ENZYMES OF TUBERCULOUS TISSUE.*

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Although a large part of the tubercle consists of cells which act as phagocytes receiving into their cell body tubercle bacilli, little effort has been made to determine what enzymes are present in tuberculous tissue. There is little direct evidence of the occurrence of enzymes capable of digesting proteid, but study of the chemistry of tuberculous tissue has indirectly suggested the possibility that autolysis may occur within the tubercle.

Friedrich Müller¹ has found that both pulmonary tissue with tuberculous pneumonia and caseous material, unlike the gray hepatized lung of acute croupous pneumonia, undergo very little autolysis. Matthes² has obtained reactions indicating the presence of deuterio-albumose and peptone in tuberculous glands which have not undergone complete caseation. Speithoff,³ continuing the studies of Matthes, has found albumose and peptone, even though in very small amount, in all of ten extracts prepared from the non-caseous, mixed with the caseous, parts of tuberculous lymphatic glands of cattle, and has suggested that their presence may be referable to autolysis. In seven of ten instances in which caseous material has been carefully freed from non-caseous tissue, these substances have not been found, whereas in the remaining three specimens incomplete evidence of their occurrence have been obtained. Schmoll⁴ has found that caseous material consists of protein with an elementary composition resembling that of albumin or globulin, but since it is almost wholly insoluble it is, he thinks, a coagulated proteid. Since phosphorus has been found to be scant

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¹ Müller, Fr., *Verhandl. d. 20 Cong. f. inner. Med.*, 1902, 192.

² Matthes, *ibid.*, discussion of address of Fr. Müller.

³ Speithoff, *Zent. f. inner. Med.*, 1904, xxv, 481.

⁴ Schmoll, *Deutsches Arch. f. klin. Med.*, 1904, lxxxi, 163.

in the caseous substance it has seemed probable that coagulative necrosis which is associated with destruction of nuclei allows the escape of products which are formed, for the change occurs in a tissue which is rich in phosphorus.

Heile⁵ has suggested that failure of caseous tuberculous lesions to undergo absorption is due to absence of enzymes rather than to insolubility of caseous material. He has found that pus-like material from a cold abscess fails to digest fibrin, but after injection of iodoform in glycerine polynuclear leucocytes appear and the exudate dissolves fibrin. Edw. Müller and Jochmann⁶ have found that pus-like fluid from a cold abscess, unlike pus of acute inflammation, is incapable of dissolving Löffler's blood serum when incubated at from 55 to 60° C., and Kolaczek and Edw. Müller⁷ have claimed that failure of a drop of pus-like fluid to cause a depression upon the surface of a plate of Löffler's serum may be used for diagnosis of uncomplicated tuberculous lesions.

Those exudates and tissues which contain in abundance polynuclear leucocytes with fine granulation contain an enzyme which is distinguishable by its ability to digest proteid in an alkaline and in a neutral medium; these cells, it is well known, constitute the predominant element of most inflammation exudates. One of us has shown that the large mononuclear phagocyte, which is abundant in the later stages of many inflammatory reactions, contains an active enzyme which is distinguishable from that of the polynuclear leucocyte by its ability to digest proteid in the presence of a weakly acid reaction.⁸ The purpose of the present study has been to determine if similar enzymes exist in tuberculous tissue.

Polynuclear leucocytes occur in scant number in the uncomplicated tuberculous lesions usually observed at autopsy and it has been believed by many observers that they have little part in the tuberculous process. Numerous experimental studies have shown that tubercle bacilli, like other bacteria, when injected into animals, cause an accumulation of polynuclear leucocytes which ingest and surround the microorganism. Benda⁹ has shown that the same pro-

⁵ Heile, *Zeit. f. klin. Med.*, 1904, lv, 508.

⁶ Müller and Jochmann, *Münchener med. Woch.*, 1906, liii, 1393.

⁷ Kolaczek and Müller, *ibid.*, 1907, liv, 253.

⁸ Opie, *Jour. of Exper. Med.*, 1905, vii, 316.

cess occurs with human tuberculosis; he has studied the formation of miliary tubercles which occur with acute miliary tuberculosis when plugs of tubercle bacilli lodge in the capillary vessels of the renal glomeruli and has observed as the first result of the presence of the microörganism accumulation of polynuclear leucocytes, about the microörganism.

Polynuclear leucocytes quickly disappear from experimental or from human tuberculous tissue and give place to mononuclear cells, the so-called epithelioid cells which are the chief constituent of the fully developed tubercle. The well-known controversy concerning the origin of these cells is as yet unsettled. Baumgarten on the one hand has maintained that they are derived from the fixed cells of the tissue, whereas Metchnikoff, who has given especial attention to their behavior as phagocytes, has believed that they are derived from leucocytes and are identical with the large mononuclear phagocyte or macrophage, which has a part in other forms of inflammation. Each view has been subjected by those who have supported it to a wide range of modification.

In order to determine if the proteolytic enzymes which occur in the polynuclear leucocytes and large mononuclear phagocytes of inflammatory exudates are present as well in tuberculous lesions it has been necessary to obtain tuberculous tissue in considerable quantity free from microörganism other than tubercle bacilli. The possibility of secondary infection of tuberculous material obtained at autopsy can be excluded by attempted cultivation of bacteria only after tissue has been prepared for the enzymic tests.

Masses of tuberculous tissue are formed in the mediastinum of the dog after injection of tubercle bacilli into one pleural cavity. At the end of two weeks the entire mediastinum and adjacent sub-pericardial membranes may be occupied by masses of pearly white translucent tissue which is firm but very succulent. At a later stage caseation occurs in this tissue which in large areas loses its translucent appearance, becomes opaque and yellowish white and is much less juicy than before the change has occurred. At a late stage of the disease caseation of the tuberculous tissue may be almost complete. The histology of the newly formed tuberculous tissue ob-

⁹ Benda, Path.-anat. Arb. J. Orth gewidmet, p. 520.

tained at various intervals after inoculation and employed in the following experiments will be described in order to determine what relation exists between the enzymic activity of the tissue and the presence of certain types of cell.

Former studies¹⁰ have shown that autolysis of tissues and cells when used in the small quantities in which most pathological products can be obtained gives an incomplete conception of the proteolytic enzymes which they contain. More accurate knowledge may be gotten by allowing a small quantity of the cells to be tested to act upon a relatively large quantity of denaturalized proteid; this method makes it possible to vary within wide limits the character of the medium in which digestion occurs. By the Kjeldahl method for the determination of nitrogen the amount of nitrogen of coagulable proteid converted into incoagulable substances is estimated. Masses of tuberculous tissue freed from fat have been finely divided by passing twice through a miniature sausage machine, which compresses the tissue and in large part squeezes out its juice. The mass of finely divided particles has been further freed from tissue juice by pressure between small pads of sterile gauze. The power of this material to cause proteolysis of heated blood serum has been tested in various strengths of acetic acid and of sodium carbonate. The method employed has been fully described in previous articles.

In the following experiments (Experiments 1 to 6) 0.3 grm. of tuberculous tissue freed from tissue-juice has been allowed to act upon denaturalized blood serum (ten cubic centimeters of a mixture of equal parts of blood serum and 0.85 per cent. sodium chloride heated one half hour at 75° C.) in the presence of acid, approximately neutral and alkaline media. The degree of digestion is indicated by cubic centimeters of one-tenth normal sulphuric acid.

EXPERIMENT 1. Eighteen days after inoculation of the right pleural cavity with an emulsion of tubercle bacilli the mediastinum contained masses of firm pearly white tissue. *Microscopical Examination.*—The tissue which is well vascularized is composed in large part of large mononuclear cells usually collected into small groups containing no blood vessels and surrounded by loose fibrous stroma. These cells have a large vesicular nucleus and abundant protoplasm. Mingled with them in small number are lymphoid and plasma cells. Polynuclear

¹⁰ Opie, *Jour. of Exper. Med.*, 1905, vii, 759; 1906, viii, 410.

leucocytes which are numerous are scattered throughout the tissue and occur in small groups. The only suggestion of caseation is the occurrence of necrosis in a few foci of large mononuclear cells; here some of the cells have lost their nuclei and the cell body is broken in fragments. In such foci polynuclear leucocytes are especially numerous.

Tuberculous Tissue from Mediastinum.

	Control.	Acetic acid.		Neutral.	Sodium carbonate.	
		0.5 per cent.	0.2 per cent.		0.2 per cent.	0.5 per cent.
0.3 grm. tuberc. tissue						
+ coagulated proteid	3.35	14.9	17.7	16.25	21.35	13.0

Studies of exudates and of various tissues of the normal animal, the pancreas excluded, have shown that active digestion in the presence of an alkaline medium is peculiar to inflammatory exudates containing polynuclear leucocytes and to tissues such as the bone marrow which contain these cells in large number. Since this enzyme is inhibited by a weakly acid medium occurrence of active digestion in the presence of acid indicates that a second enzyme is present. Evidence of the independent occurrence of this enzyme in tuberculous tissue will be furnished by subsequent experiments.

The following experiment confirms that just described and shows that tuberculous tissue obtained soon after onset of the disease can cause autolysis in the presence of acid and of alkali:

EXPERIMENT 2. An animal killed eighteen days after intrapleural injection of tubercle bacilli has exhibited advanced tuberculosis of the mediastinum and of the adjacent substernal lymphatic glands. *Microscopical Examination of Mediastinal Tissue.*—The predominant elements in the tissue are large mononuclear cells; lymphoid and plasma cells are fairly numerous. Throughout the tissue are numerous polynuclear leucocytes. The tissue is well vascularized and there is newly formed fibrous stroma. There is no caseation. *Hypertrophied Tuberculous Lymphatic Gland.*—The lymphoid tissue of the gland is in great part replaced by large mononuclear cells with vesicular nucleus and abundant protoplasm resembling those of the mediastinal tissue. Polynuclear leucocytes are very numerous throughout the gland. Fibrous stroma is increased. There is no caseation.

Tuberculous Tissue of Mediastinum and Hypertrophied Tuberculous Lymphatic Glands.

	Control.	Acetic acid, 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent.
0.3 grm. tuberc. tis. of mediastinum				
+ coagulated proteid	4.0	15.45	12.75	14.65
0.3 grm. tuberc. lymph. gland				
+ coagulated proteid	3.35	15.65	12.15	10.45

Digestion in acid and in neutral media is almost identical for the mediastinal and for the glandular tissue, but in an alkaline medium there is greater variation.

For the following experiment tuberculous tissue was obtained from an animal in which the disease was more advanced (twenty-nine days) than in the two foregoing experiments:

EXPERIMENT 3. Tuberculous tissue from the mediastinum has been obtained twenty-nine days after intrapleural inoculation with *B. tuberculosis*. This tissue is firm and in part resembles dense fibrous tissue but over more than one half of the cut surface is opaque, white, and apparently caseous. *Microscopical Examination.*—The tissue is in considerable part necrotic and nuclear stain is absent but throughout occur scattered areas of well preserved tissue which almost always surround an artery of considerable size. In these areas mononuclear cells make up the greater part of the tissue; these cells are usually somewhat smaller more irregular in shape and less clearly outlined, the one from another, than the cells seen at an earlier stage of the lesion. They resemble closely the epithelioid cells of human tubercle. Fibrous stroma is abundant. Polynuclear leucocytes are scant but a few small collections of such cells occur.

Tuberculous Tissue of Mediastinum.

	Control.	Acetic acid.		Neutral.	Sodium carbonate.		
		1 per cent.	0.5 per cent.		0.2 per cent.	0.5 per cent.	1 per cent.
0.3 grm. tuberc. tis.							
+ coag. proteid..	3.05	10.35	11.75	12.25	12.6	4.35	5.15 6.25

Digestion in the presence of acid has been active, whereas in the presence of weak alkali (0.2 per cent.) digestion has been almost wholly absent; the somewhat larger figures obtained with higher strength of alkali (0.5 and 1 per cent.) are probably referable to increased difficulty in causing complete coagulation of undigested proteid by heat in the presence of stronger alkali even after neutralization. Absence of digestion in the presence of 0.2 per cent. sodium carbonate indicates absence of leucoprotease and is in accord with the almost complete absence of polynuclear leucocytes in the tissue which has been used.

Almost identical results have been obtained with tuberculous tissue from the omentum obtained sixty-nine days after intraperitoneal inoculation with a strain of tubercle bacillus more virulent for rabbits and for dogs than that previously employed.

EXPERIMENT 4. Sixty-nine days after intraperitoneal inoculation with *B. tuberculosis* the omentum is represented by a firm flat mass of tissue about 1 cm. in thickness; this tissue is in part grayish-white but in greater part opaque and caseous in appearance. *Microscopical Examination.*—Fat of the omentum is replaced by tissue which is formed by large mononuclear cells together with a few lymphoid and plasma cells. Polynuclear leucocytes are almost wholly lacking except at the margin of caseous tissue where a few are found. Caseous areas of considerable size occur; the large mononuclear cells lose their nuclei, their protoplasm stains deeply and homogeneously and finally breaks into particles. Coarse strands of newly formed fibrous tissue are present.

Tuberculous Tissue from the Omentum.

	Control.	Acetic acid.			Neutral.	Sodium carbonate.		
		1 per cent.	0.5 per cent.	0.2 per cent.		0.2 per cent.	0.5 per cent.	1 per cent.
0.3 grm. tuberc. tis.								
+ coag. proteid..	3.8	15.85	16.5	17.15	11.9	7.15	4.9	4.45

The following experiment in which tuberculous tissue obtained after long continued tuberculosis (forty-seven days) has been used furnishes evidence that proteolytic enzymes tend to disappear after the onset of caseation:

EXPERIMENT 5. Forty-seven days after intrapleural inoculation with *B. tuberculosis* the mediastinum contains masses of tissue which has undergone almost complete caseation. The substernal lymphatic glands are greatly enlarged and homogeneously caseous. In the mediastinum behind the substernal lymphatic glands are two enlarged lymphatic glands which are translucent grayish white and succulent showing little if any caseation. An hypertrophied gland situated near the duodenum has the same appearance and together with those just mentioned has been used in the tests which follow. *Microscopical Examination of Caseous Mediastinal Tissue.*—Caseation with complete absence of nuclear stain occurs throughout the tissue save immediately below its surface where a capsule of fibrous tissue contains a few large mononuclear and lymphoid cells. *Microscopical Examination of Caseous Substernal Lymphatic Gland.*—Caseation completely obscures all structure save in a few small foci about blood vessels where large mononuclear and lymphoid cells occur. Polynuclear leucocytes are almost entirely absent. *Microscopical Examination of Hypertrophied Tuberculous Lymphatic Gland.*—Cells which resemble the large mononuclear cells of tuberculous tissue elsewhere occur in the sinuses and almost wholly replace the cords of lymphoid tissue which are well defined in only a small part of the tissue. The peripheral sinus of the gland is occupied by large mononuclear cells which resemble on the one hand the large mononuclear cells of tuberculous tissue and on the other hand the large phagocytic cells of the lymphatic gland (the so-called endothelial cells). These cells are closely packed together and in places have undergone degenerative changes; the nucleus is pale and swollen or has completely disappeared; the cell body stains deeply, loses its well-defined outline and may break into fragments. Polynuclear leucocytes are present in considerable number throughout the gland.

Caseous tissue used in the following tests has been obtained from the mediastinum and substernal lymphatic glands; the hypertrophied tuberculous glands examined for comparison are those mentioned.

Caseous Material and Hypertrophied Tuberculous Glands.

	Control.	Acetic acid, 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent
0.3 grm. caseous tuberc. tis. + coag. proteid	2.65	7.6	9.00	2.6
0.3 grm. hypertrophied tuberc. gland + coag. proteid	2.85	14.9	10.15	10.95

With advance of tuberculosis and occurrence of widespread caseation leucoprotease indicated by digestion in an alkaline medium has almost completely disappeared. In the hypertrophied gland the lesion is much less advanced and digestion still occurs in an alkaline medium. Comparison of the caseous tissue with the hypertrophied lymphatic gland and with tissue used in previous experiments in which tuberculosis has been less advanced shows that the enzyme capable of digesting in an acid medium has diminished considerably. In the following experiment caseation is almost complete and enzymes, measured by ability of the tissue to cause proteolysis, have almost wholly disappeared.

EXPERIMENT 6. After inoculation with *B. tuberculosis* fluid had accumulated in the right pleural cavity. Slightly turbid, almost colorless fluid had been withdrawn for use in experiments to be described later. The animal was killed with ether sixty-five days after inoculation.

The right pleural cavity contains 175 c.c. bloody fluid; the left cavity contains 134 c.c. reddish serous fluid. The mediastinum is occupied by bulky masses of hard, opaque, yellow tissue. *Microscopical Examination of Mediastinal Tissue.*—Tissue is wholly caseous save for several minute patches in which mononuclear cells with fairly abundant protoplasm occur in fibrous stroma; a few polynuclear leucocytes are present.

Caseous Mediastinal Tissue.

	Control.	Acetic acid.		Neutral.	Sodium carbonate.	
		0.5 per cent.	0.2 per cent.		0.2 per cent.	0.5 per cent.
0.3 grm. caseous tuberc. tis. + coag. proteid...	2.25	6.8	6.6	6.6	2.85	3.45

Tuberculous tissue of the dog during the early stages of its formation contains polynuclear leucocytes in considerable number and exhibits ability to cause proteolysis under conditions which characterize the enzyme of these cells. At a later period polynuclear leu-

cocytes disappear and power to digest in the presence of alkali is lost.

The enzyme which has been constantly found in tuberculous tissue and is present alone during the later stages of the disease is identified by its ability to cause proteolysis in an acid and in a neutral medium, whereas it is wholly inhibited by weak alkali. An enzyme of the same character, designated for convenience lympho-protease, is present in the large mononuclear phagocytes which occur in inflammatory exudates. In the dog the large mononuclear cell, the so-called epithelioid cell of the tubercle, is identical in structure with the mononuclear phagocyte or macrophage of the inflammatory exudate. This mononuclear cell of the tubercle is actively phagocytic, ingesting tubercle bacilli and occasionally taking into its substance polynuclear leucocytes and other cells.

Experiments 1 and 2 illustrate the coincident action of the two enzymes which have been mentioned. Experiments with leucoprotease described in previous articles show that this enzyme causes almost equal digestion in a neutral medium and in 0.2 per cent. sodium carbonate, but little if any in acid. Experiment 3 shows that the enzyme of tuberculous tissue when almost unaccompanied by leucoprotease (indicated by digestion in alkali) may cause nearly equal activity in neutral and in acid (0.2 per cent. acetic acid) media. Nevertheless when the two enzymes act together in the presence of a neutral medium (Experiments 1 and 2) digestion by the two in combination is not greater, but is less than that by the same tissue in alkali or in acid. This fact suggests that when the two enzymes are brought together under conditions which permit the activity of both, one partially inhibits or perhaps partially destroys the other.

Comparison of the different experiments which have been described is possible because digestion caused by tuberculous tissue has been tested under almost identical conditions. Since leucoprotease is almost wholly inactive in the presence of acid, digestion in 0.2 per cent. acetic acid may be used as an index of the content of enzyme capable of digesting in acid. In the table which follows are collected results previously obtained when 0.3 gm. of tuberculous tissue from the same situation, namely, the mediastinum, has been

allowed to act upon a constant quantity of coagulated blood serum in the presence of 0.2 per cent. acetic acid; the figure representing the control has been subtracted from that obtained after incubation during five days at 37° C. and the difference measures digestion of proteid caused by tuberculous tissue obtained at various intervals after inoculation.

Results of Experiments with Mediastinal Tissue in Acid.

Exper. 1.	Exper. 2.	Exper. 3.	Exper. 5.	Exper. 6.
18 days.	18 days.	29 days.	47 days.	65 days.
14.35	11.45	9.2	4.95	4.35

When during the third week after inoculation the figure indicates a maximum degree of digestion caseation is beginning. At this time it affects almost exclusively the large mononuclear cells which lose their nuclei, become hyaline and break into particles. The possibility is suggested that caseation may be referable to autolysis. Subsequent experiments will show that the normal power of the blood serum to check the enzymic action of tuberculous tissue may be considerably diminished in a tuberculous exudate.

After onset of caseation there is gradual and probably complete disappearance of enzymes. It is probable that the small amount of digestion recorded in Experiments 5 and 6 is referable to minute islands of living tissue rich in epithelioid still persisting in the midst of almost complete necrosis.

Various tissues of the body have been found to cause more active proteolysis in acid than in alkali. Biondi,¹¹ Hedin and Rowland¹² and other observers have shown that autolysis of tissue from the liver, spleen, kidney and other organs is more active in the presence of weak acid than in neutral or in alkaline media. Examination of the liver containing miliary tubercles in great number has afforded an opportunity of comparing under almost identical conditions the enzymic activity of tuberculous tissue with that of a parenchymatous organ. The liver tissue of four animals has been employed; one has been obtained from a normal animal and three are tuberculous, obtained eighteen and sixty-five days after inoculation.

¹¹ *Virchow's Arch.*, 1896, cxliv, 373.

¹² *Zeit. f. physiol. Chem.*, 1901, xxxii, 341.

EXPERIMENT 7.

Proteolysis Caused by Hepatic Tissue Containing Miliary Tubercles.

	Control.	Acetic acid, 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent.
Normal Liver	2.6	10.25	6.5	2.9
A Liver with small tubercles (18 days) ..	2.45	13.75	10.2	4.35
B Liver with large tubercles (18 days) ..	2.7	17.1	8.9	5.05
C Liver with large tubercles (65 days) ..	2.55	15.8	5.3	4.0

Microscopical Examination of Tuberculous Livers. A.—About half of the section is occupied by small miliary tubercles composed of large mononuclear cells loosely packed together; a considerable number of polynuclear leucocytes occur among the cells. There is no caseation. B.—Considerably more than half of the section is occupied by large miliary and conglomerate tubercles composed of loosely packed mononuclear cells together with polynuclear leucocytes in considerable number. Caseation is beginning and the centers of many tubercles are necrotic. C.—More than half of the section is occupied by large tubercles composed of closely packed mononuclear cells; a moderate number of polynuclear leucocytes are present. There is no caseation.

The presence of miliary tubercles has increased proteolytic activity in all media, but especially in acid. The increased digestion which has been observed is significant, for it is well known that the activity of digestion does not increase in direct proportion to the quantity of enzyme, but bears a closer relation to the square root of the quantity of enzyme. Furthermore it is probable that the method does not permit digestion above a certain maximum for the accumulation of products of digestion doubtless inhibits the process. Increase of digestion is greatest in the presence of acid; the following figures show the amount of digestion represented by the difference between the control and the incubated preparation.

Results of Experiments with Liver in Acid.

Normal Liver.	Liver A.	Liver B.	Liver C.
7.65	11.3	14.4	13.25

It has been shown by one of us¹³ that the serum of the blood is capable of inhibiting the enzyme which is contained in the polynuclear leucocytes. By use of the Kjeldahl method for estimation of nitrogen the influence of anti-enzyme upon a constant quantity of enzyme can be measured with considerable accuracy. This anti-enzymotic property of the blood is transmitted to the serum of an

¹³ Opie, *Jour. of Exper. Med.*, 1905, vii, 316.

inflammatory exudate and doubtless holds in check enzyme set free by disintegration of polynuclear leucocytes, thus preventing an injurious action upon tissue with which the enzyme comes in contact. A year later Stern and Eppenstein¹⁴ demonstrated the same fact by showing that leucocytes from the blood of patients suffering with spleno-myelogenous leukaemia liquefy gelatin, but lose this power in the presence of blood serum. Edw. Müller and Jochmann¹⁵ subsequently repeated the observation and showed that the power of polynuclear leucocytes from various sources to cause a depression upon the surface of coagulated blood serum is lost in the presence of fresh serum of the blood.

In an article published in 1906, one of us¹⁶ has shown that the anti-enzymotic activity of serum from the blood and from a serous inflammatory exudate is absent in the serum of a purulent exudate. Experiments have furnished evidence that the enzyme of the polynuclear leucocytes is present in serum of the abscess in such excess that the anti-enzymic power of this serum is overcome. Absence of anti-enzyme is believed to explain the solution of fibrin and of tissue which is characteristic of suppuration. The observation just described has been confirmed by Müller and Kolaczek,¹⁷ who have used coagulated serum plates.

In view of the relation of the serum of the blood and of inflammatory exudates to the enzyme of the polynuclear leucocytes, it is desirable to study the behavior of the enzymes of tuberculous tissue in the presence of the serum of a tuberculous exudate. After injection of tubercle bacilli into the pleural cavity of the dog fluid accumulates within the chest and formation of tuberculous tissue upon the pleural membranes and in the mediastinum is associated with gradual increase of fluid. Repeated attempts to cultivate bacteria from this fluid have been unsuccessful.

The following experiment shows that the serum of a tuberculous exudate inhibits in slight degree digestion caused by tuberculous tissue.

¹⁴ Stern and Eppenstein, *Vortrag in der Schles. Gesellsch. f. Vaterland. Kultur*, June 29, 1906. Quoted by Eppenstein, *Münchener med. Woch.*, 1906, liii, 2192.

¹⁵ Müller and Jochmann, *Münchener med. Woch.*, 1906, liii, 1507.

¹⁶ Opie, *Jour. of Exper. Med.*, 1906, viii, 410.

¹⁷ Müller and Kolaczek, *Münchener med. Woch.*, 1907, liv, 354.

EXPERIMENT 8. Tuberculous mediastinal tissue has been obtained fourteen days after inoculation. The exuded tuberculous serum has been obtained by centrifugalization of a tuberculous pleural exudate withdrawn from the chest cavity seventy-one days after inoculation with tubercle bacilli. As in previous tests 0.3 gram. of tissue freed from juice has been allowed to act upon the usual quantity of heated serum.

Effect of Serum of Tuberculous Pleural Exudate upon Digestion Caused by Tuberculous Tissue.

	Control.	After 5 days at 37° C.	Digestion.
Tuberc. tis. + coag. proteid	3.3	13.85	10.55
Tuberc. tis. + 0.2 c.c. exuded tuberc. ser. + coag. p. ..	3.35	11.95	8.6
Tuberc. tis. + 1 c.c. exuded tuberc. ser. + coag. p. ...	3.6	11.9	8.3

To obtain more information concerning the degree of inhibition exerted by serum of a tuberculous exudate upon enzymes contained in tuberculous tissue comparison has been made between the serum of a tuberculous pleural exudate and the serum of blood.

EXPERIMENT 9. A part of the tuberculous tissue used in the previous experiment has been ground and freed from juice; it has been suspended in physiological salt solution (1.4 gram. in 70 c.c.) and after addition of toluol (3 c.c.) incubated at 37° C. during two days. Ten cubic centimeters of this solution filtered through cheese cloth has been allowed to act upon coagulated proteid in the presence of serum of a tuberculous pleural exudate withdrawn seventy-three after inoculation and in the presence of serum of the blood.

Effect of Serum of Tuberculous Pleural Exudate and of Serum of Blood upon Digestion Caused by Extract of Tuberculous Tissue.

	Control.	After 5 days at 37° C.	Digestion.
Extract of tuberc. tis. + coag. proteid	4.8	16.95	12.15
Extract of tuberc. tis. + 2.5 c.c. exuded tuberc. ser. + c. p.	5.3	11.95	6.65
Extract of tuberc. tis. + 5 c.c. exuded tuberc. ser. + c. p.	5.	13.8	8.0
Extract of tuberc. tis. + 2.5 c.c. blood serum + c. p.	5.4	5.15	0
Extract of tuberc. tis. + 5 c.c. blood serum + c. p.	6.1	6.0	0

Two facts are noteworthy: (a) Blood serum has completely prevented digestion by the enzymes contained in the extract obtained from tuberculous tissue, whereas there has been only moderate inhibition in the presence of an equal quantity of exuded tuberculous serum; (b) increase of the quantity of tuberculous serum from 2.5 to five cubic centimeters has not increased the degree of inhibition, but has actually increased the amount of digestion. This fact has

been explained by the demonstration of unchecked enzyme in the exuded tuberculous serum; experiments showing this proteolytic activity of the serum will be described.

It has been shown (Experiments 1 and 2) that tuberculous tissue obtained between the second and third week after inoculation contains two enzymes: (*a*) leucoprotease of the polynuclear leucocytes and (*b*) an enzyme which digests in acid and resembles, if it is not identical with, the enzyme which we have previously designated lymphoprotease. Since experiments demonstrate partial inhibition of this combination of two enzymes, it has been considered desirable to examine the behavior of each of the two enzymes in the presence of the exuded tuberculous serum.

In order to test the effect of serum from a tuberculous pleural exudate upon leucoprotease, twenty milligrams of a dried powder prepared from cells of a sterile purulent exudate obtained by repeated intrapleural injection of turpentine have been allowed to act upon coagulated proteid in the presence of the serum to be tested.

EXPERIMENT 10.

Effect of Exuded Tuberculous Serum upon Leucoprotease.

	Control.	After 5 days at 37° C.	Digestion
20 mgr. powdered leucocytes (leucoprotease)			
+ coag. proteid.....	1.95	15.9	13.95
20 mgr. powdered leucocytes + 1 c.c. exuded			
tuberc. ser. + coag. proteid.....	2.2	11.2	9.0
20 mgr. powdered leucocytes + 2 c.c. exuded			
tuberc. ser. + coag. proteid.....	2.5	10.35	7.85

Since an alkaline medium is favorable to the action of leucoprotease, but inhibits other enzymes, *e. g.*, lymphoprotease, which may be present in the exuded tuberculous serum, the effect of this serum upon leucoprotease has been tested in an alkaline medium.

One cubic centimeter of the exuded tuberculous serum has completely inhibited proteolysis by the enzyme of polynuclear leucocytes in the presence of an alkaline medium; a much greater quantity of serum causes slight digestion perhaps referable to enzyme contained in the serum (see Experiment 18). The serum of the tuberculous

exudate, like the serum of the blood, inhibits that enzyme which digests in the presence of alkali (leucoprotease).

EXPERIMENT II.

Effect of Exuded Tuberculous Serum upon Leucoprotease in Presence of 0.2 Per Cent. Sodium Carbonate

	Control.	After 5 days at 37° C.	Digestion.
20 mgr. powdered leucocytes + coag. proteid..	3.3	10.4	7.1
20 mgr. powdered leucocytes + 0.2 c.c. exuded tuberc. ser. + coag. proteid.....	3.35	8.35	5.0
20 mgr. powdered leucocytes + 1 c.c. exuded tuberc. ser. + coag. proteid.....	3.6	3.6	0
20 mgr. powdered leucocytes + 5 c.c. exuded tuberc. ser. + coag. proteid.....	4.6	7.1	2.5

Attention has been next directed to the effect of tuberculous serum upon the enzyme which acts in acid. As it is difficult to obtain when needed for the experiment fresh tuberculous tissue containing the last-named enzyme alone (resembling that, for example, used in Experiment 3), the attempt has been made to determine the effect of tuberculous serum upon the similar, if not identical, enzyme of lymphatic glands. This experiment is less complicated than that with tuberculous tissue obtained at a time when polynuclear leucocytes are present, for the cells obtained from the lymphatic glands cause digestion in an acid and neutral medium, but produce little or no change in the presence of alkali, that is, leucoprotease is almost wholly wanting. The power of 0.1 gram of lymph gland prepared by the method previously described to cause digestion of coagulated serum has been as follows:

EXPERIMENT I2.

Normal Lymphatic Gland.

	Control.	Acetic acid, 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent.
0.1 gm. lymph. gland + coag. proteid..	2.35	7.65	5.5	3.35

The addition of small quantities of serum obtained by centrifugalization of a tuberculous pleural exudate causes increased rather than diminished digestion in an approximately neutral mixture. Serum from the tuberculous pleural exudates of two animals has been used.

EXPERIMENT 13.

Lymphatic Gland with Serum of a Tuberculous Exudate.

Control (0.1 grm. lym. gl. + coag. proteid).....	2.35
	After 5 days at 37° C.
0.1 grm. lym. gl. + coag. proteid	5.5
0.1 grm. lym. gl. + 0.2 c.c. exuded tuberc. ser. No. i + coag. proteid...	8.3
0.1 grm. lym. gl. + 1.0 c.c. exuded tuberc. ser. No. i + coag. proteid...	12.55
0.1 grm. lym. gl. + 0.2 c.c. exuded tuberc. ser. No. ii + coag. proteid...	6.75
0.1 grm. lym. gl. + 1.0 c.c. exuded tuberc. ser. No. ii + coag. proteid...	8.35

Tuberculous sera No. i and No. ii have been obtained from pleural exudates withdrawn fifty days after inoculation of two animals with *B. tuberculosis*. Exudate No. i contains very few cells; no tubercle bacilli are found in a stained specimen. Fluid is alkaline to litmus. Exudate No. ii contains cells in moderate number and polynuclear leucocytes are predominant; no tubercle bacilli are found. Fluid is alkaline to litmus.

The following experiment confirms that just described.

EXPERIMENT 14.

Lymphatic Gland with Serum of a Tuberculous Exudate.

Control (0.1 grm. lym. gl. + coag. proteid).....	2.1
	After 5 days at 37° C.
0.1 grm. lym. gl. + coag. proteid	4.0
0.1 grm. lym. gl. + 0.2 c.c. exuded tuberc. ser. + coag. proteid.....	6.25
0.1 grm. lym. gl. + 1.0 c.c. exuded tuberc. ser. + coag. proteid.....	11.55

Exuded tuberculous serum used in this experiment has been obtained forty-three days after inoculation from the same animal as serum No. i, Experiment 13. Cells are scant; one large mononuclear cell contains a tubercle bacillus. The fluid is alkaline to litmus.

Since the serum of the tuberculous exudate in association with enzyme of the lymphatic gland causes much more digestion than this enzyme is capable of causing alone, the possibility is suggested that the exuded serum itself may have proteolytic power.

In the following experiment measured quantities of serum obtained by centrifugalization of a tuberculous exudate have been allowed to act upon the usual quantity of coagulated proteid and at the same time comparison has been made with the digestive power of an equal quantity of fresh blood serum.

EXPERIMENT 15. The tuberculous exudate used in the following experiment has been obtained from the pleural cavity fifty-six days after inoculation with

tubercle bacilli. It is almost colorless and only slightly turbid. Microscopical examination shows that cells are present in very small numbers; a few well-preserved polynuclear leucocytes are found and mononuclear cells which are present are often vacuolated and possess poorly-stained nuclei. No tubercle bacilli are found in the stained specimen. After prolonged centrifugalization almost clear serum has been obtained and has been used for the following test.

Serum of Tuberculous Pleural Exudate.

	Control.	After 5 days at 37° C.	Digestion
0.2 c.c. exuded tuberc. ser. + coag. proteid.....	2.6	4.1	1.5
1.0 c.c. exuded tuberc. ser. + coag. proteid.....	2.9	10.95	8.05
5.0 c.c. exuded tuberc. ser. + coag. proteid.....	4.0	11.55	7.55
0.2 c.c. blood serum + coag. proteid.....	2.6	2.9	0.3
1.0 c.c. blood serum + coag. proteid.....	2.8	3.4	0.6
5.0 c.c. blood serum + coag. proteid.....	3.9	4.2	0.3

Whereas active digestion is caused by tuberculous serum digestion referable to the action of blood serum is almost wholly wanting. The results of digestion with tuberculous serum alone are in harmony with those obtained when tuberculous serum has been allowed to act in combination with enzyme from tuberculous tissue or from lymphatic gland. Digestion is not always so active as that just described (Experiment 15), but similar results have been obtained in Experiments 17 and 18 which will be described for another purpose. In the following experiment tuberculous exudate removed from the pleural cavity twelve and fourteen days after inoculation has been employed.

EXPERIMENT 16. Exudate (15 c.c.) removed from the pleural cavity twelve days after inoculation with tubercle bacilli is very turbid and contains cells in considerable number. Polynuclear leucocytes constitute about 60 per cent. of the cells present; large mononuclear leucocytes, usually collected into clumps, 38 per cent.; lymphocytes, 2 per cent. The proteolytic activity of the serum obtained by centrifugalization is as follows:

Serum of Tuberculous Pleural Exudate.

	Control.	After 5 days at 37° C.	Digestion.
1 c.c. exuded tuberc. ser. + coag. proteid.....	2.2	7.35	5.15
5 c.c. exuded tuberc. ser. + coag. proteid.....	3.25	8.9	5.65

Two days later the animal has been killed with ether. The right pleural cavity contains 30 c.c., the left 26 c.c. of turbid fluid; masses of tuberculous tissue are found in the usual situations. The exudate contains cells in the following proportions: polynuclear leucocytes, 74.5 per cent.; large mononuclear cells, 17

per cent.; lymphocytes, 8.5 per cent. One large mononuclear cell contains a tubercle bacillus. The proteolytic activity of the serum obtained by centrifugalization of the exudate has been compared with that of blood serum from the same animal.

Serum of Tuberculous Pleural Exudate Compared with Serum of Blood.

	Control.	After 5 days at 37° C.	Digestion.
0.2 c.c. exuded tuberc. ser. + coag. proteid.....	1.65	3.35	1.7
1.0 c.c. exuded tuberc. ser. + coag. proteid.....	1.8	5.6	3.8
5.0 c.c. exuded tuberc. ser. + coag. proteid.....	2.95	5.55	2.6
0.2 c.c. blood serum + coag. proteid.....	1.7	2.2	0.5
1.0 c.c. blood serum + coag. proteid.....	2.0	2.45	0.45
5.0 c.c. blood serum + coag. proteid.....	3.6	3.8	0.2

The second test made two days after the first has shown diminished proteolytic activity, perhaps as the result of the previous removal of a considerable part of the fluid in the chest; nevertheless digestion occurs, although the blood serum of the same animal is almost wholly inactive.

The experiments have shown that the serum obtained from a tuberculous pleural exudate possesses proteolytic activity which is almost wholly wanting in the serum of the blood. Absence of anti-enzymotic action exerted by the exuded serum allows the action of an enzyme contained in the tuberculous exudate. The behavior of this enzyme has been tested in acid, in an approximately neutral and in an alkaline medium, by allowing the same quantity of tuberculous serum to act upon coagulated proteid in the presence of various media.

EXPERIMENT 17.

Action of Exuded Tuberculous Serum in Various Media.

	Control.	Acetic acid, 0.2 per cent.	Neutral.	Sod. carb., 0.2 per cent.
1 c.c. exuded tuberc. ser. + coag. proteid.....	1.9	3.0	7.75	2.0

The serum used has been obtained from a tuberculous pleural exudate withdrawn from the pleural cavity sixty-two days after inoculation.

Repetition of the experiment has given almost identical results.

EXPERIMENT 18.

Action of Exuded Tuberculous Serum in Various Media.

	Control.	Acetic acid, 0.2 per cent.	Neutral.	Sod. carb., 0.2 per cent.
2.5 c.c. exuded tuberc. ser. + coag. proteid....	2.0	4.8	10.2	3.0

The serum used has been obtained from the animal of Experiment 17 seventy-three days after inoculation.

Digestion is active in the presence of an approximately neutral medium, comparatively slight in the presence of acid and almost absent in a neutral medium. This relation to change of reaction does not accurately correspond with that exhibited by either of the two enzymes which have been found in tuberculous tissue, since one digests with almost equal activity in acid and neutral media, whereas the other digests with approximately equal activity in an alkaline and in a neutral medium.

Since the exuded tuberculous serum possesses an ability to cause proteolysis, which is absent in the serum of the blood, it is important to know if the serum of an inflammatory exudate obtained by other means possesses the same property. For the purpose of comparison an inflammatory exudate has been obtained by injecting turpentine into the pleural cavity of the dog. Samples of fluid have been removed on the third and on the fifth day after injection; the serum obtained by centrifugalization of this exudate has been employed in the following experiment:

EXPERIMENT 19.

Serum of Exudate Obtained on the Fifth Day after Injection of Turpentine.

	Control,	After 5 days at 37° C.	Digestion.
1 c.c. exuded serum + coag. proteid.....	2.75	4.35	1.6
5 c.c. exuded serum + coag. proteid.....	2.0	2.85	0.85

Serum of Exudate Obtained on the Third Day after Injection of Turpentine.

	Control,	After 5 days at 37° C.	Digestion.
1 c.c. exuded serum + coag. proteid.....	1.65	2.0	1.25
2.5 c.c. exuded serum + coag. proteid.....	2.1	3.7	1.6

Comparison of these results with digestion caused by the serum of a tuberculous exudate shows that proteolysis occurs in the presence of serum of the acute inflammatory exudate produced by turpentine, but is slight.

CONCLUSIONS.

Epithelioid cells which form the chief element of tuberculous tissue contain an enzyme which causes active digestion of proteid in an approximately neutral or in a weakly acid medium, but is inactive in the presence of weak alkali. This enzyme resembles that

which occurs in the large mononuclear cells of an inflammatory exudate and is more active than the similar enzyme of parenchymatous organs such as the liver.

The enzyme which digests in the presence of acid exhibits greatest activity at a time when caseation is beginning. With advance of caseation its activity diminishes so that tissue which has undergone almost complete caseation exhibits trivial evidence of the presence of enzyme. It is probable that complete caseation is followed by total disappearance of enzymes.

Tuberculous tissue contains an enzyme capable of digesting proteid in the presence of alkali (leucoprotease) only during the early stages of its development. This enzyme, present at a time when the tissue contains numerous polynuclear leucocytes, quickly disappears so that when enzyme digesting in acid is still active, leucoprotease has disappeared.

The serum of a tuberculous pleural exudate obtained by intrapleural inoculation with tubercle bacilli causes slight inhibition of the mixture of enzymes contained in tuberculous tissue shortly after inoculation. The serum of blood causes complete inhibition of the enzymes contained in the same tuberculous tissue. Analysis of this difference indicates that the exuded tuberculous serum, like the serum of the blood, inhibits proteolysis caused by leucoprotease, but fails to inhibit digestion caused by an enzyme acting in the presence of acid. In testing this property of the exuded tuberculous serum lymphatic gland has been used because suitable tuberculous tissue has not been available.

The serum of the tuberculous pleural exudate produced experimentally not only fails to exert the anti-enzymotic power which is exhibited by the serum of the blood, but is itself capable of causing active digestion of coagulated proteid. Normal blood serum does not digest proteid and the serum of a sterile inflammatory exudate obtained by injection of turpentine into the pleural cavity has caused very little digestion. The tests which have been made indicate that loss of anti-enzymotic power and ability to cause proteolysis increase with the age of the exudate.

The foregoing facts offer suggestions which may serve to explain in part the nature of the tubercle and the changes which occur

within it. The so-called epithelioid cells of the tubercle resemble the large mononuclear phagocytes of inflammatory exudates and both contain an enzyme of the same character. It is not improbable that caseation which, like autolysis, is accompanied by disappearance of nuclei is in part dependent upon the presence in the cells of this active proteolytic enzyme which is for a time held in check. Injury to cells by products of the tubercle bacillus or partial anæmia, the result of imperfect vascularization of the tuberculous tissue, may have a part in rendering these cells susceptible to self-digestion. Changes which have been observed in serum of the tuberculous exudate show that the anti-enzymotic property of the normal blood may be absent in the exudate of a tuberculous lesion. This loss of anti-enzymotic action, perhaps referable to changes caused by products of the tubercle bacillus, may favor self-digestion of the enzyme-containing cells and diffusion of their enzyme.