

ENZYMES OF TUBERCULOUS EXUDATES.¹

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In a former publication² we have shown that tuberculous tissue contains an enzyme which digests protein with considerable activity in the presence of an acid medium. This enzyme is present in the tubercle at all stages of its development and disappears only after caseation has occurred. During the formation of the lesion polynuclear leucocytes are abundant and for the first weeks after inoculation the newly formed tissue exhibits the enzymotic activity characteristic of these cells, namely, an ability to digest protein in the presence of an alkaline medium. At the end of about four weeks polynuclear leucocytes have in great part disappeared and the tissue has almost wholly lost its power to digest protein in the presence of alkali. Since the tissue at this time consists almost wholly of mononuclear epithelioid cells it may be assumed that they contain the enzyme which digests in an acid or in an approximately neutral medium but fails to digest in the presence of an alkaline medium. The cells of the tubercle are similar in appearance to the large mononuclear phagocytes of an inflammatory exudate and to the phagocytic cells which are abundant in the sinuses of lymphatic glands in the neighborhood of a focus of inflammation, and all these cells cause digestion of protein under the same conditions. The enzyme of such cells differs from the autolytic enzyme of various organs, for example of the liver, in its greater activity.³

The demonstration of an active proteolytic enzyme in the epithelioid cells of the tubercle derives importance from the fact that these cells ingest and apparently dissolve tubercle bacilli and other

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³*Jour. of Exper. Med.*, 1906, viii, 410; and 1908, x, 645.

cells. It is noteworthy that Bergel⁴ has recently obtained evidence that the same cells contain an enzyme which digest soft wax and such fatty substances as neutral butter fat and neat's-foot oil. The following observations which in large part confirm those of our previous study have been made during the course of experiments undertaken for another purpose.

The serum of the blood even in small quantity inhibits the action of the enzyme contained in the polynuclear leucocytes, (leucoprotease) and this antienzymotic action is exerted as well by the serum of an inflammatory exudate. Our former study has shown that the serum of a tuberculous exudate obtained by injecting tubercle bacilli into the pleural cavity of the dog exhibits the same anti-enzymotic action upon leucoprotease. Moreover, the serum of the blood likewise inhibits that enzyme which, constantly present in tuberculous tissue, digests protein in the presence of acid, but experiments recorded in our former publication have shown that the power of the serum of the tuberculous exudate to inhibit the enzymes of tuberculous tissue may be much less than that of the blood. Nevertheless these experiments were inconclusive because the tuberculous tissue employed for the test was obtained only two weeks after inoculation at a time when polynuclear leucocytes were numerous and leucoprotease (inhibited by the serum) was present in abundance. Partial inhibition may have been referable to the action of the serum on leucoprotease. In the following experiment, although the tuberculous tissue employed exhibited some digestion in alkali (indicating the presence of leucoprotease), digestion in acid was predominant. The methods employed have been fully described in the article mentioned above.

EXPERIMENT I.—Tuberculous tissue from the mediastinum of a dog forty-two days after inoculation of the right pleural cavity with *Bacillus tuberculosis* (human), was passed through a miniature sausage machine and, suspended in salt solution with addition of toluol in small quantity, was allowed to stand in the ice chest. The fluid freed from large solid particles by filtration through gauze was incubated (5 c.c. being used for each test) during five days with coagulated protein in the presence of 0.2 per cent. acetic acid, 0.2 per cent. sodium carbonate and in approximately neutral medium (the volume of each mixture being 25 c.c.), in order to test its proteolytic activity.

⁴ *Münchener med. Woch.*, 1909, lvi, 64.

The suspension of tuberculous tissue (enzyme) plus coagulated protein contains nitrogen in incoagulable substances represented by 2.6 c.c.

| | 0.2 per cent. Acetic acid. | Neutral. | 0.2 per cent. Sodium carbonate |
|---|-------------------------------|----------|-----------------------------------|
| Extract of tuberculous tissue + coagulated protein after 5 days at 37° C..... | 9.9 | 6.6 | 5.5 |
| Digestion (control being 2.6)..... | 7.3 | 4.0 | 2.9 |

The same suspension of tuberculous tissue was allowed to act upon the same quantity of coagulated protein in the presence of various quantities both of blood serum and of serum from a tuberculous exudate removed from the pleural cavity of a dog forty-seven days after inoculation.

| | After 5 days at 37° C. |
|--|---------------------------|
| Ext. tuberculous tissue + coag. protein, | 6.6 |
| Ext. tuberculous tissue + coag. protein + 2.5 exuded tuberculous serum, | 9.85 |
| Ext. tuberculous tissue + coag. protein + 1 exuded tuberculous serum, | 7.55 |
| Ext. tuberculous tissue + coag. protein + 0.5 exuded tuberculous serum, | 6.35 |
| Ext. tuberculous tissue + coag. protein + 0.25 exuded tuberculous serum, | 5.3 |
| Ext. tuberculous tissue + coag. protein + 0.1 exuded tuberculous serum, | 4.0 |
| Ext. tuberculous tissue + coag. protein + 2.5 blood serum, | 3.9 |
| Ext. tuberculous tissue + coag. protein + 1 blood serum, | 3.55 |
| Ext. tuberculous tissue + coag. protein + 0.5 blood serum, | 3.5 |
| Ext. tuberculous tissue + coag. protein + 0.25 blood serum, | 3.45 |
| Ext. tuberculous tissue + coag. protein + 0.1 blood serum, | 3.4 |

The action of the exuded tuberculous serum (1 and 0.5 c.c.) employed in this experiment upon coagulated protein under conditions similar to those described above is shown by the following tests:

Exuded tuberculous serum (1 c.c.) plus coagulated protein contains nitrogen in incoagulable substances represented by 1.65 c.c. *N/10* sulphuric acid (control).

| | After 5 days at 37° C. | Digestion. |
|--|---------------------------|------------|
| 1 c.c. exuded tuberculous serum + coag. protein, | 4.3 | 2.65 |
| 0.5 c.c. exuded tuberculous serum + coag. protein, | 1.9 | 0.25 |

The foregoing experiment shows that the addition of 1 or 2.5 c.c. of exuded tuberculous serum not only fails to inhibit digestion by tuberculous tissue in a neutral medium but actually increases it. Digestion caused by this tuberculous tissue in a neutral medium is due to the action of two enzymes one of which digests in acid and neutral media but fails to digest in alkali (enzyme of epithelioid cells), whereas the other digests in alkaline and in neutral media but fails to digest in acid (leucoprotease). Inhibition of the latter

enzyme alone may explain the diminished digestion in the presence of very small quantities (0.1 to 0.5 c.c.) of exuded tuberculous serum—quantities too small to exhibit the proteolytic activity which is exerted by 1 c.c. but not by 0.5 c.c. of serum (see experiment).

In the experiments just described serum was separated from the cells of the exudate by centrifugalization. Since the cells, though scant in quantity, contain additional enzyme, the whole exudate will probably exert greater proteolytic activity than the serum alone. This suggestion is confirmed by the following test:

EXPERIMENT 2.—Turbid fluid was withdrawn from the right pleural cavity on the seventy-sixth day after inoculation with *Bacillus tuberculosis*.

| | Control. | After 5 days at 37°. | Digestion. |
|---|---------------|-------------------------|----------------|
| 1 c.c. tuberculous exudate + coag. protein, | 1.2 (approx.) | 7.25 | 6.05 (approx.) |
| 1 c.c. ser. of tuberc. exudate + coag. protein, | 1.2 | 4.85 | 3.65 |

The same test was repeated with exudate withdrawn two days latter.

| | Control. | After 5 days at 37°. | Diges- tion. |
|--|----------|-------------------------|-----------------|
| 1 c.c. tuberculous exudate + coag. protein, | 1.45 | 4.75 | 3.3 |
| 1 c.c. serum of tuberculous exudate + coag. protein, | 1.2 | 3.05 | 1.85 |

In this experiment proteolytic action exerted by the whole exudate has been relatively weak because cells have been present in scant amount. Since the whole exudate digests with greater activity than its serum alone it is evident that the presence of serum increases the enzymotic action of the cells.

Unusual opportunity to study the proteolytic action of cells of a tuberculous exudate was afforded by a fluid removed from the pleural cavity fifty-seven days after intrapleural infection with a virulent (bovine) tubercle bacillus. The exudate which was small in amount was thick and resembled pus. Cells, in great part large mononuclear cells, were present in great abundance and serum was scant in amount.

EXPERIMENT 3.—Fifty-seven days after intrapleural inoculation with 1 c.c. of a translucent suspension of *Bacillus tuberculosis* (bovine) death has occurred and the pleural surfaces, mediastinum and adjacent lymphatic glands exhibit abundant newly formed tuberculous tissue which is in part caseous. The right pleural cavity contains 3 c.c. of thick yellow pus-like fluid; agar-agar inoculated with this fluid has remained sterile. Microscopic examination of the exudate

shows that it contains an immense number of cells. Large mononuclear cells with round oval or indented nuclei, and abundant protoplasm, form the greater bulk; these cells which resemble the epithelioid cells of tuberculous tissue in the dog often contain tubercle bacilli. Polynuclear leucocytes occur in large number and lymphocytes are numerous. It is not possible to determine the relative number of the different kinds of cells because the large mononuclear cells in considerable part occur in clumps, whereas the other cells are scattered.

Nitrogen of incoagulable substances in 0.25 c.c. exudate plus coagulated protein is represented by 2.4 c.c. *N/10* sulphuric acid.

| | Acetic acid. 0.2 per cent. | Neutral. | Carbonate. 0.2 per cent. |
|---|-------------------------------|----------|-----------------------------|
| 0.25 c.c. tuberculous exudate + coag. protein | | | |
| after 5 days at 37° C..... | 14.4 | 14.45 | 6.2 |
| Digestion | 12.0 | 12.05 | 3.8 |

Whereas in Experiment 2, 1 c.c. of serous tuberculous exudate has caused digestion represented by 7.25 c.c. of *N/10* sulphuric acid, in the foregoing experiment 0.25 c.c. of the tuberculous exudate (without addition of acid or of alkali) has caused digestion represented by 14 c.c. *N/10* sulphuric acid. The polynuclear leucocytes of pus cause maximum digestion in the presence of alkali, but this pus-like exudate which consists in great part of large mononuclear cells, digests with far greater activity in a neutral or acid than in an alkaline medium. Nevertheless in the exudate used in Experiment 3 polynuclear leucocytes are present and there is referable to them some digestion in an alkaline medium.

Former experiments have shown that the enzyme of polynuclear leucocytes digests in neutral as well as in alkaline media. In the foregoing experiment digestion in neutral medium is referable to the two enzymes acting together. When allowance is made for this fact it is evident that digestion in acid caused by the mononuclear cells which are predominant must be more active in an acid than in neutral medium—a conclusion in accord with previous observations made with tuberculous tissue.

Our former study has shown that the serum of a pleural tuberculous exudate (obtained by experimental inoculation) unlike the serum of the blood or the serum of an inflammatory exudate obtained by intrapleural injection of a sterile irritant causes fairly active digestion of protein. An attempt has been made to determine the conditions which influence this exceptional proteolytic activity.

Fractional precipitation of the proteins of the blood serum have shown that the globulin contains an enzyme or combination of enzymes which is somewhat more active in a neutral than in an alkaline medium⁵; the albumin fraction contains antienzyme. In the whole serum the enzyme of the globulin is restrained by the anti-enzyme of the albumin, so that the serum of the blood when brought into contact with denaturalized protein fails to cause digestion. The enzymotic action of the tuberculous exudate digesting more energetically in neutral than in acid or alkaline medium resembles that of the globulin fraction of the blood. It is not improbable that proteolysis in both instances is brought about by a combination of two enzymes. Such a combination of two enzymes, one of which—leuco-protease—digests in ~~acid and~~ neutral media, whereas another, *e. g.*, the enzyme of tuberculous tissue, digests in neutral and acid media may produce greater digestion in neutral than in either acid or alkaline medium. Of essential importance in the present study is the observation that enzymes may exist in tuberculous exudate unrestrained by anti-enzyme.

A considerable number of observations have been tabulated (Table I) in order to show the relationship of the enzymotic activity of exuded tuberculous serum to the duration and course of the infection, and to inoculation with different strains of tubercle bacilli (human and bovine).

In every instance one cubic centimeter of exuded serum obtained by centrifugalization of the tuberculous exudate has been allowed to act upon five cubic centimeters of coagulated protein during five days at 37° C. The figures in the table represent the amount of digestion which has occurred, and have been obtained by subtracting the figure representing the control, *i. e.*, incoagulable nitrogen in the mixture before incubation, from the figure representing incoagulable nitrogen after incubation during five days at 37° C.

With the progress of the infection, following inoculation with the human type of bacillus there is no increase of proteolytic activity; there is in general a slight decrease. This observation is contrary to the impression mentioned in our former publication on the subject.

⁵ Opie and Barker, *Jour. of Exper. Med.*, 1907, ix, 207.

TABLE I.

| No. | Type of B. tuberculosis. | First week. | Second week. | Third week. | Fourth week. | Fifth week. | Sixth week. | Seventh week. | Eighth week. | Ninth week. | Tenth week. | Eleventh week. | Twelfth week. | Remarks. |
|-----|-----------------------------|----------------|-----------------|----------------|-----------------|----------------|----------------|------------------|-----------------|----------------|----------------|-------------------|------------------|---|
| 1 | Human | | 6.2 | | | | | | | | | | | Fluid tested was ob- tained after death. When fluid was ob- tained animal was very sick; killed two days later. Last test was made two days after pre- ceding test. |
| 2 | Human | | 5.5 | | o | | | | | | | | | |
| 3 | Human | | 5.7 | | | | | | | | | | | |
| 4 | Human | | | | | | | | | | | | | |
| 5 | Human | | 5.15 | 3.73 | | | | 0.6 | | | | | | |
| 6 | Human | | | | | | | 0.6 | | | | | | |
| 7 | Human | | | | | | | | 4.85 | | | 3.6 | 1.8 | |
| 8 | Human | | 6.8 | 3.35 | 4.8 | 4.15 | 4.9 | | | | | | | |
| 9 | Bovine | | | | 0.4 | 0.25 | | | | | | | | |
| 10 | Bovine | | | | | | | | 0.25 | | | 0.6 | | |

In two instances serum of exudates obtained by inoculation with the human type of bacillus failed to cause noteworthy digestion. In one such instance fluid was removed after death; in the second the animal had lost much weight and was very sick.

In both instances in which the bovine type of tubercle bacillus has been used there is an almost complete absence of proteolysis, whereas animals inoculated with human tubercle bacilli have shown fairly active proteolysis at corresponding periods after inoculation. It is not improbable that this difference bears some relation to the virulence of the two strains, for the bovine type kills dogs with much greater certainty and after a shorter interval.

Attempts have been made to determine if the serum of human tuberculous exudates causes proteolysis similar to that obtained after inoculation of dogs. Such tests have been uniformly negative. The following examples are cited from a number of almost identical observations for which we are indebted to Dr. A. R. Dochez.

Tuberculous Pleurisy.—McC., Presbyterian Hospital of New York. Yellowish slightly turbid fluid was withdrawn October 21 from the pleural cavity. Serum was obtained by centrifugalization. No bacteria were grown from the fluid; tubercle bacilli were not found. Clinical diagnosis is tuberculous pleurisy.

| | Control. | After 5 days at 37°C. |
|--|----------|--------------------------|
| 1 c.c. exuded tuberculous (?) serum + coag. protein, | 1.5 | 1.7 |
| 2.5 c.c. exuded tuberculous (?) serum + coag. protein, | 1.8 | 1.9 |

Tuberculous Pleurisy.—Q., Presbyterian Hospital. Coagulable fluid was withdrawn from the pleural cavity; a guinea-pig inoculated with this fluid died with generalized tuberculosis. Serum obtained by centrifugalization of the pleural exudate gave the following result, tests being made in duplicate:

| | Control. | After 5 days at 37°C. |
|--|----------|--------------------------|
| 1 c.c. exuded tuberculous serum + coag. protein, | 2.1 | 2.3 |
| 2.5 c.c. exuded tuberculous serum + coag. protein, | 2.2 | 2.4 |
| | | 2.35 |

Tuberculous Peritonitis.—H. G., Presbyterian Hospital. Slightly turbid orange yellow fluid was removed by tapping from the peritoneal cavity; a small quantity of blood was present and a coagulum which formed was somewhat blood stained.

| | Control. | After 5 days at 37°C. |
|--|----------|--------------------------|
| 1 c.c. exuded tuberculous serum + coag. protein, | 1.6 | 1.8 |
| 2.5 c.c. exuded tuberculous serum + coag. protein, | 2.25 | 2.65 |

At autopsy the peritoneal cavity contained 150 c.c. of yellow fluid; the omentum was thickened, retracted and partly caseous. The peritoneal surfaces were studded with large tubercles, undergoing caseation.

The observations which have been described confirm those previously recorded and show that the mononuclear epithelioid cells of tuberculous tissue contain an enzyme which digests protein actively in an acid and with slightly less rapidity in an approximately neutral medium, but is almost wholly inactive in the presence of alkali. This enzyme unlike the enzyme of the polynuclear leucocytes is not inhibited by the serum of a tuberculous exudate obtained by injection of tubercle bacilli (human) into the pleural cavity of the dog, although it is inhibited by the blood serum. The serum of such an exudate, moreover, unlike the serum of the blood is capable of digesting denaturalized protein. Two observations indicate that this proteolytic activity may disappear just before death. Such proteolytic activity is exhibited by exuded serum of animals inoculated with an organism to which they are relatively insusceptible, and is absent in animals inoculated with a more virulent type (bovine) of tubercle bacillus. Power to digest protein has not been demonstrable in the serum of human tuberculous exudates.