SOLUTION OF TISSUE WITH ABSCESS.

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In a former publication [1] I have shown that the two types of cells which especially act as phagocytes, namely the polynuclear leucocytes with fine granulation and the large mononuclear phagocyte or macrophage of Metschnikoff, are each characterized by a distinct proteolytic enzyme. That enzyme which is contained in the polynuclear leucocytes and as I have previously shown [2] is manufactured in the bone marrow is capable of causing proteolytic digestion in the presence of a neutral or alkaline reaction and is almost wholly incapable of action when placed in an acid medium. For this enzyme I have suggested the name leuco-protease, since it is characteristic of leucocytes contained in pus and other inflammatory exudates. The large mononuclear phagocytes of inflammatory exudates are especially concerned with the ingestion and destruction of cellular elements; the same cells are found in lymphatic glands adjacent to the site of inflammation and are doubtless identical with those which, for example, are abundant in the spleen lymphatic glands and other situations with typhoid fever. These cells contain an enzyme which is incapable of digesting proteid in an alkaline medium but is active in the presence of a weak acid; I have suggested for this enzyme the name lympho-protease.

The serum of an exudate produced by injecting aleuronat into the pleural cavity of a dog inhibits the proteolytic activity of leuco-protease [3]. The serum of the blood has the same property. Since this enzyme contained in the polynuclear leucocytes is incapable of digesting proteid in the presence of the blood serum its action is limited to these cells;

[1] *Jour. Exper. Med.*, 1906, viii, 410.
[2] *Ibid.*, 1905, vii, 759.
[3] *Ibid.*, 1905, vii, 316.

it can act only upon substances which have been ingested by these phagocytes and thus removed from the influence of the serum. Doubtless the same property of the serum serves to protect the tissues from the proteolytic enzyme contained in the polynuclear leucocytes, for even should it be set free by disintegration of these cells or otherwise its activity would be inhibited. Since lympho-protease does not cause proteolysis in a neutral or alkaline medium, the alkalinity of the blood serum would check its action should it be set free by disintegration of mononuclear phagocytes.

An experiment in which increasing quantities of serum are allowed to act upon the same quantity of enzyme demonstrates very clearly this anti-enzymotic property of the serum. Previous experiments have shown that the cells of an exudate obtained by injecting aleuronat into the pleural cavity of a dog preserve their power to cause proteolysis when dried after treatment with absolute alcohol and ether. Leuco-protease alone is preserved by this method. A weighed quantity of the dry enzyme has been allowed to act during five days at 37° C. upon a measured quantity of diluted blood serum (10 c.c.) heated to 75° C. for one half-hour and thus partially coagulated and denaturalized. The methods employed have been described in previous articles.

Nitrogen in substances uncoagulable by heat has been estimated by the Kjeldahl method and for convenience is represented by cubic centimeters of 1/10 N sulphuric acid.

20 mgr.	powdered leucocytes	+	coagulated proteid						24.2 c.c.
20 "	" "	+	"	"	+ 0.25c.c.	serum			22.15 "
20 "	" "	+	"	"	+ 0.5 "	"			18.8 "
20 "	" "	+	"	"	+ 1.0 "	"			10.6 "
20 "	" "	+	"	"	+ 2.5 "	"			7.55 "

Control, 2.85c.c.

Two and a half cubic centimeters of serum has almost completely inhibited the proteolytic action of twenty milligrams of the powdered cells (by subtracting nitrogen in uncoagulable form contained in the control mixture, digestion is found to be represented by 3.28 c.c.) while the addition of only a quarter of a cubic centimeter materially hinders digestion.

By adding increasing quantities of the enzyme-containing powder to the same quantity of serum it has been found that a given quantity of serum can inhibit the action of only a limited quantity of enzyme.

10 mgr.	powdered	leucocytes	+	coagulated	serum	+ 2.5	c.c.	serum	6.15 c.c.
20 "	"	"	+	"	"	+ 2.5	"	"	6.5 "
40 "	"	"	+	"	"	+ 2.5	"	"	10.4 "
80 "	"	"	+	"	"	+ 2.5	"	"	19.2 "
160 "	"	"	+	"	"	+ 2.5	"	"	25.15 "

Nitrogen in uncoagulable form in coagulated serum + 2.5 c.c. serum is represented by 6.0 c.c.; in 160 mgr. powdered leucocytes, by 2.4 c.c.

The enzymotic activity of the powder used in the preceding experiment is shown by the following test:

20 mgr. powdered leucocytes + coagulated serum	18.9 c.c.
Control	4.3 "

Two and a half cubic centimeters of serum have completely inhibited twenty milligrams of the dried cells but have failed to prevent proteolysis when a larger quantity of enzyme is employed.

In a former publication I have shown that exudates removed from the pleural cavity of a dog one or two days after the injection of aleuronat fail to undergo autolysis because the anti-enzyme of the serum inhibits the proteolytic enzymes of the leucocytes. In exudates removed three days after injection of the inflammatory irritant slight autolysis occurs. Variation in the enzymotic activity of the serum of the exudates doubtless explains the inconstant results of Schutz [4] and of Zak,[5] who failed to find in human exudates any relation between the autolysis of an exudate and the abundance of its cellular elements.

Since an abscess is characterized by softening and solution of tissue it has seemed not improbable that the fluid obtained by centrifugalization from purulent exudates would fail to exhibit the anti-enzymotic action which is characteristic of sterile exudates produced in the pleural cavity by aleuronat, and, doubtless, according to the previously mentioned observations of Schutz and of Zak, of certain human exudates. As the result of the experimental pleurisy produced by aleuronat, at least, the chest wall is uninjured and the pleural cavity, save for a few adhesions, may return to a normal condition.

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

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

A sterile purulent exudate was obtained by injecting a small quantity (one cubic centimeter) of turpentine into the subcutaneous or intermuscular tissue of the flank of the dog. At the end of four or five days a large cavity distended with fairly thick purulent fluid is formed. Such purulent fluid contains polynuclear leucocytes in immense number together with mononuclear cells, fat globules, and particles of degenerated cells. Agar-agar inoculated with this purulent exudate has in every instance remained sterile. A measured quantity (5 c.c.) of this exudate was diluted with four times its volume of 0.85 per cent. sodium chloride and after addition of toluol subjected for five days to a temperature of 37° C.

5 c.c. pus at 37° C. for 5 days	12.6 c.c.
Control	7.05 "
Digestion	5.55 "

The following experiment demonstrates that the same purulent exudate is capable of digesting coagulated proteid under the conditions just mentioned.

2.5 c.c. pus + 5 c.c. coagulated serum	17.45 c.c.
Control	6.4 "
Digestion	11.05 "

In order to determine if the proteolysis caused by the purulent exudate is due to the absence of anti-enzyme in the serum of the exudate, cells were separated from the serum by centrifugalization. A cloudy fluid containing globules of fat but no leucocytes was obtained. The effect of this serum upon a given quantity of enzyme preserved as a dry powder was compared with the anti-enzymotic action of the blood serum from the same animal. In the following experiments the dry enzyme was prepared from the cells of a purulent exudate obtained after injecting turpentine into the subcutaneous tissue of a dog. Twenty milligrams of the powdered leucocytes were allowed to act upon coagulated serum in the presence of 2.5 c.c. of serum of the pus from which the dried enzyme was obtained or in the presence of serum of the blood of the same animal.

	Serum of pus	Serum of blood
With 2.5 c.c. of serum	14.15 c.c.	5.15 c.c.

The proteolysis produced by the same quantity of enzyme acting upon the same quantity of proteid without addition of serum is represented by 16.5 c.c.

The following experiment, in which the conditions do not differ from those just described save that the preserved enzyme was obtained from an exudate produced by injecting aleuronat into the pleural cavity, further demonstrates the almost total absence of anti-enzymotic action in the serum from the abscess.

	Serum of pus	Serum of blood
With 1.5 c.c. of serum	17.0 c.c.	9.6 c.c.
With 2.5 c.c. of serum	17.6 "	9.4 "

Proteolysis produced by the same quantity of enzyme alone is represented by 17.95 c.c., the control containing nitrogen in uncoagulable form represented by 3.95 c.c.; 2.5 c.c. of serum of pus contains nitrogen in uncoagulable form represented by 2.1 c.c.; 2.5 c.c. of blood serum, by 1.7 c.c.

A similar experiment in which serum from pus obtained five days after the injection of turpentine was used, confirms those just described. A measured quantity of coagulated proteid was subjected to the action of twenty milligrams of powdered leucocytes in the presence of one and of two and a half cubic centimeters of serum of pus or, for the sake of comparison, of blood.

	Serum of pus	Serum of blood
With 1. c.c. of serum	17.6 c.c.	6.7 c.c.
With 2.5 c. c. of serum	18.65 c.c.	7.35 "

Proteolysis produced by the same quantity of enzyme without the addition of serum is represented by 15.65 c.c., the control containing uncoagulable nitrogen represented by 4.3 c.c.; 2.5 c.c. of serum of pus contains nitrogen in uncoagulable form represented by 3.3 c.c., and the same amount of serum of blood by 1.7 c.c.

When allowance is made for the quantity of nitrogen in uncoagulable form in the various mixtures before digestion, it is evident that one cubic centimeter of blood serum completely inhibits the activity of twenty milligrams of the powdered leucocytes, but more than twice as much serum from the purulent exudate has no inhibitory action.

Since the purulent exudates used in the experiments just described have been obtained by injecting turpentine into the

subcutaneous tissue, it is possible that the absence of anti-enzyme in the serum of these exudates may be due to the action of this substance. In the following experiment the ability of 2.5 c.c. of serum to inhibit the digestion of coagulated proteid caused by forty milligrams of powdered leucocytes was tested in the presence and in the absence of turpentine. For the sake of comparison the same amount of enzyme was allowed to act upon coagulated proteid with and without turpentine in the presence of 2.5 c.c. of blood serum heated to 75° C in order to destroy its anti-enzymotic activity:

	Without turpentine	With turpentine
With 2.5 c.c. heated serum	31.2 c.c.	28.05 c.c.
With 2.5 c.c. serum	5.55 "	5.4 "
Control	3.55 "	4.3 "

Leuco-protease is capable of causing proteolysis in the presence of turpentine and the power of the blood serum to inhibit this digestion is unaffected by turpentine.

It is not improbable that an increasing quantity of proteolytic enzyme set free by disintegration of polynuclear leucocytes has so far overcome the anti-enzymotic action of a limited quantity of exuded serum that the entire pus is capable of active autolysis and has the power of digesting foreign proteid. The limited ability of a given quantity of blood serum to inhibit the action of increasing quantities of enzyme, demonstrated by the experiment already described, gives confirmation of this view. A purulent exudate is characterized by its ability to dissolve fibrin and necrotic tissue because its serum, unlike that of the non-purulent exudate, does not check the activity of the proteolytic enzyme furnished in great abundance by the polynuclear leucocytes.