THE ENZYMES OF FIBRINOUS EXUDATES—THE EFFECT OF ONE ENZYME UPON ANOTHER.*

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In a previous study[1] I have found that fibrin of the blood contains two enzymes which are capable of acting on a foreign proteid, such as heated blood serum. Proteolysis occurs in the presence of 0.2 per cent. sodium carbonate and is inhibited by greater strengths of the alkali. Digestion also occurs in the presence of acid, though to a smaller extent, and with less constancy. Since polynuclear leucocytes have been shown to contain an enzyme which digests only in the presence of an alkaline or neutral medium, the facts just mentioned have been believed to give evidence that two enzymes exist in the fibrin of the blood, one, leucoprotease, peculiar to the polynuclear leucocytes, and a second, an enzyme which digests in the presence of acid, and is similar to the enzyme present in the large mononuclear cells of an inflammatory exudate.

It has been believed that a study of an inflammatory fibrinous exudate might better define the peculiarities of these two enzymes, and afford opportunity of studying changes which they undergo during the progress of an inflammatory reaction.

Fibrinous exudates are readily obtained by injection of turpentine into the pleural cavity.[2] After injection, fluid rapidly accumulates in the chest. This fluid is coagulable, and fibrin is deposited abundantly. Accumulation of fluid reaches a maximum in from two to four days and quickly disappears so that after six days the cavity contains no fluid. Fibrin, however, remains, gradually diminishes in amount, and in about two weeks has in most cases entirely disappeared, the cavity returning to normal. It has seemed probable that disappearance of fibrin is due to action of enzymes.

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[1] *Jour. of Exper. Med.*, 1908, x, 343.

[2] *Jour. of Exper. Med.*, 1907, ix, 391.

Exuded fibrin suspended in various media undergoes autolysis; solution, indicated by disappearance of fibrin and presence of biuret reaction after coagulation, occurs with constancy when the fibrin is incubated in an acid medium, but is less constant in neutral, or in alkaline media. In the early stages of the inflammatory reaction, after from two to four days, fibrin undergoes autolysis in the presence of sodium carbonate, but at a later period the power of digestion is completely lost. Disappearance of the fibrin of the blood when suspended in various solutions has been found an inaccurate measure of the proteolytic enzymes which it contains. A far more accurate method is to determine by use of the Kjeldahl method for nitrogen the power of a weighed quantity of the enzyme-containing substance to act on denaturalized proteid.

It has been considered advisable to apply the same method to exuded fibrin, with the purpose of determining more accurately what changes occur during the course of the inflammatory reaction. This study has shown that conditions occur, under which each of the two enzymes previously defined, exists alone, and has given opportunity to determine the character of digestion caused by combination of these enzymes.

Of the washed, pressed and shredded fibrin of the sterile exudate, 0.3 gram has been added to flasks containing ten cubic centimeters of diluted and heated blood serum, with sufficient salt solution to make a volume of twenty-five cubic centimeters, after the flasks have received the necessary amount of acetic acid, or sodium carbonate to yield the required strength. Strengths of acid above 0.2 and 0.5 per cent. have been used, with the purpose of obtaining evidence concerning the optimum medium for the action of the enzyme.

Comparison between experiments in which digestion has occurred in the presence of acid and those in which such digestion has been almost completely absent has shown two facts. First, with active digestion in presence of acid, a maximum occurs in strength of 0.2 per cent., or occasionally 0.5 per cent., and is less with greater concentration. Second, in the presence of a strength of acid greater than 1 per cent., complete coagulation of proteid by heat is difficult, so that the figures obtained are somewhat larger than is con-

sistent with entire inhibition of the enzyme. It is moreover by no means improbable that slight hydrolysis may occur in the absence of enzyme, as the result of the action of acid alone. In later experiments it was considered unnecessary to use higher strengths of acid, 0.2 and 0.5 per cent. giving a sufficiently clear indication of the enzymic contents of the fibrin tested. For the same reason, the higher strengths of carbonate were later omitted.

The following table gives the results of a systematic study of the enzymes present in fibrin removed from two to six days after a single injection of one cubic centimeter of turpentine:

TABLE I.

Experiment.	Age of Exuded Fibrin.	Control.	Acetic Acid.						Neutral.	Sodium Carbonate.				
			5.0 Per Cent.	3.0 Per Cent.	2.0 Per Cent.	1.0 Per Cent.	0.5 Per Cent.	0.2 Per Cent.		0.2 Per Cent.	0.5 Per Cent.	1.0 Per Cent.	2.0 Per Cent.	3.0 Per Cent.
1	2 day	2.6		5.30	5.50	7.25	7.95	7.15	23.50	8.35	9.00	9.20		
2		1.8					8.4	8.55	15.6	13.75	8.55			
3	3 day	2.85		5.8	5.6	6.2	7.55	8.35	13.2	3.0	3.6	3.65	5.1	4.85
4		2.4		3.95	4.3	5.1	6.8	5.9	7.5	2.8	3.1	3.7	4.25	4.2
5	4 day	1.95			5.1	6.0	7.75	9.00	8.75	2.3	2.45	3.0		
6		1.95			4.9	5.3	6.65	7.7	12.7	5.7	5.2	6.3		
7	5 day	1.85		3.85	3.15	4.8	5.8	5.5	17.05	4.0	3.3	3.75	4.2	3.4
8	6 day	2.0	5.05		3.13		8.45	9.05	17.2	4.05	4.5	4.7		

Of special significance is the fact that fibrin of two days (that is, removed at the end of two days after injection) digests with considerable activity in the alkaline media, but after this period digestion is for a time (three and four days), almost completely absent, the figures obtained in 0.2 per cent. being little greater than the control; again digestion is slightly increased at the end of four, five or six days.

In an acid medium digestion occurs in all the specimens examined. In fibrin of two days it may be assumed that at least two enzymes are present, for it has already been pointed out that the enzyme which acts in an alkaline medium can cause little if any digestion in an acid medium.[3] The enzyme which acts in an acid medium is further shown by Experiments 3, 4 and 5 to be practically inactive in the presence of alkali. Both enzymes are, however, capable of

[3] *Jour. of Exper. Med.*, 1905, vii, 316.

active proteolysis in a neutral medium, and when both are combined as in Experiments 1 and 2, digestion is far more active in a neutral medium than in either an acid or an alkaline fluid. Nevertheless the table shows that maximum activity in a neutral medium is not uniformly dependent upon combination of the two enzymes, and in only one experiment, No. 5, is digestion more active in the presence of acid than in a neutral medium. Again, after four, five and six days, with slight activity in the presence of alkali, digestion in a neutral medium far exceeds that in acid. Fibrin of three days, and one of the specimens of four days, agree in showing an almost complete absence of digestion in the presence of sodium carbonate, yet their behavior in neutral and acid media is not constant, for whereas digestion in fibrin of three days is far more active in a neutral medium, in that of four days (Experiment 5) digestion is more active in the presence of acid. This fact has remained unexplained.

The two members of each group, containing animals killed after two, three and four days respectively, have been so arranged within the individual group that there is at first a continuous decrease and later a continuous increase of digestion in a neutral medium. Such an arrangement shows that digestion in the neutral medium which is very active at the end of two days, diminishes to a minimum between three and four days, and again gradually increases after the fourth day.

In the foregoing table, fibrin has been obtained from sero-fibrinous exudates produced by a single injection of turpentine. In a study of experimental pleurisy,[4] Dr. Opie has found that repeated injections of turpentine cause continued emigration of polynuclear leucocytes which give the fibrin increased power of autolysis in the presence of alkali. The fibrin becomes more succulent and softer. The serous exudate becomes more turbid and finally assumes the appearance of pus. Such fibrin, unlike that previously employed, when suspended in an alkaline medium has been found to undergo very active autolysis. The Kjeldahl method used in the foregoing experiments has been employed to determine the power of a weighed quantity of this fibrin to cause digestion of coagulated serum. The following table represents the digestion of coagulated blood serum

[4] *Jour. of Exper. Med.*, 1907, ix, 415.

under conditions analogous to those of the experiments recorded in Table I.

TABLE II.

Experiment.	No. of Days After First Injection of Turpentine.	Control.	Acetic Acid.						Neutral.	Sodium Carbonate.		
			5.0 Per Cent.	3.5 Per Cent.	2.0 Per Cent.	1.0 Per Cent.	0.5 Per Cent.	0.2 Per Cent.		0.2 Per Cent.	0.5 Per Cent.	1.0 Per Cent.
9	5	2.8				4.25	4.7	4.45	21.00	21.5	9.85	8.9
10	5	3.2	4.5	4.35	4.45		3.7	3.25	16.1	17.55		7.9

Comparison of Tables I and II shows that great increase in leucoprotease, as the result of a second injection of the inflammatory irritant, has been associated with almost complete disappearance of ability to digest in an acid medium. In both experiments of Table II, the second injection of turpentine has been received three days after the first and the fibrin removed from the chest two days later; comparison with Table I shows that the enzyme digesting in acid and present during this period may be completely replaced, perhaps destroyed by leucoprotease.

Experiments 3, 4 and 5 of Table I are examples of digestion caused by that ferment which acts in acid and is inhibited by alkali; digestion in alkali is almost wholly absent. The two experiments of Table II on the contrary exhibit the action of that ferment which acts in the presence of alkali and is inhibited by acid. Especially noteworthy is the fact that both enzymes are capable of acting in an approximately neutral medium. This fact best explains their action in the body. Each is capable of exerting an almost maximum activity in a neutral medium, whereas a slight change of reaction favors one and inhibits the other.

Should an exudate contain both enzymes, the method which has been employed would demonstrate proteolysis in both acid and alkali. In a neutral medium the two enzymes acting simultaneously might cause far greater digestion. Such combination of enzymes with maximum activity in the neutral medium is illustrated by Experiments 1, 2, 6, 7 and 8. In the following experiment the simultaneous occurrence of two enzymes has been demonstrated by using a method which previous experiments have shown preserves one enzyme and injures the other. After three injections of turpentine

into the right pleural cavity, fibrinous exudate has been obtained in great part from the left cavity. This fibrin has been tested in the fresh state under the usual conditions; a part has been treated with alcohol and ether, dried and powdered and a weighed quantity approximately corresponding to the usual amount of fresh fibrin has been used. It has been previously found that this method preserves almost exclusively leucoprotease and fails to preserve the enzyme which has been designated as lymphoprotease.[5]

TABLE III.

Experiment.	Amount of Fibrin Used.	Control.	Acetic Acid.					Neutral.	Sodium Carbonate.		
			3.0 Per Cent.	2.0 Per Cent.	1.0 Per Cent.	0.5 Per Cent	0.2 Per Cent.		0.2 Per Cent.	0.5 Per Cent.	1.0 Per Cent.
11	0.3 gms. fresh.	2.25	3.85	4.2	6.05	6.5	7.4	19.35	8.05	6.4	6.85
12	20 mgr. powd.	2.7	4.05	3.6	4.25	4.55	4.7	20.05	14.35	5.3	6.7

The experiment shows that drying of fibrin has markedly diminished its activity in the presence of acid, and at the same time has increased its activity in the presence of alkali (0.2 per cent. sodium carbonate). Digestion in a neutral medium has remained almost unimpaired. Table II on the one hand gives evidence that leucoprotease may destroy the enzyme which acts in acid (lymphoprotease); Table III on the other suggests that the last-named enzyme may under certain conditions destroy or inhibit leucoprotease, since removal of its influence increases the activity of digestion in alkali.

CONCLUSIONS.

By a study of the enzymes contained in fibrinous exudates produced by injection of a sterile inflammatory irritant (turpentine) conditions have been found in which each of two enzymes occurs alone. The one, leucoprotease, digests in the presence of alkali; the other, resembling lymphoprotease, digests in the presence of acid, yet both exhibit almost maximum activity in an approximately neutral medium. It is probable that both enzymes, in the body, exert their greatest activity in an approximately neutral medium, slight changes in reaction increasing digestion by the one, and suspending digestion by the other.

[5] *Jour. of Exper. Med.*, 1907, ix, 415.

The enzyme digesting in acid, present in the fibrinous exudate obtained after a single injection of turpentine, disappears when repeated injection of the same irritant transforms the sero-fibrinous into a purulent exudate, and causes accumulation of leucoprotease in great quantity.

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