ENZYMES AND ANTI-ENZYMES OF INFLAMMATORY EXUDATES.

By EUGENE L. OPIE, M.D.

(*From the Rockefeller Institute for Medical Research, New York.*)

Many substances insoluble in the fluids of the body when injected into the tissues undergo solution through the agency of phagocytic cells which surround or engulf them. Much study has been devoted to the factors which influence phagocytosis but comparatively little attention has been given to the process of intracellular digestion.

The occurrence of pepton in pus was demonstrated by Eichwald [1] as early as 1864, and later confirmed by Maixner [2] and Hofmeister.[3] In association with a variety of diseases characterized by formation and accumulation of pus, such as empyæma, purulent peritonitis, abscess, epidemic cerebro-spinal meningitis, and pyelitis, Maixner found pepton in the urine. Pepton was also observed in the urine during the resolution of croupous pneumonia. Finding that the amount of pepton present in purulent fluid bore a relation to the number of cells which were present, and was more abundant in the cellular sediment than in the overlying serum or in the filtrate from such an exudate, Hofmeister reached the conclusion that living pus cells have the power of chemically or mechanically binding pepton, and peptonuria is a symptom of the destruction of pus cells.

To explain the constant presence of pepton in purulent sputum, Kossel [4] cites hitherto apparently unpublished observations of Fr. Müller, who had found that a glycerine extract of purulent

[1] *Würzb. med. Zeit.*, 1864, 335. Cited by Hofmeister.
[2] *Prager Vierteljahrschrift*, cxliii, 78. Cited by Hofmeister.
[3] *Zeit. für phys. Chem.*, 1880, iv, 253, 268.
[4] *Zeit. für klin. Med.*, 1888, xiii, 149.

phthisical sputum is capable of digesting in a weakly alkaline medium fibrin and coagulated albumin if decomposition is prevented. Other purulent sputa have the same power, but purely mucous sputum and the sputum of pneumonia before the crisis do not exhibit it. As soon as crisis occurs and the sputum has assumed a white pus-like appearance, the presence of a ferment is demonstrable. In the fresh pus obtained from an abscess, a similar ferment can be demonstrated, but is absent in the thin pus-like fluid of a cold abscess. Achalme [5] has studied the effect of pus upon gelatin, fibrin, coagulated egg-albumin, and casein, and has found that it is capable of liquefying or dissolving these substances. Similar observations have been made by Leber.[6]

Ascoli and Mareschi [7] subjected the cellular sediment of sterile exudates, obtained by injecting aleuronat into the peritoneal cavity, to autolysis under chloroform, and determined before and after digestion, by means of the Kjeldahl method, the amount of nitrogen not precipitable by tannin. A well marked digestion occurred but was absent when the cellular sediment had been previously heated to 60° C. for three hours.

Attempts to study the autolysis of fluids withdrawn from the serous cavities of individuals suffering with peritoneal or pleural effusions have given inconstant results. Estimating by means of the Kjeldahl method the amount of proteid converted by digestion into non-coagulable nitrogen-containing substances, Umber [8] showed that the peritoneal fluid from two patients suffering with abdominal tumors underwent autolysis. In ascitic fluid from five patients and in pleural exudates from two, Schütz [9] found by the same method no measurable destruction of proteids. The fluid in two instances contained a considerable number of leucocytes. The autolysis of exudates withdrawn from serous cavities of twelve patients suffering with hydro-

[5] *Compt. rend. de la Soc. de Biol.*, 1899, li, 568.

[6] *Ueber die Entstehung der Entzündung*, Leipzig, 1891.

[7] *Eleventh Italian Congress for Internal Medicine*, Pisa, October 27–31, 1901. Ref. Maly's *Jahresber.*, 1902, xxxii, 291.

[8] *Münchener med. Woch.*, 1902, xlix, 1169.

[9] *Cent. für inner. Med.*, 1902, xxiii, 1161.

thorax, pleurisy, and ascites has been studied by Zak.[10] In one-half of the cases, including two cases of pleurisy, no autolysis was demonstrable, while in only four instances was there noteworthy digestion of coagulable albumin. Zak found no relation between the abundance of cells and the degree of autolysis.

Methods.—The present series of experiments was undertaken with the purpose of studying the capacity for proteolytic digestion possessed by inflammatory exudates at various stages. Exudates, obtained by injecting suspensions of plasmon or of aleuronat into the pleural cavities of dogs or rabbits, were, with aseptic precautions, removed from the animal killed by bleeding or were withdrawn from the etherized animal by aspiration. A suspension of plasmon in ten times its weight of 0.5 % potassium hydroxide was at first used, but aleuronat meal in from seven to ten times its weight of water, to which was added 3 % of starch, proved more useful. Of this suspension 10 cubic centimeters were injected into a pleural cavity. The exudate removed at the end of from one to three days was introduced in carefully measured quantities into small flasks and diluted with four times its volume of 0.85 % salt solution. In most instances 1 cubic centimeter of toluol was added to each flask, which was then placed in the thermostat at 37° C. At the end of two or more days the flasks were removed and their contents were heated to 100° C., after acidification with acetic acid.

Complete precipitation of the coagulable proteids was at first effected with some difficulty, but was later much facilitated by the addition of an equal volume of 16 % solution of magnesium sulphate. Should precipitation be incomplete, filtration is difficult and the filtrate is turbid. By adjusting the reaction to slight acidity with acetic acid (1 %), or with sodium hydroxide ($\frac{1}{10}$ N.) if acid has been added in excess, albumin still in solution may be coagulated by heat and again removed by filtration. The filtrate from the coagulum repeatedly washed was received directly into a Kjeldahl flask and the amount of nitrogen present was estimated by the Kjeldahl method.

[10] *Wiener klin. Woch.*, 1905, xviii, 376.

The filtrate from an equal amount of exudate diluted in the same way and immediately coagulated by heat served as a control. The difference between the autolyzed exudate and its control represents in terms of $\frac{1}{10}$ N. sulphuric acid the nitrogen of the coagulable proteid converted by digestion into soluble form. I am indebted to Dr. Flexner and to Dr. P. A Levene for numerous suggestions which have aided me in the application of the chemical methods employed.

Autolysis of Sterile Inflammatory Exudates.—In the following experiments, sterile exudates, obtained by injecting a suspension of aleuronat into the right pleural cavity of dogs, were removed aseptically and kept in sterile flasks without addition of toluol or other antiseptic for forty-eight hours at body temperature. That no autolysis had occurred during this time is shown by the following figures which represent in terms of $\frac{1}{10}$ N. acid the nitrogen of substances in solution after coagulation by heat.

EXPERIMENT 1.—Reddish turbid fluid containing many leucocytes and a considerable number of red blood corpuscles was removed from the right pleural cavity of a dog twenty-four hours after the injection of aleuronat. Nitrogen present in the filtrates from the coagulated fluid is represented by c. c. of $\frac{1}{10}$ N. acid.

5 c. c. exudate at 37° C. 2 days	3.1 c. c.
Control	3.7 "

EXPERIMENT 2.—Exudate was removed forty-eight hours after the injection of aleuronat into the right pleural cavity of a dog. The exudate contained 6.5 % of cells.

5 c. c. exudate at 37° C. 2 days	3.05 c. c.
Control	3.75 "

In both experiments less nitrogen was found in the control subjected to immediate coagulation than in the flask kept forty-eight hours at body temperature. The significance of this fact, confirmed by subsequent experiments, will be discussed later.

In similar experiments the exudates obtained from rabbits by injecting aleuronat into the pleural cavity were used. The diluted exudate was kept at body temperature four days.

EXPERIMENT 3.—Exudate was removed twenty-four hours after the injection of 10 c. c. of aleuronat suspension into the right pleural cavity of a rabbit. The cells present formed 6 % of its volume.

3 c. c. exudate at 37° C. 4 days (aseptic)	0.7 c. c.
Control	0.9 "

EXPERIMENT 4.—Exudate was removed two days after the injection of 5 c. c. of aleuronat suspension. Cells form 5.5 % of the volume.

3 c. c. exudate at 37° C. 4 days (with toluol)	1.65 c. c.
Control	1.7 "

EXPERIMENT 5.—Exudate was removed three days after the injection of 10 c. c. of aleuronat. Cells form 2.5 % of the volume.

1 c. c. exudate at 37° C. 4 days (aseptic)	2.10 c. c.
Control	1.55 "

No autolysis was obtained except with the exudate removed three days after the injection of the inflammatory irritant. Since it was suspected that autolysis occurred only after the death of cells contained in the exudate, longer periods of digestion were considered necessary, and at the same time the effect of toluol, which presumably destroys the cells, was studied. A considerable number of flasks containing equal quantities of exudate, some aseptically prepared, others prepared with toluol, were kept at body temperature for varying periods as indicated below.

EXPERIMENT 6.—An exudate containing 4 % cells was obtained twenty-four hours after injecting 10 c. c. aleuronat suspension into the right pleural cavity of a dog. Each flask contained 3 c. c. exudate diluted as in previous experiments.

	Aseptic.	With toluol.
Control	2.0 c. c.	
At 37° C. 2 days	2.0 "	1.3 c. c.
" " " 4 "	2.1 "	2.15 "
" " " 6 "	1.7 "	2.5 "
" " " 8 "	1.7 "	2.55 "

EXPERIMENT 7.—An exudate containing 2.5 % cells was removed from the right pleural cavity of a dog three days after the injection of 10 c. c. aleuronat. Each flask contained 3 c. c. exudate.

	Aseptic.	With toluol.
Control	7.9 c. c.	7.7 c. c.
At 37° C. 2 days	7.8 "	7.7 "
" " " 4 "	9.9 "	10.25 "
" " " 6 "	12.9 "	10.9 "

Exudates removed both from the dog and from the rabbit one or two days after the injection of aleuronat underwent little if any digestion, while that removed three days after injection autolyzed to an appreciable though slight extent. The presence of toluol did not very materially affect the result in either case, and greatly facilitates the performance of the experiment, since

it obviates the necessity of preserving the strictest asepsis when, as in subsequent experiments, centrifugalization of the exudate is necessary. In the preceding experiments, cultures made from each flask at the end of digestion remained sterile.

It is noteworthy that Schütz, attempting to autolyze exudates obtained by tapping the pleural or peritoneal cavities, obtained negative results, while those of Zak were inconstant. Zak found no relation between autolysis and the cellular contents of the exudate.

Autolysis of Leucocytes.—In several instances the quantity of cells in the exudates employed in the preceding experiments was estimated by means of the hæmatocrit and varied by volume from 2.5 % to 6.5 %. There was no relation between the proportion of cells and the occurrence of autolysis. In the exudates obtained from rabbits, those removed one and two days after injection contained respectively 6 % and 5.5 % of cells, while the exudate which was removed on the third day and which underwent slight digestion contained 2.5 %. In order to determine if autolysis affected only the cells, in the following experiments cells of an exudate twenty-four hours after injection of aleuronat were separated from the serum by centrifugalization and suspended in nineteen times their volume of .85 % solution of sodium chloride (5 % suspension). The suspension was diluted with four times its volume of salt solution and subjected to autolysis during five days. An equal amount of serum was kept during the same time at 37° C. and compared with a control flask coagulated immediately.

EXPERIMENT 8.—In 5 c. c. of a 5 % suspension of cells and in 5 c. c. of serum, obtained from an exudate removed twenty-four hours after the injection of aleuronat into the pleural cavities of a dog, the amount of nitrogen contained in substances not coagulable by heat before and after digestion is represented in terms of $\frac{1}{10}$ N. acid by the following figures:

5 c. c. cells at 37° C. 5 days	2.35 c. c.
Control	.75 "
5 c. c. serum at 37° C. 5 days	3.7 "
Control	4.15 "

With cells alone well marked autolysis occurred, although with the entire exudate removed at the same stage of inflam-

mation and containing approximately the same number of cells, none was demonstrable (e.g., Experiment 2, in which the percentage of cells in the exudate used was 6 %). It is noteworthy that the serum kept at 37° C. for five days, like the whole exudate in previous experiments, contained less nitrogen in soluble form than the control coagulated immediately.

Anti-enzymotic Action of the Serum of Sterile Inflammatory Exudates.—The experiment just described has suggested that the serum of the inflammatory exudate inhibits the autolysis of the leucocytes which it contains. Obviously this possibility may be tested by separating, by centrifugalization, cells and serum and digesting separately and after recombining.

In the following experiment, cells from an exudate removed from the pleural cavity twenty-four hours after the injection of aleuronat were suspended in nine times their volume of salt solution. In order to test the autolysis of these cells, 5 cubic centimeters of the suspension were diluted with 20 cubic centimeters of salt solution. To the same quantity of cells was added 1 cubic centimeter, and in another flask 5 cubic centimeters, of the serum of the exudate, and in every instance the total volume of fluid was brought to 25 cubic centimeters by the addition of salt solution. In another flask 5 cubic centimeters of serum, diluted with 5 cubic centimeters of salt solution, were heated for twenty minutes in boiling water; to this coagulated serum were added 5 cubic centimeters of the suspension of cells. After addition of toluol the various mixtures were kept at 37° C. for five days. The nitrogen in uncoagulable compounds, contained in 5 cubic centimeters of serum from the exudate diluted to 25 cubic centimeters and allowed to remain in the thermostat for the same time, was, for the sake of comparison, estimated by the usual method.

EXPERIMENT 9.—Cells and serum, obtained from an exudate removed from the pleural cavities of a dog twenty-four hours after the injection of aleuronat, were tested as follows:

5 c. c. cells at 37° C. 5 days	8.2	c. c.
Control	1.3	"
Digestion	6.9	"

5 c. c. serum at 37° C. 5 days	4.35	c. c.
5 c. c. cells + 1 c. c. serum at 37° C. 5 days	3.45	"
Uncoagulable nitrogen in 5 c. c. cells (1.3 c. c.) and 1 c. c. serum (approximately 0.87 c. c.) represented by	2.17	"
Digestion	1.28	"
5 c. c. cells + 5 c. c. serum at 37° C. 5 days	7.75	" *
Uncoagulable nitrogen in 5 c. c. cells (1.3 c. c.) and 5 c. c. serum (approximately 4.35 c. c.) represented by	5.65	"
Digestion	2.1	"
5 c. c. cells + 5 c. c. coagulated serum at 37° C. 5 days	30.65	"
Uncoagulable nitrogen in 5. c. c cells and 5 c. c. serum represented by	5.65	"
Digestion	25.00	"

* Culture from this flask showed bacterial contamination; the flask had been insecurely closed and the toluol used had escaped.

The autolysis of the leucocytes contained in the suspension is inhibited by the addition of serum, so that the nitrogen derived by digestion from coagulable proteids is diminished to less than one-third. When, however, the serum is heated to approximately 100° C. no inhibition occurs and digestion proceeds far beyond that referable to autolysis of cells. The intracellular ferments, the action of which is now unrestrained, attacks the proteids furnished by the heated serum.

The following experiments confirm the results just described:

EXPERIMENT 10.—Cells obtained twenty-four hours after the injection of aleuronat into the pleural cavities of a dog were suspended in nineteen times their volume of salt solution.

5 c. c. cells at 37° C. 5 days	4.55	c. c.
Control	2.05	"
Digestion	2.5	"
5 c. c. serum at 37° C. 5 days	3.85	"
Control (coagulated immediately)	4.5	"
Digestion	——	
5 c. c. cells + 5 c. c. serum at 37° C. 5 days	5.4	"
Uncoagulable nitrogen in 5 c. c. cells (2.05 c. c.) and 5 c. c. serum (4.5 c. c.) represented by	6.55	"
Digestion	——	

5 c. c. cells + 5 c. c. coagulated serum at 37° C. 5 days....	14.2	c. c.
Uncoagulable nitrogen in 5 c. c. cells and 5 c. c. serum represented by.....	6.55	"
Digestion.....	7.65	"

EXPERIMENT 11.—Cells from an exudate removed from the pleural cavities of a dog three days after the injection of aleuronat were suspended in nine times their volume of salt solution.

5 c. c. cells at 37° C. 5 days.....	9.3	c. c.
Control.....	3.6	"
Digestion.....	5.7	"
5 c. c. serum at 37° C. 5 days.....	6.5	"
Control (coagulated immediately).....	7.25	"
Digestion.....	——	
5 c. c. cells + 1 c. c. serum at 37° C. 5 days.....	10.2	"
Uncoagulable nitrogen in 5 c. c. cells (3.6 c. c.) and 1 c. c. serum (1.45 c. c.) represented by.....	5.05	"
Digestion.....	5.15	"
5 c. c. cells + 1 c. c. coagulated serum at 37° C. 5 days.....	11.05	"
Uncoagulable nitrogen in 5 c. c. cells and 1 c. c. serum represented by.....	5.05	"
Digestion.....	6.00	"
5 c. c. cells + 5 c. c. serum at 37° C. 5 days.....	10.95	"
Uncoagulable nitrogen in 5 c. c. cells (3.6 c. c.) and 5 c. c. serum (7.25 c. c.) represented by	10.85	"
Digestion.....	.1	"
5 c. c. cells + 5 c. c. coagulated serum at 37° C. 5 days	23.1	"
Uncoagulable nitrogen in 5 c. c. cells and 5 c. c. serum represented by.....	10.85	"
Digestion.....	12.25	"

It is noteworthy that the inhibitory action of the serum of an exudate used in the last experiment and removed three days after the injection of the imflammatory irritant is less than that of the serum from the exudates employed in the two preceding experiments and withdrawn only twenty-four hours after injection. One cubic centimeter of the serum in this experiment had little effect upon the digestion of the cells mixed with it (digestion of cells alone, 5.7 cubic centimeters; of cells with 1

cubic centimeter serum, 5.15 cubic centimeters), while in the preceding experiments the same amount of serum completely prevented or materially retarded digestion. This observation is in agreement with the fact already noted, that the whole exudate removed one or two days after the onset of inflammation undergoes little if any autolysis, while that of exudates three days old is considerable.

That the digestion of other proteids by enzymes contained in the leucocytes is inhibited by the serum of the exudate has been shown by several readily performed experiments in which gelatin and fibrin have been subjected to the action of cells and to cells with serum.

EXPERIMENT 12.—To test-tubes containing 6 c. c. 6 % gelatin was added 1 c. c. 20 % suspension of cells from an exudate obtained twenty-four hours after the injection of aleuronat. To a second gelatin tube were added 1 c. c. suspension of cells and 1 c. c. serum from the same exudate. After remaining twenty hours at 37° C., the gelatin containing cells alone remained liquid after cooling while that with cells and serum solidified.

EXPERIMENT 13.—To one of two tubes containing balanced quantities of fibrin obtained from dog's blood and cut into small pieces, were added 2 c. c. 20 % suspension of cells from an exudate removed two days after injection of aleuronat; to the second, 2 c. c. suspension and 2 c. c. serum from the exudate, the volume being made in both instances 8 c. c. with salt solution. At the end of forty-eight hours under toluol at 37° C. fibrin in the tube containing cells had almost completely disappeared, while that mixed with cells and serum was apparently unchanged.

Effect of Temperature upon the Anti-enzymotic Action of the Exuded Serum.—It has already been shown that the anti-enzymotic action exerted by the serum of a sterile exudate upon the proteolytic ferment of the leucocytes is destroyed by a temperature of 100° C. In order to determine at what point of an ascending scale the inhibitory power is destroyed, equal quantities of serum, diluted with an equal volume of salt solution, were heated to 45°, 55°, 65°, 75°, and 100° C. for one-half hour and then mixed with a suspension of cells, the entire mixture being brought to a volume of 25 cubic centimeters by addition of salt solution.

EXPERIMENT 14.—Cells from an exudate obtained three days after the injection of aleuronat into the pleural cavity of a dog were, after repeated washing

suspended in nine times their volume of salt solution. Mixtures prepared as follows were kept in the thermostat at 37° C. for five days:

(5 c. c. cells + 5 c. c. serum) coagulated as control.....	8.45 c. c.
" " + "	9.9 "
" " + " " heated to 45° C.....	8.5 "
" " + " " " " 55° C.....	8.8 "
" " + " " " " 65° C.....	7.8 "
" " + " " " " 75° C.....	25.05 "
" " + " " " " 100° C.....	27.0 "

A temperature of 75° C. evidently suffices to destroy the anti-enzymotic action of the serum. Lower temperatures apparently cause a slight increase of the inhibition exerted by the serum, but the explanation of the slight change which occurs is not clear. It is not improbable that a temperature lower than 75° C. produces changes in the serum which exert an unfavorable influence upon the ferment or ferments contained in the leucocytes, or, as will subsequently appear, upon a proleolytic enzyme contained in the serum and destroyed perhaps by 65° C.

Effect of Reaction upon the Enzymes and Anti-enzymes of Sterile Inflammatory Exudates.—It was next considered desirable to study the influence of reaction upon this anti-enzymotic action of the serum of sterile exudates. This exuded serum is strongly alkaline to litmus. To mixtures of cells and serum, prepared as in previous experiments, was added sodium bicarbonate or acetic acid to the strength of 0.2%. For comparison the same amount of alkali or acid was added to similar mixtures of cells and coagulated serum.

EXPERIMENT 15.—Cells from an exudate removed twenty-four hours after the injection of aleuronat were suspended in nine times their volume of salt solution. Mixtures containing 5 c. c. of this suspension and 5 c. c. of serum were kept in the thermostat at 37° C. for five days.

	Cells + serum.	Cells + coagulated serum.
With 0.2 % sodium bicarbonate..	10.1 c. c.	35.05 c. c.
Reaction unchanged.....	12.9 "	24.0 "
With 0.2 % acetic acid.....	27.05 "	19.55 "

The leucocytes of the exudate are capable of digesting proteids in both an alkaline and in an acid medium, but are more efficient in the former. The inhibition exerted by the serum

is favored by an increase of the normal alkalinity of this fluid but is completely lost with the acidity produced by 0.2 % acetic acid. In this acid medium there is, indeed, more digestion of proteid with unheated than with heated serum. The possibility that the serum of the exudate contains an enzyme active in an acid medium is suggested. The preceding experiment was repeated and at the same time to serum alone was added alkali or acid in the quantity previously used.

EXPERIMENT 16.—Cells obtained from an exudate three days after the injection of aleuronat were suspended in nine times their volume of salt solution. Mixtures containing 5 c. c. suspension of cells and 5 c. c. serum, 5 c. c. suspension and 5 c. c. coagulated serum, and 5 c. c. serum alone were prepared as follows and kept in the thermostat at 37° C. for five days:

	Cells + serum.	Cells + coagulated serum.	Serum.
With 0.2 % sodium bicarbonate..	8.0 c. c.	35.15 c. c.	6.25 c. c.
Reaction unchanged.....	9.9 "	27.0 "	4.6 "
With 0.2 % acetic acid.....	33.8 "	26.3 "	13.75 "

The nitrogen of uncoagulable substances contained in 5 c. c. of the serum before digestion is represented by 6.3 c. c. $\frac{1}{10}$ N. acid.

The experiment confirms the results previously obtained. An increased alkalinity favors the inhibition of the intracellular ferments, which are, when this inhibition is destroyed by heat, themselves most active in an alkaline medium. The addition of acid to a mixture of unheated serum and cells destroys the anti-enzymotic action of the serum, but in this acid medium digestion is more active than in a mixture of cells and serum of which the anti-enzymotic action has been destroyed by heat. This increased digestion is doubtless due to the action of a ferment contained in the serum and made active by the addition of acetic acid. For no proteolysis occurs when alkali is added to serum alone, and, when the reaction is unchanged, the same diminution of nitrogen in uncoagulable form, noted in preceding experiments, is found, but in an acid medium there is well marked digestion of proteid (represented by 13.75 minus 6.3 equals 7.45 cubic centimeters). The serum contains a ferment which, being destroyed by heat, explains the greater proteolysis obtained with cells and unheated serum than with

cells and heated serum (represented by 33.8 minus 26.3 equals 7.5 cubic centimeters).

In the serum of the blood Delezenne and Pozerski [11] have demonstrated the existence of a proteolytic enzyme capable of exerting its action on gelatin and casein when the serum is treated with chloroform. Under ordinary conditions, they believe, anti-bodies which may be destroyed by chloroform inhibit the action of the ferment. Hedin [12] has more recently found in the blood serum of the ox a weak proteolytic enzyme which acts in an alkaline medium. The enzyme is precipitated with the globulin of the serum; anti-bodies which inhibit its action are, he finds, contained mainly in the albumin fraction of the serum.

Destruction of the anti-enzymotic action of the blood serum by acetic acid is in accord with observations of Levene,[13] who has shown that the resistance which uncoagulated egg-albumin and normal blood serum offer to digestion by an extract of fresh pancreatic tissue is overcome by exposing these substances to the action of acetic acid before addition of the extract.

Anti-enzymotic Action of the Serum of the Blood.—The well-known fact that certain anti-ferments are present in the normal blood serum has suggested the probability that the anti-body which in the serum of a sterile inflammatory exudate inhibits the activity of ferment in the leucocytes is derived from the serum of the blood. The action of blood serum of the dog was therefore compared with the serum of an exudate obtained by injecting aleuronat into the pleural cavity.

EXPERIMENT 17.—An exudate was obtained by injecting aleuronat into the pleural cavity of a dog, and the cells removed by centrifugalization were suspended in nineteen times their volume of salt solution. In order to increase the amount of digestible proteid, into each flask were put 5 c. c. of diluted blood serum coagulated by heating to 100° C. The carefully sealed flasks containing toluol were kept at 37° C. for five days. Blood serum was obtained by centrifugalization of the defibrinated blood.

(Cells + blood serum) coagulated as control.....	3.45 c. c.
Cells + coagulated blood serum at 37° C. 5 days.....	10.95 "

[11] *Compt. rend. de la Soc. de Biol.*, 1903, lv, 327, 690, 693.

[12] *Jour. of Phys.*, 1904, xxx, 195.

[13] *Jour. of Med. Research*, 1903, x, 217.

Cells + coagulated blood serum + 5 c. c. exuded serum at 37° C. 5 days	8.5 c. c.
Cells + coagulated blood serum + 5 c. c. blood serum at 37° C. 5 days	8.25 "

When it is recalled that the serum of the exudate contains a considerable amount of nitrogen in a form not coagulable by heat, it is evident that inhibition of digestion is more marked than that indicated by these figures. The inhibitory action of blood serum and of serum from the inflammatory exudate is, it appears, approximately equal. In order to compare more accurately the effect of serum from the blood and from the exudate, another series of mixtures was prepared from the blood serum and exuded serum of an animal which had received an intrapleural injection of aleuronat.

EXPERIMENT 18.—Washed cells from an exudate obtained two days after the injection of aleuronat were suspended in nine times their volume of salt solution. Each flask contained 5 c. c. suspension of cells and 5 c. c. exuded serum or 5 c. c. blood serum, and was kept at 37° C. for five days.

(Cells + exuded serum) coagulated as control	6.4 c. c.
Cells + exuded serum at 37° C. 5 days	7.9 "
Cells + coagulated exuded serum at 37° C. 5 days	21.55 "
(Cells + blood serum) coagulated as control	6.25 c. c.
Cells + blood serum at 37° C. 5 days	8.35 "
Cells + coagulated blood serum at 37° C. 5 days	31.6 "

The experiment demonstrates that the anti-enzymotic action exhibited by the serum of the exudate is exerted by the serum of the blood as well, and in the case of a sterile exudate two days after the onset of inflammation in approximately the same degree.

Anti-enzymotic Action of the Serum of Bacterial Exudates.—An accidental infection of the pleural cavity of the dog afforded an opportunity for the study of an exudate containing bacteria. Following aspiration of the right pleural cavity twenty-four hours after the injection of aleuronat, breathing became laborious and the animal appeared sick. By aspiration three days later, about 150 cubic centimeters of opaque grayish-white fluid were withdrawn from the right pleural cavity. Cultures from the

exudate showed the presence of streptococcus pyogenes in immense numbers, accompanied by a few colonies of a coccus which proved to have the cultural characters of staphylococcus pyogenes albus. Cover-slip preparations from the exudate contained cocci in great numbers, situated largely outside of the leucocytes. The animal died shortly after the removal, by aspiration, of 150 cubic centimeters of exudate.

EXPERIMENT 19.—Cells from the exudate containing streptococcus pyogenes and staphylococcus pyogenes albus were separated from the serum by centrifugalization and suspended in approximately nine times their volume of salt solution. Mixtures of cells and serum were made, and to each were added 5 c. c. toluol. Cultures made from each flask at the end of incubation remained sterile.

5 c. c. cells at 37° C. 5 days	6.05 c. c.
Control	2.85 "
Digestion	3.20 "
5 c. c. serum at 37° C. 5 days	8.25 "
Control (coagulated immediately)	7.7 "
5 c. c. cells + 1 c. c. serum at 37° C. 5 days	7.25 "
Uncoagulable nitrogen in 5 c. c. cells (2.85 c. c.) and 1 c. c. serum (1.54 c. c.) represented by	4.39 "
Digestion	2.86 "
5 c. c. cells + 1 c. c. coagulated serum at 37° C. 5 days	8.65 "
Uncoagulable nitrogen in 5 c. c. cells and 1 c. c. serum represented by	4.39 "
Digestion	4.26 "
5 c. c. cells + 5 c. c. serum at 37° C. 5 days	12.15 "
Uncoagulable nitrogen in 5 c. c. cells (2.85 c. c.) and 5 c. c. serum (7.7 c. c.) represented by	10.55 "
Digestion	1.60 "
5 c. c. cells + 5 c. c. coagulated serum at 37° C. 5 days	21.85 "
Uncoagulable nitrogen in 5 c. c. cells and 5 c. c. serum represented by	10.55 "
Digestion	11.30 "

The anti-enzymotic action exhibited by the serum of a sterile inflammatory exudate has been demonstrable in an exudate containing bacteria in considerable number. Inhibition of en-

zymotic action is, however, less complete than in many of the experiments previously recorded. This difference may be partially referable to the fact that the leucocytes in this experiment were less thoroughly washed with salt solution than were those used in other experiments.

In the following experiment an exudate obtained by injecting bacillus coli into the pleural cavity of a dog was used. The serum of the exudate exerted an inhibitory effect upon the cellular ferments, but in the amount previously used the serum failed to prevent completely digestion.

EXPERIMENT 20.—Two entire cultures of bacillus coli grown forty-eight hours on agar-agar surfaces 7 × 14 cm. were injected into the right pleural cavity of a dog. Twenty-two hours later 30 c. c. of turbid reddish fluid were withdrawn by aspiration. Carefully washed cells in nine times their volume of salt solution were subjected to autolysis under toluol in the following mixtures:

5 c. c. cells at 37° C. 5 days	7.1	c. c.
Control	2.25	"
Digestion	4.85	
5 c. c. cells + 5 c. c. serum at 37° C. 5 days	7.35	"
(5 c. c. cells + 5 c. c. serum) coagulated immediately as control	4.55	"
Digestion	2.80	"
5 c. c. cells + 5 c. c. coagulated serum at 37° C. 5 days	18.7	"
(5 c. c. cells + 5 c. c. serum) coagulated immediately as control	4.55	"
Digestion	14.15	"

Cultures made from each mixture at the end of incubation remained sterile, although the exudate on removal from the pleural cavity contained bacillus coli, recovered by culture.

Changes Produced by Incubation in the Serum of Inflammatory Exudates.—A fact repeatedly observed during the course of these experiments requires some notice. When exudates removed one or two days after the onset of inflammation are subjected to autolysis, less nitrogen is demonstrable in the uncoagulable filtrate after digestion for from two to five days than in the control prepared by coagulating the fresh exudate. This fact is illustrated by Experiments 1, 2, 3, 4, 6, 7, in which this difference is at times considerable, representing in Experiments 1, 2, 3, and

6 (aseptic digestion) from one-fifth to one-eighth of the nitrogen present in the filtrate from the fresh exudate. Subsequent experiments (Nos. 8, 10, 11, and 14) show that this difference is referable to the serum alone.

	Uncoagulable nitrogen in fresh serum of exudate represented by	Uncoagulable nitrogen in serum of exudate after incubation at 37° C. represented by
Experiment 8....	4.15 c. c.	3.7 c. c.
" 10....	4.5 "	3.85 "
" 11....	7.25 "	6.5 "
" 14....	6.3 "	4.6 "

In Experiment 10, a mixture of cells and serum confirmed the result obtained with serum alone. An exception to the facts just described is afforded by Experiment 14, in which the exudate was removed from the pleural cavity three days after the onset of inflammation, and by Experiment 19, in which the serum of the exudate contained streptococci in great numbers. The explanation of this disappearance of uncoagulable nitrogen is not evident. There is little probability that nitrogen has escaped as ammonia formed by digestion. It is not inconceivable that proteids have been less completely coagulated in the control than in the specimen incubated at 37° C., but there is no direct evidence of such a difference. Transformation of soluble nitrogen-containing substances into a form precipitable by heat is obviously not demonstrable by the methods employed.

Anti-enzymes.—Anti-bodies to several enzymes have been found in the normal blood serum. Hammarsten and Röden [14] demonstrated that the blood serum of the horse inhibits the action of lab-ferment and thus prevents coagulation of milk. The phenomenon has been studied by Briot,[15] Morgenroth,[16] and Fuld and Spiro.[17] The anti-tryptic action of the blood serum was first noted by M. Hahn [18] and later studied by a considerable

[14] *Upsala Läkareforenings forhandlingar*, xxii, 546; Ref. Maly's *Jahresb.*, 1887, xvii, 160.

[15] *Compt. rend. de l'Acad. des Sciences*, 1899, cxxviii, 1359.

[16] *Cent. für Bakt.*, 1899, xxvi, Abt. i, 349; 1900, xxvii, Abt. i, 721.

[17] *Zeit. für phys. Chem.*, 1900, xxxi, 132.

[18] *Berliner klin. Woch.*, 1897, xxxiv, 499.

number of observers, Landsteiner,[19] Glaessner,[20] and Cathart [21] having sought to determine to what fraction of the serum the anti-body is attached. Ascoli and Bezzola [22] have described an increase of the anti-tryptic action of the blood serum during the early stage of pneumonia, followed by a gradual return to normal after the crisis. Delezenne [23] maintains that the anti-tryptic action of the blood serum is referable to an anti-enterokynase preventing the amboceptor-like action of enterokynase. Bayliss and Starling,[24] believing with Pawlow that enterokynase is a ferment converting trypsinogen into trypsin, oppose the view of Delezenne and furnish evidence to prove the existence of a true anti-trypsin. In a limited number of individuals they find that the serum of the rabbit contains anti-enterokynase.

Hedin [25] has demonstrated the presence in the spleen of two ferments, one acting in an acid the other in an alkaline medium; in the serum of the ox he has found an anti-body inhibiting the action of the latter and attached mainly to the albumin fraction of the serum.

The foregoing experiments show that the ferments contained in the leucocytes are incapable of acting in the serum of the exudates employed. The activity of these ferments is apparently limited to the cells which produce them. Solid particles ingested by the cells and thus separated from the serum are acted upon by these ferments. By phagocytosis substances insoluble in the fluids of the body are brought into contact with powerful ferments capable if unchecked of destroying tissues. Destruction of tissue occurs within an abscess when with the formation of pus, as the result perhaps of loss of anti-enzymotic action, fibrin and necrotic tissue undergo solution. In the later stages of sterile inflammation and with bacterial infection of a serous cavity some loss of anti-enzymotic power has been demonstrable.

[19] *Cent. für Bakt.*, 1900, xxvii, Abt. i, 357.
[20] Hofmeister's *Beiträge zür chem. Phys.*, 1903, iv, 79.
[21] *Jour. of Phys.*, 1904, xxxi, 497.
[22] *Berliner klin. Woch.*, 1903, xl, 391.
[23] *Compt. rend. de la Soc. de Biol.*, 1903, lv, 132.
[24] *Jour. of Phys.*, 1905, xxxii, 129.
[25] *Ibid.*, 1904, xxx, 155.

It is not improbable that the number of anti-enzymes is greater than that now recognized. Facts already known suggest that such bodies accompany many ferments, holding them in check and limiting their action to those situations in which their proper function is accomplished.

CONCLUSIONS.

The serum of an inflammatory exudate has the power of inhibiting the action of proteolytic ferments contained in the leucocytes. This anti-enzymotic power is possessed by the blood serum from which it doubtless passes into the exudate.

In the later stages of inflammation there is some diminution of this anti-enzymotic action.

The anti-body contained in the serum is destroyed by a temperature of 75° C.

The proteolytic ferments of the leucocytes act both in an acid and in an alkaline medium but are most efficient in the latter. The anti-enzymotic action of the serum is favored by an alkaline reaction, but is completely lost in an acid medium.