granulation, corresponding to the neutrophile leucocytes of human blood. Mononuclear cells include not only the large mononuclear phagocytes but smaller cells resembling the lymphocytes of the blood; the latter form only a very small proportion, rarely so much as two or three per cent. of the number of cells.

It has been desirable to separate the experiments into several groups since in all instances the conditions of the experiment have not been the same.

Series A.—In the following experiments inflammatory exudates were obtained by injecting 10 c.c. of suspension of aleuronat into each pleural cavity of dogs which were killed at the end of one, two, four, and five days respectively. The washed leucocytes were suspended in nineteen times (1:20) their volume of salt solution. At the time these experiments were performed the method of comparison finally adopted was not contemplated so that the record is in some instances incomplete. The following figures represent the digestion of 5 c.c. heated serum produced by 5 c.c. of cell suspension kept at 37°C during five days (save in Experiment XII in which digestion was stopped at the end of four days) in 0.2% sodium carbonate, in an approximately neutral medium, and in 0.2% acetic acid.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age of</th>
<th>Reaction of medium</th>
<th>Cells of exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>exudate</td>
<td>Alkaline</td>
<td>Neutral</td>
</tr>
<tr>
<td>XI</td>
<td>1 day</td>
<td>19.7 c.c.</td>
<td></td>
</tr>
<tr>
<td>XII</td>
<td>2 days</td>
<td>0.6 c.c.</td>
<td>15.8 c.c.</td>
</tr>
<tr>
<td>XIII</td>
<td>4 d.</td>
<td>21.85 c.c.</td>
<td></td>
</tr>
<tr>
<td>XIV</td>
<td>5 d.</td>
<td>24.05 c.c.</td>
<td>20.55 c.c.</td>
</tr>
<tr>
<td>XV</td>
<td>5 d.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In Experiment XV (See Experiment II) digestion occurred in 1/10 N sodium carbonate (approximately 0.2%) and in 1/10 N acetic acid (approximately 0.2%).

The ability of the fresh cells to digest in an alkaline medium shows considerable irregularity which has not been repeated in
subsequent experiments; the almost total absence of digestion in Experiments XII and XIII has not been explained. In only two experiments was the power to digest in a neutral medium tested. With proteolysis in an acid medium there was an almost uniform increase as the time between the injection of the inflammatory irritant and the removal of the exudate increased.

A second series of two experiments is not comparable with those just described because the cell suspensions used were prepared by suspending cells in nine times their volume of salt solution (1:10).

**Series B.**—In the two experiments which follow the conditions are the same as those in Series A, save that washed leucocytes were suspended in nine times their volume of salt solution.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age of exudate</th>
<th>Reaction of medium</th>
<th>Cells of exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alkaline</td>
<td>Neutral</td>
</tr>
<tr>
<td>XVI</td>
<td>1 day</td>
<td>28.0 c.c.</td>
<td>16.95 c.c.</td>
</tr>
<tr>
<td>XVII</td>
<td>5 days</td>
<td>26.7 &quot;</td>
<td>18.55 &quot;</td>
</tr>
</tbody>
</table>

Although the ability of the cells to digest in an alkaline or neutral medium has undergone little change, their efficiency in an acid medium has materially increased. At the same time the proportion of mononuclear cells has increased. It may be recalled on the one hand that the proportion of mononuclear cells does not accurately represent their relation to those with polymorphous nucleus since along with an increase in number the mononuclear phagocytes have undergone a great increase in volume, often having a diameter from three to five times as great as that of the polymuclear cells. On the other hand the amount of digestion during a given time does not increase in direct proportion to the amount of ferment contained in the cells but is more closely related to the square root of its quantity.

The exudates used in the preceding experiments were obtained by injecting a large amount of aleuronat, ten cubic centimeters of the suspension, into each pleural cavity. At the end of five days aleuronat is still present in great quantity adherent to the parietal pleura and embedded within the tissue of the anterior mediastinum. Doubtless as the result of the presence of the
foreign body in large amount irritation is continued and the exudate fails to undergo resolution; at the end of five days there may be present in each pleural cavity 150 or 200 cubic centimeters of fluid. In the following experiments a smaller quantity of aleuronat, five cubic centimeters of the suspension, was injected into the right pleural cavity alone in the hope that long continued exudation of polynuclear leucocytes might be avoided. In an animal killed at the end of four days the right pleural cavity contained 75 cubic centimeters of fluid in which however the proportion of polynuclear leucocytes was still large.

Series C.—Cells obtained one and five days after the injection of 5 c.c. of aleuronat suspension into the right pleural cavity of dogs were suspended in nineteen times their volume of salt solution (1:20).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age of exudate</th>
<th>Reaction of medium</th>
<th>Cells of exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alkaline</td>
<td>Neutral</td>
</tr>
<tr>
<td>XVIII</td>
<td>1 day</td>
<td>26.35 c.c.</td>
<td>22.0 c.c.</td>
</tr>
<tr>
<td>XIX</td>
<td>5 days</td>
<td>25.75 &quot;</td>
<td>25.1 &quot;</td>
</tr>
</tbody>
</table>

Series D.—In two experiments the conditions were identical with those just described save that washed leucocytes were suspended in twenty-nine times their volume of salt solution (1:30).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age of exudate</th>
<th>Reaction of medium</th>
<th>Cells of exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alkaline</td>
<td>Neutral</td>
</tr>
<tr>
<td>XX</td>
<td>1 day</td>
<td>19.4 c.c.</td>
<td>16.95 c.c.</td>
</tr>
<tr>
<td>XXI</td>
<td>4 days</td>
<td>17.2 &quot;</td>
<td>20.6 &quot;</td>
</tr>
</tbody>
</table>

The results of the experiments of Series B, C, and D are uniform; with the progress of the inflammatory reaction there is an increase in the proportion of mononuclear cells and diminution in the percentage of polynuclear leucocytes, while corresponding with these changes there is a slight diminution of proteolysis in an alkaline medium and a well marked increase in an acid medium. On the one hand the polynuclear leucocytes have decreased from approximately eighty-five to seventy-five per cent, and on the other hand the mononuclear cells have almost doubled in number and at the same time have increased much in bulk. Evidence has already been produced to show that the cells of inflammatory exudates contain two enzymes characterized like pepsin and tryp-
sin by their relation to acids and alkalis. The preceding experiments afford evidence that the polynuclear leucocytes contain that enzyme which is capable of active proteolysis both in an alkaline and in an almost neutral medium while the large mononuclear phagocytes produce that enzyme which digests proteid in an acid medium. It is not improbable that the latter enzyme produces slight proteolysis in a neutral medium since in Series B, C, and D there is with a marked increase of digestion in an acid medium a slight increase of digestion in a neutral medium.

Further evidence that the power to digest in an acid medium is dependent upon the mononuclear phagocytes is afforded by the following experiments in which were employed exudates produced by the injection of red blood corpuscles of the rabbit into the pleural cavity of the dog. Metschnikoff has shown that an exudate rich in mononuclear cells is obtained within twenty-four hours after the injection of red blood corpuscles of one species into an animal of another species. The large proportion of mononuclear cells noted by Gengou, who found the exuded cells almost wholly of this type, was not obtained.

**Series E.**—Into each pleural cavity of two dogs were injected 5 c.c. of washed red blood corpuscles of the rabbit. In one animal, killed at the end of nineteen hours, the two cavities contained about 50 c.c. of deep red fluid from which 2.9 c.c. of cells were obtained by centrifugalization. In the two pleural cavities of the second animal, killed after three days, were 10 c.c. of thick pinkish-gray fluid from which were obtained 5.9 c.c. of cells. Suspensions of the leucocytes in both instances diluted in the proportion of 1:20 were allowed to act upon heated serum under the usual conditions.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age of exudate</th>
<th>Reaction of medium</th>
<th>Cells of exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alkaline</td>
<td>Neutral</td>
</tr>
<tr>
<td>XX II</td>
<td>19 hours</td>
<td>23.9 c.c.</td>
<td>20.65 c.c.</td>
</tr>
<tr>
<td>XX III</td>
<td>3 days</td>
<td>21.65 &quot;*</td>
<td>19.5 &quot;</td>
</tr>
</tbody>
</table>

* Digestion occurred in presence of 0.1% sodium carbonate.

The previously observed increase of proteolysis in an acid medium associated with an increase in the age of the exudate has not occurred when the proportion of mononuclear phagocytes has not undergone noteworthy change.

*Changes in the Proteolytic Activity of Cells of Lymphatic Glands*

* Annal. de l'Inst. Pasteur, 1901, xv, 68.*
near the Site of Inflammation.—The enlarged lymphatic glands which are situated in the neighborhood of the focus of inflammation afford further opportunity to study the enzymotic action of the large mononuclear phagocytes. Aleuronat injected into the pleural cavity of the dog rapidly accumulates, in part perhaps as the result of gravity, upon and within the anterior mediastinum, which at the end of from three to five days is converted into a thick firm mass of tissue within which aleuronat is still recognizable. Microscopical examination shows that masses of aleuronat are surrounded by polynuclear leucocytes which in the immediate neighborhood of the substance have not infrequently undergone necrosis. Mononuclear cells of the type previously described are numerous. In the anterior mediastinum above the level of the heart aleuronat is much less abundant, the tissue is moderately edematous, and there are present at least a half dozen enlarged succulent lymphatic glands. Two larger than the others are situated immediately behind the upper part of the sternum and at the end of about three days after injection of the irritant measure one and a half centimeters in length.

Microscopical examination of these mediastinal lymphatic glands show that their sinuses are distended with fluid and contain even within twenty-four hours after the injection of aleuronat large mononuclear cells which do not differ from those found in the pleural exudate and are capable of ingesting red blood corpuscles and polynuclear leucocytes. These cells increase progressively in size and number and in some instances are so closely packed within the sinuses that the organ has the microscopic appearance of a mesentric lymphatic gland during typhoid fever. Not only within the sinuses but in the medullary cords as well are found such cells containing numerous partially disintegrated polynuclear leucocytes. In the sinuses and in the medullary cords free polynuclear leucocytes are numerous; the germinating centers of the follicles are enlarged and do not contain polynuclear leucocytes.

Since these lymphatic glands contain large mononuclear phagocytes massed together in large number it was thought not improbable that they would exhibit some change in their ability
to produce proteolysis. Jacoby 39 has shown that the thymus gland undergoes autolysis and Hedin and Rowland 40 have found that autolysis of lymphatic glands proceeds more rapidly in an acid than in an alkaline medium. In a previous publication I have shown that an emulsion of cells prepared from the mesenteric lymphatic glands of the dog is capable of digesting heated blood serum.

By scraping the gland with a knife and forcing the tissue through a small sieve made with fine wire gauze, cells were completely separated from stroma. After washing the cells with salt solution they were measured in a graduated centrifugal tube and suspended in a definite quantity of salt solution. Since the normal mediastinal glands of the dog are too small to furnish cells in sufficient quantity for use it was necessary to compare the proteolytic activity of the inflamed mediastinal glands with glands distant from the site of inflammation. For this purpose the mesenteric glands were suitable. In the sinuses of these glands are often found a considerable number of large mononuclear cells resembling those found in the inflamed gland. In every instance the mesenteric glands were examined microscopically and compared with sections from one or more of the mediastinal glands which were used. The sinuses of the latter were often packed with mononuclear phagocytes in diameter several times that of a polymuclear leucocyte while those of the mesenteric glands contained a moderate number of similar cells only slightly larger than polymuclear leucocytes and very rarely containing ingested material.

Experiment XXIV.—Into each pleural cavity were injected 10 c.c. of aleuronat. At the end of three days when the animal was killed there was no fluid in either cavity. The substernal lymphatic glands were large and succulent and together with several bronchial glands were used to prepare a suspension diluted with salt solution in the proportion of 1: 30. The proteolytic action of 5 c.c. of this suspension and of the same amount of a similarly prepared suspension from the mesenteric glands upon 5 c.c. of heated serum when kept two days at 37° C. is represented by the following figures:

19 Beiträge z. chem. Physiol. u. Path., 1902, i, 147.
The Enzymes in Phagocytic Cells of Inflammatory Exudates

<table>
<thead>
<tr>
<th></th>
<th>Mediastinal</th>
<th>Mesenteric</th>
</tr>
</thead>
<tbody>
<tr>
<td>With 0.2% acetic acid</td>
<td>11.45 c.c.</td>
<td>9.9 c.c.</td>
</tr>
<tr>
<td>Without addition</td>
<td>4.9 &quot;</td>
<td>5.2 &quot;</td>
</tr>
<tr>
<td>With 0.2% sodium carbonate</td>
<td>5.1 &quot;</td>
<td>5.05 &quot;</td>
</tr>
<tr>
<td>Control</td>
<td>5.05 c.c.</td>
<td></td>
</tr>
</tbody>
</table>

EXPERIMENT XXV.—Suspensions, diluted 1:30, were prepared from the substernal and mesenteric lymphatic glands removed four days after the injection of 5 c.c. of aleuronat into the right pleural cavity of a dog.

<table>
<thead>
<tr>
<th></th>
<th>Mediastinal</th>
<th>Mesenteric</th>
</tr>
</thead>
<tbody>
<tr>
<td>With 0.2% acetic acid</td>
<td>11.95 c.c.</td>
<td>8.75 c.c.</td>
</tr>
<tr>
<td>With 0.2% sodium carbonate</td>
<td>4.25 &quot;</td>
<td>3.8 &quot;</td>
</tr>
<tr>
<td>Control (approximately*), 3.3 c.c.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The quantity of cells obtainable from the lymphatic glands was insufficient for the preparation of a control flask. The amount of nitrogen in uncoagulable form contained in 5 c.c. of serum with 5 c.c. of a suspension of cells from the pleural exudate was determined. The amount of such nitrogen in this quantity of cells is insignificant.

The preceding experiments have shown that suspensions of cells from the inflamed mediastinal glands and from the relatively normal mesenteric glands cause proteolysis in an acid medium but are capable of causing little if any digestion in a neutral or alkaline medium. In both experiments greater proteolysis was caused by the inflamed than by the normal gland. The two experiments which follow confirm this observation.

EXPERIMENT XXVI.—The proteolytic activity of the cells from the mediastinal glands of a dog killed (A) nineteen hours after the injection of aleuronat was compared with that of cells from a dog allowed to live (B) five days after injection. From the latter animal a similarly prepared suspension (1:30) was obtained from the mesenteric lymphatic glands.

<table>
<thead>
<tr>
<th></th>
<th>Mediastinal</th>
<th>Mesenteric</th>
</tr>
</thead>
<tbody>
<tr>
<td>A—with 0.2% acetic acid</td>
<td>7.6 c.c.</td>
<td>9.2 c.c.</td>
</tr>
<tr>
<td>B—&quot;</td>
<td>12.2 &quot;</td>
<td></td>
</tr>
<tr>
<td>Control, A (approximately), 2.9 c.c.; B (approximately), 2.9 c.c.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the following experiment an inflammatory reaction was caused by the injection of red blood corpuscles of the rabbit into the pleural cavities of the dog. The resulting histological changes in the mediastinal lymphatic glands do not differ materially from those produced by aleuronat. The sinuses contain in great number large mononuclear phagocytes actively engaged in ingesting polymonuclear leucocytes and red blood corpuscles.
Experiment XXVII.—A suspension of cells obtained from the mediastinal lymphatic glands of the dog used in Experiment XXIII was diluted with salt solution in the proportion of 1:40 and compared with a similarly prepared suspension from the mesenteric glands. The result of digestion of heated serum during five days at 37° C. was as follows:

<table>
<thead>
<tr>
<th></th>
<th>Mediastinal</th>
<th>Mesenteric</th>
</tr>
</thead>
<tbody>
<tr>
<td>With 0.2% acetic acid</td>
<td>7.85 c.c.</td>
<td>5.85 c.c.</td>
</tr>
<tr>
<td>Control (approximately), 1.9 c.c.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The foregoing experiments have shown that the proteolytic action of lymphatic glands containing large mononuclear phagocytes, which do not differ from those present in the exudate during the late stages of inflammation, is exhibited in the presence of acid and is almost wholly absent in a neutral or alkaline medium. In the dried powder prepared from washed leucocytes a proteolytic enzyme digesting in a neutral or alkaline medium was found almost wholly free from activity in the presence of acid. It has been possible therefore to obtain separately each of two enzymes demonstrable in the phagocytic cells which have a part in the inflammatory process.

As the length of time after injection of the inflammatory irritant increases mononuclear phagocytes increase in number and size and the exuded cells exhibit an increased ability to digest proteids in the presence of acid. In the neighboring lymphatic glands where cells of similar character accumulate there is an increased proteolytic activity in the presence of acid. The progressively increasing activity of similarly prepared suspensions from mediastinal glands at various intervals after the injection of aeluronat is indicated by the following figures obtained from Experiments XXV and XXVI (in which the conditions are the same) by subtracting the figure representing the control determination of nitrogen in uncoagulable form from that found at the end of digestion.

<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>4 days</th>
<th>5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>With 0.2% acetic acid</td>
<td>4.7 c.c.</td>
<td>8.05 c.c.</td>
<td>9.3 c.c.</td>
</tr>
</tbody>
</table>

When it is recalled that the rapidity of digestion does not vary directly with the amount of ferment but is more closely related to the square root of this quantity the significance of these figures is increased: an increase in the number of mononuclear
The Enzymes in Phagocytic Cells of Inflammatory Exudates

Phagocytosis is accompanied by an increase in the amount of the enzyme which digests proteid in the presence of acid.

The Occurrence of Two Proteolytic Enzymes in the Cells of an Inflammatory Exudate and their Relation to Bone Marrow and Lymphoid Tissue.—It has not been the purpose of the present study to determine the nature of the enzymes which have been found in the leucocytes of inflammatory exudates nor their relation to other somewhat similar bodies. The resistance of both enzymes to a temperature of 70°C indicates that they are not identical with the alexine or complement, which is destroyed at approximately 56°C. Although the existence of two enzymes has been established, the one present in the polynuclear leucocytes, the other, in the large mononuclear phagocytes or macrophages, there is no evidence that they are identical with the bactericidal complement or microcystase and the hemolytic complement or macrocystase which Metschnikoff thinks have their origin in these two types of cell. The experiments which have been described have not been concerned with the varieties of the complement nor with its relation to the leucocytes.

That enzyme of which the activity is limited to an acid medium resembles in this respect pepsin, while that which digests in an alkaline medium is more closely related to trypsin. On the one hand the ferment which limits its action to an acid medium is destroyed by means which are used to preserve pepsin, namely precipitation and drying with alcohol and ether, and fails to act in that concentration of hydrochloric acid which is most favorable to the action of pepsin, namely 0.2% (see Experiment II., \( \frac{1}{10} \% \ N = 0.15\% \)). On the other hand that enzyme which is contained in the polynuclear leucocytes and digests in an alkaline medium is apparently far less active than trypsin, obtained from the pancreas by similar methods.

It is not improbable that the enzyme of the mononuclear phagocytes is closely related to if not identical with the autolytic enzyme which is contained in various parenchymatous organs and acts more efficiently in an acid than in an alkaline medium. Repeated experiment has shown that the cells of an apparently normal lymphatic gland are capable not only of autolysis but
of digesting foreign protein (heated serum) in the presence of acid. Of especial significance, however, is the fact that the amount of enzyme increases when the gland contains an increased number of mononuclear cells capable of intracellular proteolysis.

Recent observers who have studied the morphology and histogenesis of the various types of leucocyte found in the blood with few exceptions support the view long maintained by Ehrlich that the lymphocytes and granular leucocytes differ in origin, one type having its origin in the lymphatic glands and other lymphatic structures, the other in the bone marrow. Concerning the nature of the large mononuclear leucocytes there has been greater divergence of opinion though most observers maintain with Ehrlich and Lazarus that they are closely related to the lymphocytes and have their origin in great part at least in lymphoid tissue. Marchand for example believes that the large mononuclear phagocytes which accumulate in the peritoneal cavity after the injection of various irritant substances are derived from large mononuclear cells situated in the adventitia of the small blood vessels of the omentum and other structures. These cells he designates leucocytoid since they resemble similar cells found within the blood vessels and by division produce not only large phagocytes but he thinks smaller cells which do not differ from lymphocytes. Maximow maintains that the large phagocytes which appear during the course of an inflammatory reaction are derived from lymphocytes which migrate from the blood vessels and has brought forward much experimental evidence in favor of this view. He believes that the adventitial cells of Marchand are of the same nature. Helly, studying the exudates produced by the injection of bacteria into the peritoneal cavity, has reached conclusions similar to those of Maximow.

Corresponding with the two types of phagocyte, one the

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43 Ziegler's *Beiträge*, 1905, xxxvii, 171.
polynuclear leucocyte with fine granulation, the other the large non-granular cell derived from lymphoid tissue, there are two enzymes characterized by their ability to digest proteid, the one in a neutral or alkaline medium, the other in an acid medium. I have shown that the enzyme which is active in the presence of alkali occurs in the bone marrow and that the bone marrow, unlike the liver, spleen, kidney, and other organs, digests proteid more actively in an alkaline than in an acid medium. Since the polynuclear leucocytes with fine granulation—the neutrophile leucocytes of man—are formed in the bone marrow this enzyme doubtless has the same origin and may be designated leuco-protease. The name lympho-protease may be given to that enzyme which is contained in the large mononuclear phagocytes since these cells have their origin in lymphoid tissue.