LEUCOPROTEASE AND ANTI-LEUCOPROTEASE OF MAMMALS AND OF BIRDS.

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Studies of Fr. Müller[1] and subsequent observers have shown that the polynuclear leucocytes of man and other mammals contain an enzyme which digests proteid and is particularly active in the presence of a weak alkaline reaction. One of us[2] has shown that a suspension of cells from an inflammatory exudate causes proteolysis both in an alkaline and in an acid medium, though digestion is more active in the former. It has further been possible to obtain from such cells a dry powder[3] which digests only in the presence of an alkaline reaction; this enzyme has been designated leucoprotease.

Proteolysis caused by cells of an inflammatory exudate in the presence of acid is more active in proportion to the number of large mononuclear phagocytes or macrophages which are present, while an emulsion made from the lymphatic glands which are situated near the seat of inflammation and contain such cells in immense number causes proteolysis in an acid medium and fails to digest in a neutral or alkaline solution; for convenience, this enzyme has been designated lympho-protease. Hence, each of the two types of phagocytic cells which are capable of ingesting and dissolving within their substance micro-organisms and other proteid-containing bodies is characterized by an enzyme, namely, the polynuclear leucocyte contains leucoprotease, which digests in a neutral or alkaline medium while the large mononuclear phagocyte, which is most abundant in the inflammatory exudate during the later stages of inflammation and often attacks and digests the smaller polynuclear cell, contains lymphoprotease which digests only in the presence of acid.

[1] Kossel, *Zeit. f. klin. Med.*, 1888, xiii, 149.
[2] *Jour. of Exper. Med.*, 1905, vii, 316.
[3] *Ibid.*, 1906, viii, 410.

Leucoprotease, like trypsin, digests in the presence of an alkaline reaction, while lympho-protease, like pepsin, requires an acid medium. Lymphoprotease is active in the presence of very weak hydrochloric acid (1/100 N.) but fails to act in the presence of that concentration of hydrochloric acid which is most favorable to the action of pepsin.[4] Leucoprotease, moreover, has been found to be far less active than trypsin. Further knowledge concerning the relation of the enzymes of leucocytes to the enzymes of the digestive tract is wanting.

The studies of Salkowski[5] have shown that organs kept at body temperature under conditions which prevent bacterial growth undergo self-digestion. Bondi[6] found that self-digestion of liver tissue is more active in an acid than in an alkaline medium. Hedin and Rowland[7] have shown that juice expressed from the spleen of the beef, horse, pig and sheep undergo especially active autolysis in the presence of an acid reaction. The reaction of the expressed juice is acid, but the addition of 0.1 per cent. hydrochloric acid increases proteolysis. In the presence of an alkaline reaction produced by adding from 0.2 to 0.37 per cent. of sodium bicarbonate, the degree of autolysis is diminished, but is, nevertheless, considerable. Subsequent observations[8] have shown that lymphatic glands, the kidneys and the liver contain proteolytic enzymes which are more efficient in an acid than in an alkaline medium. The juice expressed from voluntary muscle undergoes only slight autolysis which is not increased either by addition of acid or of alkali. The heart muscle contains an enzyme which resembles that of other organs and is more active in the presence of acid. Levene and Stookey[9] found that autolysis of nerve tissue and of testis is increased by acid.

Hedin[10] succeeded in separating from the spleen two enzymes, one of which, designated by him lieno-*a*-protease, acted in alkaline

[4] *Ibid.*, 1906, viii, 418.
[5] *Zeit. f. klin. Med.*, 1890, Suppl. zum xvii, 77.
[6] *Virchow's Archiv.* 1896, cxliv, 373.
[7] *Zeit. f. physiol. Chem.*, 1901, xxxii, 341.
[8] *Ibid.*, 1901, xxxii, 531.
[9] *Jour. Med. Research*, 1903, x, 212.
[10] *Jour. of Physiol.*, 1904, xxx, 155.

medium, while a second enzyme, which he called lieno-β-protease, acted in the presence of acid. After spleen pulp had been digested in the presence of 0.1–0.2 per cent. acetic acid, that enzyme which digested in the presence of acid was found in solution, while from the residue, after extraction with three per cent. sodium chloride, precipitation with acetic acid and subsequent neutralization, an enzyme was obtained which acted rather strongly in an alkaline medium and much less in an acid. Hedin showed that the enzymes which he isolated not only caused autolysis, by digesting the substance of the cells which contained them, but were capable of breaking down other proteids, such as fibrin, casein and coagulated blood serum.

The cells of an inflammatory exudate obtained by injecting aleuronat into the pleural cavity of the dog, unlike all of the organs which have been mentioned cause more active proteolysis in an alkaline than in an acid medium. Another tissue has been found by one of us[11] to share this property, namely, the bone-marrow. A suspension of cells from the spleen, lymphatic glands, liver or kidneys caused much more active proteolysis of heated serum with acid than with alkali, while similarly prepared suspensions of cells from the bone-marrow were far more active in an alkaline medium. It is not improbable that the enzyme which is present in large amount in the cells of the exudate rich in polynuclear leucocytes and in the tissue from which the polynuclear lucocytes arise is identical with the similar enzyme which Hedin obtained from the spleen.

The Relation of Anti-leucoprotease to the Globulins and Albumin of the Blood Serum.—Hahn[12] first showed that normal blood serum has the power of inhibiting or wholly preventing the action of trypsin. According to Landsteiner[13] this anti-enzymotic action is not possessed by the serum globulin, precipitated by half saturation with ammonium sulphate, but is present in the albumin precipitated by complete saturation with ammonium sulphate after removal of the globulin. Glaessner[14] failed to confirm this observation, main-

[11] *Jour. of Exper. Med.*, 1905, vii, 759.
[12] *Berliner klin. Woch.*, 1897, xxxiv, 499.
[13] *Cent. f. Bakt.*, 1900, xxvii, Abt. i, 357.
[14] *Hofmeister's Beiträge*, 1904, iv, 79.

taining that the euglobulin fraction precipitated by one third saturation with ammonium sulphate inhibited the action of trypsin on coagulated proteid contained in Mett's tubes, while the pseudo-globulin subsequently precipitated by half saturation exhibited little, and the albumin fraction, no anti-enzymotic action. The results obtained by Landsteiner have been confirmed by Cathcart,[15] who found anti-tryptic action with the albumin, but not with the globulin fraction. This anti-enzymotic action of the serum is destroyed by a temperature of 70° C. but that of the isolated albumin fraction is destroyed by 55° C.

That enzyme of the spleen which acts in an alkaline medium and has been designated by Hedin lieno-a-protease is inhibited by the blood serum. Hedin found that the substance in the serum of the ox which checked this enzyme was contained in the albumin and pseudo-globulin fractions, whereas, in one experiment the euglobulin fraction slightly increased, in another slightly diminished, its activity. That part of the euglobulin fraction which was precipitated by dialysis of the serum was found to increase rather than diminish proteolysis caused by the splenic enzyme.

It has been shown by one of us[16] that proteolysis in an alkaline or approximately neutral medium caused by a suspension of cells from an inflammatory exudate is prevented by small quantities of the serum of the exudate or of the serum of the blood. This anti-enzymotic action is destroyed by a temperature of 75° C. but is unaffected by heating to 70° C. during an half hour. Baer and Loeb[17] observed that the serum of the blood exerted a similar action upon the autolytic enzyme contained in the liver, but since, as they believed, this property was little, if at all, altered by heat, even at the temperature of boiling, they did not think it attributable to a true anti-enzyme. The same inhibiting action was exerted by the albumin of the serum but was wholly lacking in the globulin.

The purpose of the following experiments has been to determine primarily if the power to resist the action of the leucoprotease of the polynuclear leucocytes is common to all proteids of the blood

[15] *Jour. of Physiol.*, 1904, xxxi, 497.
[16] *Jour. of Exper. Med.*, 1905, vii, 316.
[17] *Arch. f. exper. Path. u. Phar.*, 1905, liii, 1.

serum or if this anti-body is localized in a particular fraction of the serum. The somewhat discordant results obtained with anti-bodies for other proteolytic enzymes have been cited for comparison.

To separate the globulins and albumin of the blood, the method of fractioning the serum employed by Freund and Joachim[18] has been used. The blood serum after centrifugalization was diluted with three times its volume of distilled water. A small amount of precipitate, euglobulin, was obtained by adding a saturated solution of ammonium sulphate in quantity to cause one third saturation. The precipitate was washed, dissolved in distilled water, and dialyzed; it was again precipitated by one third saturation with ammonium sulphate and again dialyzed. To the filtrate obtained after one third saturation was added a saturated solution of ammonium sulphate in quantity sufficient to cause one half saturation. The bulky precipitate thus obtained was dissolved in water, dialyzed and reprecipitated. The filtrate was completely saturated with ammonium sulphate by the addition of dry salt. A bulky precipitate, albumin, was obtained and further purified as before. The fractions of serum obtained by this method were dissolved in a volume of water approximately equal to or in some instances double that of the serum employed.

Dried and powdered leucocytes prepared by the method previously described (leucoprotease), in weighed quantities, were allowed to act at 37° C. during five days on a measured quantity of coagulated proteid (five cubic centimeters of heated serum) in the presence of the various fractions which had been isolated. Digestion took place in closely stoppered flasks; the volume of each mixture was brought to twenty-five cubic centimeters by addition of 0.85 per cent. salt solution, and one cubic centimeter of toluol was added. The amount of nitrogen in substances incoagulable by heat has been measured by the Kjeldahl method, and for the sake of comparison is given in terms of cubic centimeters of 1/10 N. sulphuric acid. Since previous experiments had shown that the anti-enzymotic activity of the serum was slightly greater when the alkalinity of the medium was increased, to each mixture in the following experiment was added 0.2 per cent. of sodium carbonate;

[18] *Zeit. f. physiol. Chem.*, 1903, xxxvi, 407.

in subsequent experiments this addition was not made. Former experiments having shown that the anti-enzymotic action of the serum is destroyed by heat, the effect of the unheated fraction was compared with that of the same fraction previously heated to 75° C. during one half hour.

20 mgr. leucoprotease+coagulated proteid+10 c.c. euglobulin	20.5 c.c.
20 mgr. leucoprotease+coagulated proteid+10 c.c. euglobulin heated	16.1 c.c.
20 mgr. leucoprotease+coagulated proteid+10 c.c. pseudo-globulin	27.2 c.c.
20 mgr. leucoprotease+coagulated proteid+10 c.c. pseudo-globulin heated	21.05 c.c.
20 mgr. leucoprotease+coagulated proteid+10 c.c. albumin	13.8 c.c.
20 mgr. leucoprotease+coagulated proteid+10 c.c. albumin heated	29.7 c.c.

In order to test the action of the various fractions in the absence of leucoprotease, quantities of pseudo-globulin and of albumin equal to those previously employed were allowed to act on heated serum under conditions similar to those just described, addition of alkali being omitted. The control represents the amount of nitrogen in uncoagulable form contained in a given mixture before digestion.

10 c.c. pseudo-globulin	+ coagulated proteid	21.1 c.c.
Control		6.95 c.c.
10 c.c. albumin	+ coagulated proteid	10.8 c.c.
Control		9.6 c.c.

The experiment demonstrates that digestion is increased when the euglobulin and pseudo-globulin fractions are added to a mixture of leucoprotease and heated serum. Digestion is inhibited on the contrary by the albumin fraction. The second half of the experiment shows that the pseudo-globulin fraction contains an active proteolytic enzyme, while the albumin fraction fails to cause noteworthy digestion.

The following experiments confirm the results of that just described. The conditions of the experiments are the same, save that euglobulin and pseudo-globulin were not separated but were precipitated together by half saturation with ammonium sulphate. The globulin fraction was dissolved in a volume of water approximately equal to that of the serum from which it was obtained. The albumin fraction was dissolved in twice its volume of water.

20 mgr. leucoprotease + coagulated proteid + 15 c.c. globulin	33.45 c.c.
20 mgr. leucoprotease + coagulated proteid + 15 c.c. globulin heated	24.2 c.c.
20 mgr. leucoprotease + coagulated proteid + 15 c.c. albumin	7.5 c.c.
20 mgr. leucoprotease + coagulated proteid + 15 c.c. albumin heated	18.1 c.c.

The action of the two fractions on coagulated proteid in the absence of leucoprotease was further tested.

Coagulated proteid + 15 c.c. globulin	28.1 c.c.
Control	10.3 c.c.
Coagulated proteid + 15 c.c. albumin	7.05 c.c.
Control	6.55 c.c.

The globulin and albumin fractions used in the next experiment were dissolved in volumes of water approximately equal to that of the serum from which they were obtained.

20 mgr. leucoprotease + coagulated proteid + 5 c.c. globulin	25.2 c.c.
20 mgr. leucoprotease + coagulated proteid + 5 c.c. globulin heated	23.85 c.c.
20 mgr. leucoprotease + coagulated proteid + 5 c.c. albumin	13.1 c.c.
20 mgr. leucoprotease + coagulated proteid + 5 c.c. albumin heated	22.6 c.c.

The same quantities of the globulin and of the albumin were allowed to act upon coagulated proteid at 37° C. during five days in the presence of an approximately neutral, alkaline, and acid reaction:

Coagulated proteid + 5 c.c. globulin	16.3 c.c.
Coagulated proteid + 5 c.c. globulin + 0.2 per cent. sodium carbonate	6.5* c.c.
Coagulated proteid + 5 c.c. globulin + 0.2 per cent. acetic acid	3.7 c.c.
Control	2.3 c.c.
Coagulated proteid + 5 c.c. albumin	2.4 c.c.
Coagulated proteid + 5 c.c. albumin + 0.2 per cent. sodium carbonate	2.55 c.c.
Coagulated proteid + 5 c.c. albumin + 0.2 per cent. acetic acid	2.15 c.c.
Control	2.4 c.c.

* Since this figure differed markedly from that obtained when the reaction of the medium remained approximately neutral (16.3 c.c.) it was suspected that an error had occurred in making the Kjeldahl determination; repetition of the test gave the figure, 13.2 c.c.

These experiments which show that the anti-enzyme for leucoprotease is present in the albumin of the serum and absent in the globulins are analogous to those of Landsteiner and of Cathcart who found anti-trypsin only in the albumin fraction of the serum. Hedin, it has been mentioned, found an anti-body for his lieno-α-protease in the albuimn fraction, but present also, according to his observations, in the pseudo-globulin.

The globulin of the serum not only possesses no anti-leucoprotease but contains a proteolytic enzyme which is active under conditions similar to those which favor the action of leucoprotease. Delezenne and Pozerski[19] had observed that serum treated with chloroform

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

digests proteid, and believed that the serum contained an anti-body which normally held this enzyme in check. Hedin[20] showed that a weak proteolytic enzyme which digests in an alkaline medium is present in the globulin of the serum and is inhibited by an anti-body which is mainly contained in the albumin. It is not improbable that this enzyme is identical with that which is present in the polynuclear leucocytes and in the bone marrow and with the similar enzyme of the spleen. In the blood serum this enzyme is held in check by the anti-body which is precipitated with the albumin; this anti-body which in given quantity doubtless holds in check only a limited quantity of enzyme,[21] is present in the serum in excess, so that the whole serum is capable of further anti-enzymotic action.

Action of Anti-leucoprotease of Different Mammalian Species upon Leucoprotease of the Same and of Different Species.—By study of the anti-enzymotic action of sera from animals of various species upon leucoprotease derived from different animals, it was hoped that evidence concerning the identity or multiplicity of such enzymes might be obtained; for if each of two enzymes bears a specific relationship to its own serum, it is improbable that the two enzymes are identical. Glaessner believed that the anti-tryptic action of the blood serum bears a specific relation to trypsin from the same species; he found that trypsin from the ox was more strongly inhibited by ox serum and trypsin from the pig by pig's serum than by other sera. The experiments of Cathcart[22] did not confirm this view, but were not decisive.

In order to test the inhibiting action of sera obtained from a variety of species upon the leucoprotease of the dog, a measured quantity (twenty milligrams) of the dry powder prepared from leucocytes, obtained either by injecting aleuronat into the pleural cavity or turpentine into the subcutaneous tissue, was allowed to act during five days at 37° C. upon a measured quantity of coagulated proteid in the presence of various sera. In the following experiment three cubic centimeters of the serum of dog, man, and ox were employed. To determine for comparison the proteolytic activity of

[20] *Jour. of Physiol.*, 1904, xxx, 195.
[21] *Jour. of Exper. Med.*, 1906, viii, 538.
[22] *Loc. cit.*

the enzyme, twenty milligrams were allowed to act upon coagulated proteid in the presence of three cubic centimeters of the various sera previously heated to 75° C. during one half hour, in order to destroy their anti-enzymotic action. The control represented the amount of nitrogen in uncoagulable substances present in the mixtures before digestion.

	Human serum.	Dog's serum.	Ox's serum.
Control	3.35 c.c.	4.65 c.c.	3 c.c.
With 3 c.c. heated serum	25.25 c.c.	26.5 c.c.	21.8 c.c.
With 3 c.c. unheated serum	7.4 c.c.	8.7 c.c.	7.6 c.c.

The sera of man and of ox act like that of the dog, and hinder digestion caused by the dog's enzyme. It is not improbable that a maximum degree of inhibition has been caused by these sera when three cubic centimeters of each were employed. In order to test more accurately the relative activity of the different sera, smaller quantities were employed; 0.5 and 0.25 cubic centimeters of the serum of dog, cat, goat, and pigeon were added to mixtures containing twenty milligrams of leucoprotease and a measured quantity of coagulated proteid.

	Dog's serum.	Cat's serum.	Goat's serum.	Pigeon's serum.
With 0.5 c.c. serum	9.9 c.c.	4.3 c.c.	4.75 c.c.	16.8 c.c.
With 0.25 c.c. serum	10.85 c.c.	4.25 c.c.	6.4 c.c.	17.6 c.c.

The proteolysis caused by twenty milligrams of the enzyme acting on the quantity of coagulated proteid used in the above mixtures is represented by 18.2 c.c., the control being 3.0 c.c.

The serum of the cat causes more complete inhibition of the dog's enzyme than the serum of the goat, but both sera are more actively anti-enzymotic than the dog's own serum. Digestion in the presence of equal quantities of pigeon's serum is little less than that caused by the unrestrained enzyme. In the following experiment the sera of dog and rabbit were compared, the effect of increasing quantities of these sera on twenty milligrams of dog's leucoprotease being tested. Since the coagulated proteid used for digestion (five cubic centimeters of heated dog's serum) was not the same in the two series, the results are not accurately comparable, but demonstrate that the inhibiting power of the rabbit's serum is considerably greater than that for the dog.

	Dog's serum.	Rabbit's serum.
With 0.25 c.c. serum	22.15 c.c.	7.35 c.c.
With 0.5 c.c. serum	18.8 c.c.	5.35 c.c.
With 1 c.c. serum	10.6 c.c.	4.3 c.c.
With 2.5 c.c. serum	7.55 c.c.	4.9* c.c.
Without the addition of serum	24.2 c.c.	21.45 c.c.
Control	2.85 c.c.	2.85 c.c.

* This figure is larger than that obtained when a smaller quantity of rabbit's serum was used because the mixture before digestion contained more nitrogen in uncoagulable form, 2.5 c.c. of dog's serum being represented by 1.45 c.c. and 2.5 c.c. rabbit's serum by 1.75 c.c.

In the second experiment the figures obtained are comparable, the same coagulated proteid being used for digestion in all of the tests.

	Dog's serum.	Rabbit's serum.
With 0.25 c.c. serum	12.5 c.c.	6.45 c.c.
With 1.0 c.c. serum	6.5 c.c.	5.15 c.c.

Digestion caused by the enzyme used in this experiment, in the absence of serum, is represented by 20.05 c.c., the control being 4.05 c.c.

Since the enzyme of the dog's leucocytes is more markedly inhibited by the serum of man, ox, cat, goat, and rabbit than by the dog's own serum, it is necessary to determine if the corresponding enzyme of other animals bears the same relation to foreign mammalian sera. For this purpose, the attempt was made to obtain polynuclear leucocytes of the rabbit in quantities sufficient for the tests required. By injection of aleuronat into the pleural cavity of the rabbit, only a small quantity of exudate was obtained, and this exudate was so poor in cells that their suspension in salt solution completely failed to digest coagulated proteid. Injections of turpentine into the subcutaneous tissue of the rabbit caused exudation of serum and accumulation of dry friable material of opaque white color forming a layer adherent to the necrotic tissues which had been in contact with the injected turpentine. This material consisted in great part of polynuclear leucocytes held together by fibrin; typical suppuration with softening and solution of tissue was entirely lacking. The exudate was scraped from the surface of the exposed tissues and dried by the method previously described. The dry powder thus obtained was found to have weak proteolytic action when allowed to act at body temperature on heated serum. The anti-

enzymotic action of rabbit's, dog's and hen's serum was tested with this enzyme.

20 mgr. leucoprotease + coagulated proteid + 0.5 c.c. rabbit's serum	3.1 c.c.
20 mgr. leucoprotease + coagulated proteid + 0.5 c.c. dog's serum	3.7 c.c.
20 mgr. leucoprotease + coagulated proteid	4.15 c.c.
Control	2.5 c.c.

Since the amount of digestion in the experiment, even in the absence of serum, was insignificant, a larger quantity of the ferment was employed.

50 mgr. leucoprotease + coagulated proteid + 0.5 c.c. rabbit's serum	3.45 c.c.
50 mgr. leucoprotease + coagulated proteid + 0.5 c.c. dog's serum	4.05 c.c.
50 mgr. leucoprotease + coagulated proteid	7.6 c.c.
Control	2.45 c.c.

These two experiments have shown that leucoprotease derived from the rabbit bears to the two sera with which it has been tested a relation which is identical with that of dog's leucoprotease to the same sera; the enzyme from both animals is inhibited in greater degree by the serum of the rabbit than by that of the dog. Since the anti-body of one species has no specific relation to the leucoprotease of the same species, the experiments tend to support the belief that the leucoprotease of different mammalian species is identical.

The enzyme from the rabbit is much weaker than the similarly prepared enzyme from the dog; the anti-enzyme of the rabbit's serum is, on the contrary, much more active. Since experiments previously described have shown that suppuration with solution of fibrin and of necrotic tissue in the dog is associated with loss of anti-enzyme in the purulent exudate, it is not improbable that the well-known absence of typical suppuration with liquefaction of tissue in the rabbit is due to the weakness of the enzyme present in the polynuclear leucocytes and to the strength of the anti-body which opposes it.

Anti-leucoprotease in the Serum of Birds.—An experiment in which the anti-enzymotic power of pigeon's serum was compared with that of dog's, cat's, and goat's serum showed that the serum of the pigeon failed to prevent active proteolysis caused by dog's leucoprotease. The inhibiting action of hen's serum was further

tested with leucoprotease of dog and was compared with that of dog's serum. The following experiment shows the effect of 0.5 and of 1 cubic centimeter of these sera on twenty milligrams of dog's leucoprotease when allowed to digest coagulated serum under the conditions already described:

	Dog's serum.	Hen's serum.
With 0.5 c.c. serum	9.5 c.c.	14.65 c.c.
With 1 c.c. serum	6.05 c.c.	13.75 c.c.

Twenty milligrams of enzyme in the absence of serum caused digestion of coagulated proteid represented by 16.8 c.c. of 1/10 N. sulphuric acid, the control being 2.1 c.c.

It is evident that the anti-enzymotic action of the serum of both pigeon and of hen for leucoprotease of dog, though appreciable, is slight. Since sera of these birds differ from the mammalian sera examined, it has suggested itself that the enzymes present in the leucocytes of the bird might have peculiarities corresponding to this difference. To obtain inflammatory exudates containing leucocytes, aleuronat was injected into the peritoneal cavity of the hen, but from the resulting exudate a sufficient quantity of cells could not be obtained. Turpentine injected into the subcutaneous tissue caused necrosis and accumulation of a considerable number of leucocytes, but suppuration with softening of the tissue did not occur. A powder prepared by the method previously mentioned from the white fibrinous exudate at the seat of inoculation caused very weak proteolysis, when allowed to act at body temperature upon coagulated proteid in the presence of acid, but failed to digest in the presence of an alkaline reaction. Since previous experiments[23] have shown that the bone marrow of the dog contains that enzyme, namely, leucoprotease, which is characteristic of the polynuclear leucocytes, the proteolytic action of a suspension of cells of the bone marrow from the hen was tested in the presence both of acid and of alkali. The bone marrow removed from the bones was shaken violently in salt solution and forced through a fine sieve; the cells thus obtained were washed several times by centrifugalization and suspended in nine times their volume of salt solution. Five cubic centimeters of this suspension were allowed to act on coagulated proteid at body temperature in

[23] *Loc. cit.*

the presence of 0.2 per cent. acetic acid and of 0.2 per cent. sodium carbonate, and with the reaction of the medium unchanged. The experiments of Hedin and of one of us have shown that while the spleen of various mammals undergoes more active autolysis and digests foreign proteid more energetically in an acid medium, it contains at the same time no inconsiderable quantity of an enzyme which digests in the presence of alkali. The proteolytic action of a suspension of splenic cells from the hen was compared with the similarly prepared suspension of bone marrow from the same bird; for further comparison, a suspension prepared from the liver was used. In the following table the digestion of coagulated proteid produced by these suspensions is represented by centimeters of 1/10 N. acid.

	Bone marrow.	Spleen.	Liver.
With reaction unchanged	3.0 c.c.	3.45 c.c.	2.55 c.c.
With 0.2 per cent. acetic acid	10.7 c.c.	12.15 c.c.	8.6 c.c.
With 0.2 per cent. sodium carbonate	2.85 c.c.	3.05 c.c.	2.35 c.c.
Control	2.15 c.c.		1.8 c.c.

These figures show that the bone marrow of the hen, unlike that of the dog, fails almost completely to cause digestion of proteid in the presence of an approximately neutral or alkaline reaction, but causes active proteolysis in an acid medium. An enzyme similar to leucoprotease of the dog, if present, occurs only in very small quantity. The spleen likewise causes very trivial digestion in an alkaline or neutral medium, but is active in an acid medium; the control for the spleen which is lacking doubtless closely approximates that of the bone marrow. The liver which in an acid medium causes less digestion than bone marrow or spleen almost completely fails to digest in a neutral or alkaline medium. An enzyme which digests in an alkaline medium, and occurs in abundance in the organs of mammals is according to the foregoing observations almost wholly wanting in the tissues, notably in the blood-forming organs of the bird.

The absence in the blood-forming organs of the hen of this enzyme is apparently due to its absence in the polynuclear leucocytes. By injecting turpentine into the peritoneal cavity of the hen, a sterile inflammation results: in two experiments at the end of three

and of four days the cavity was found to contain yellowish fluid, but the intestines are loosely matted together by a white or pale greenish, gelatinous material in which are numerous opaque yellow spots. This exudate consists of fibrin holding serum in its meshes; the characteristic polynuclear leucocytes of the bird are present in large number. Bits of this fibrinous exudate washed with salt solution as free as possible of serum were suspended in acid, neutral, and alkaline solutions of which the volume was brought to five cubic centimeters by addition of .85 per cent. salt solution. After digestion at 37° C. during seven days the result of the experiment was as follows:

	Condition of fibrin.
With 0.2 per cent. acetic acid.	Fine powdery sediment.
With 0.1 per cent. acetic acid.	Much eroded.
In normal salt solution.	Unchanged.
With 0.1 per cent. sodium carbonate.	Unchanged.
With 0.2 per cent. sodium carbonate.	Unchanged.

While the experiment does not demonstrate with certainty that solution of fibrin in the presence of acid is due to enzymotic action, the result agrees with that obtained with bone marrow and demonstrates that autolysis of fibrinous exudate containing polynuclear leucocytes fails to occur in a neutral or alkaline medium. The fibrinous exudate obtained several days after injecting turpentine into the pleural cavity of the dog when washed free from serum undergoes complete autolysis, if immersed in 0.2 per cent. sodium carbonate and kept at body temperature.

CONCLUSIONS.

The inhibiting action of the blood serum upon the enzyme of the polynuclear leucocytes, leucoprotease, is exerted by the albumin fraction of the serum. The albumin fraction contains no proteolytic enzymes.

The globulin fraction of the serum contains no anti-enzyme for leucoprotease; it contains, on the contrary, an enzyme which digests proteids in a neutral or alkaline medium. This enzyme resembles leucoprotease which is present in the polynuclear leucocytes of an inflammatory exudate and in the bone marrow from which these cells are derived, and is doubtless identical with the similar enzyme

occurring in smaller quantity in the spleen. This enzyme which is present in the blood serum is held in check by its anti-enzyme, but the latter is in such excess that the serum as a whole is capable of checking the action of leucoprotease when added in considerable quantity.

Leucoprotease of one mammalian species is inhibited by sera of other mammalian species, but the anti-enzymotic activity of various sera differs; the anti-enzyme of the rabbit's serum is stronger than that of dog's serum, when tested either with dog's or with rabbit's leucoprotease. The co-existence in the rabbit of leucoprotease with feeble strength and anti-body of great activity may explain the absence in these animals of typical suppuration with liquefaction of tissues.

The serum of birds which have been tested, namely, pigeon and hen, almost completely fails to inhibit mammalian leucoprotease (of dog). The polynuclear leucocytes, the bone marrow and the spleen of the hen do not contain an enzyme resembling leucoprotease of mammals. The absence of anti-enzyme in the serum is associated with absence of a corresponding enzyme in the leucocytes.