TISSUE-DIGESTING ENZYME (HISTASE) OF STREPTOCOCCI.

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In the fall of 1925, while making a study of beta type hemolytic streptococci from a variety of sources, a strain was isolated from impetigo-like lesions which was found to be capable of digesting a considerable portion of the meat in tubes of Holman's (1) modification of Robertson's cooked meat medium. It did not liquefy gelatin and in every other respect resembled *S. pyogenes*.

Digestion of animal tissues by streptococci of the type represented by *S. pyogenes* has not been described before, as far as can be ascertained from a search of the available literature. Digestion of casein has been observed and reference to this will be made later on.

Description of the Tissue Digestion.

Tubes of cooked meat medium with vaseline seals were inoculated with the proteolytic strain of streptococcus referred to above. The level of the chopped meat in the tubes was carefully marked with a red wax pencil. After 24 hours at 37°C. no change in the level of the meat had occurred. After 48 hours, however, the level slowly descended. Smears made at this time showed pure cultures of the streptococci. After 5 days at 37°C. about 30 per cent of the meat had completely disappeared. The meat continued to be liquefied during 3 subsequent weeks at room temperature. At the end of 1 month about 75 per cent of the meat had disappeared and the remainder had the finely granular appearance sometimes seen in cooked meat cultures of very proteolytic anaerobic bacilli. No gas or foul odor was produced.

Occurrence in Filtrates.

Several tubes of cooked meat were inoculated and allowed to incubate for 5 days at 37°C. Part of the tissue having liquefied, the supernatant fluid was removed and passed through a Berkefeld filter. The filtrate was incubated along with subcultures from it. After 2 weeks both were found sterile.

The level of the meat in three tubes of cooked meat medium was carefully marked as above. The first tube received 1 cc. of the sterile filtrate. The second received 0.5 cc. and the third tube was not opened. All three were incubated at 37°C. After 18 hours the meat level in the two tubes containing the filtrate was about 2 mm. below the mark. The control tube was unchanged. After 5 days the meat level in the filtrate-containing tubes was about 8 mm. below the mark while the control was still unchanged. It appears that a true ectoenzyme is responsible for the tissue digestion. The name histase is proposed for this enzyme.

Effect of Heat on the Enzyme.

The meat level in six tubes of cooked meat medium was carefully marked. To the first was added 1 cc. of the sterile filtrate described above. A tube containing 5 cc. of the filtrate was then immersed in a water bath held at 60°C. After waiting 4 minutes for the temperature to become uniform, 1 cc. of the filtrate was removed at 15 minute intervals and placed in one of the marked tubes of cooked meat. All tubes were then incubated at 37°C. with an unopened control and a sterility control. Tissue in all tubes containing filtrate was digested, but not at the same rate. After 5 days visible digestion had taken place at about equal rates in tubes heated not longer than 30 minutes. About 2 days later, liquefaction was observed in the 45 minute tube and about 3 days subsequent to this, digestion was discernible in the tube heated for 1 hour. After 2 weeks all tubes showed about the same degree of digestion. Exposure to 60°C. for 1 hour, therefore, seems to retard the action of the enzyme but not completely to destroy it. It may be that most of the enzyme was destroyed but that sufficient remained to digest a few mm. of the meat.

Action of the Enzyme on Human Tissues.

In preparing the cooked meat medium referred to above, the method of Holman (1) was adopted, with the exception that beef hearts were used and vaseline seals were added to the tubes. Similar tubes were prepared with human instead of bovine heart. The human heart was taken from an uninfected case at autopsy. Nine strains of hemolytic streptococci were studied in this medium. Of these, three did not digest bovine or human heart, while six digested both.

In several tubes inoculated with streptococci and sealed with vaseline, digestion failed to occur. Smears were made to determine whether growth had occurred. It was found to be so sparse as to leave the ability of the organisms to digest meat in doubt. The vaseline seals were removed with sterile pipettes and the cultures reincubated. After 24 hours good growth had occurred and after 5 days extensive digestion was observed. These strains were evidently fairly strict aerobes. This peculiarity has since been observed in several other cases. A difference in the appearance of the meat in tubes treated in this manner deserves mention. In such tubes, after growth has occurred, the color of the meat changes from the usual red-brown to a greenish grey most intense at the top surface. The meat in which growth has occurred without the removal of the vaseline seal does not change color, or at most becomes a slightly darker brown. Strains which digest meat in the presence of the vaseline seal, do not produce any color change when cultivated in tubes from which the seal has been removed, although they still digest the meat.

Relation to Hemolysin.

Julianelle (2), in a study of the hemolytic staphylococci, obtained data suggesting that proteolysis and hemolysis by those organisms are somewhat closely related. With this in mind twenty-two strains of hemolytic streptococci from various sources were tested for ability to digest meat and to produce hemolysin. For the hemolysin test the organisms were cultivated for 18 hours in infusion bouillon at 37°C. In each test 0.5 cc. of culture was mixed, with 0.5 cc. of 5 per cent suspension of washed rabbit corpuscles. The mixtures were shaken and placed in the water bath at 37°C. Readings were made at the

end of 90 minutes. The results of the comparisons are shown in Table I. This series would seem to indicate that proteolysis and hemolysis by this type of organism are not closely related. It is

TABLE I.

Relation between Hemolysis and Proteolysis.

Strain No.	Source	Tube hemolysis* (90 min.)	Meat digestion
8556	Septicemia	4+	−
3606	Certified milk	−	−
Reed.	Infected ear	4+	−
8674	Septicemia	−	+
Mort. 23	Septic sore throat	4+	+
Lexley	Infected ear	4+	+
X 40	Septic sore throat	4+	+
8616	Erysipelas	4+	+
S 2	Scarlet fever	4+	+
X 32	Septic sore throat	2+	+
X 39	Septic sore throat	4+	−
X 41	Septic sore throat	4+	+
Cow 108	Mastitis	4+	+
3641	Certified milk	+	±
Hamer	Sore throat	4+	+
2735	Certified milk	4+	+
8576	Septicemia	+	±
2082	Certified milk	4+	+
3639	Certified milk	+	−
2081	Certified milk	4+	−
Hebron	Sore throat	4+	+
2600	Certified milk	4+	−

*4+ indicates complete hemolysis.

Summary:

Strains digesting meat and producing soluble hemolysin	12
Strains digesting meat and not producing soluble hemolysin	1
Strains not digesting meat but producing soluble hemolysin	6
Strains not digesting meat and not producing soluble hemolysin	1
Doubtful	2

possible that the method used for determination of hemolysin production is too crude. De Kruif and Ireland (3) have found that hemoly-

sin may disappear from cultures more than 14 hours old. It is evident however that some strains of streptococci may produce a large amount of hemolysin yet fail to digest meat.

TABLE II.

Comparison of the Proteolytic Activities of Various Species of Streptococci.

Strain No.	Source	Species*	Meat digestion†	Casein digestion‡	Liquefaction of gelatin†	Digestion of blood serum
8616	Erysipelas	*Pyogenes*	+	+	−	−
X 40	Epidemic sore throat	*Pyogenes*§	+	−	−	−
3639	Certified milk	*Pyogenes*	−	+	−	−
8591	Bronchopneumonia	*Pyogenes*	−	−	−	−
3056	Certified milk	*Pyogenes*	+	−	−	−
2735	Certified milk	*Pyogenes*	+	−	−	−
2082	Certified milk	*Infrequens*	+	−	−	−
S 2	Scarlet fever	*Pyogenes*	+	−	−	−
Lexley	Infected ear	*Pyogenes*	+	+	−	−
Lanc.	Impetigo-like lesion	*Equi*	−	−	−	−
8674	Septicemia	*Pyogenes*	+	+	−	−
3636	Certified milk		−	+	−	−
3052	Certified milk	*Infrequens*	+	−	−	−
8556	Postabortion septicemia	*Infrequens*	−	−	−	−
8741	Bronchopneumonia	*Pyogenes*	+	+	−	−
8576	Septicemia	*Pyogenes*	−	−	−	−
Hamer	Sore throat	*Pyogenes*	+	+	−	−

* Holman's classification (12).

† 7 days at 37°C.

‡ 48 hours at 37°C.

§ *S. epidemicus* of Davis (13).

Relation of Tissue Digestion to Liquefaction of Gelatin, Coagulated Blood Serum and Casein.

Eighteen strains of hemolytic streptococci were tested for ability to digest meat, casein, coagulated blood serum and gelatin. To determine gelatin liquefaction, tubes of infusion gelatin were melted in the incubator. These were inoculated with 1 drop of 24 hour bouillon cultures. The coagulated serum was prepared in the form of Loeffler's slants. These were inoculated with 1 drop of the bouillon

cultures above mentioned. The tests for casein digestion were at first made by inoculating tubes of brom-cresol purple milk. Most of these promptly coagulated, and it was difficult to distinguish between small amounts of digestion and separation of the whey. The method was abandoned.

Following the procedure of Eijkman (4), infusion agar was melted in tubes and to each tube, containing about 12 cc. of agar, was added 1 cc. of sterile milk. The mixtures were poured into plates and the surfaces were streaked with the streptococci to be tested. Hydrolysis of the casein is evidenced by a clear area about the colonies which fails to become clouded upon flooding the plate with 25 per cent acetic acid. The results of these tests are recorded in Table II.

From an inspection of this table it is seen that there is no relation between ability to digest tissue and liquefaction of gelatin. This of course applies to the method and strains used. Ability to digest bovine or human tissue does not imply ability to liquefy coagulated bovine serum. Ability to hydrolyze casein is not paralleled by ability to digest tissue, serum or gelatin. The enzyme which digests tissues appears therefore to be distinct from other proteolytic enzymes produced by streptococci.

Nature of the Enzyme.

Ten cultures of hemolytic streptococci in cooked meat medium, all of about the same age (1 month) were selected. Five of these showed considerable digestion. Two were doubtful, the meat level being only about 1 mm. below the line, while the remaining three were distinctly negative. Two were cultures which had shown proteolytic activity only after removal of the vaseline.

Being careful to avoid stirring up the meat, 1 cc. of the clear supernatant fluid was removed from each tube and formol titrations made by Brown's modification (14) of Sörensen's method. This modification is particularly adapted for use with bacteriological media. Table III shows the results of the titrations. It is to be seen in Table III that the cultures showing extensive digestion after 5 days growth at 37°C. also contain large amounts of substances determinable by the formol titration. The extent of the digestion roughly parallels the increase in such substances. The doubtful cultures as well as the

frankly negative ones show little increase in these bodies, the doubtfuls containing little more than the negatives. These results seem to show that the enzyme resembles trypsin in its action.

Further Studies.

To date about 58 strains of hemolytic streptococci have been studied. These represent a variety of sources, bovine, human and otherwise. At present it is sufficient to state that about 30 of them

TABLE III.

Formol Titration of the Supernatant Fluid in Tubes of Cooked Meat Medium Containing Cultures of Various Hemolytic Streptococci.

Strain No.	Approximate per cent of meat digested in 5 days at 37°C.	Quantity of N/20 NaOH needed for formol titration of 1 cc. of the culture
Sterile No. 1	—	0.25
Sterile No. 2	—	0.12
8616	30	4.23
3052*	15	0.55
8741	25	4.85
X 40	25	4.13
8576	5?	0.47
8591	5?	0.41
Lanc.	0	0.40
2082*	12	0.57
4d ign.	0	0.31
S. H.	0	0.40

* Digested meat only after removal of the vaseline seal.

digest meat vigorously. There seems to be no relation between this power and source, pathogenicity or fermentative reactions. A more detailed report of this will be made later.

DISCUSSION.

Proteolysis by streptococci has been observed before. Casease has been frequently described both in culture filtrates and in extracts of washed cells (5, 6). The presence of peptase is well known to be of common occurrence. Prevot (7) has described certain types of

strictly anaerobic streptococci which are capable of breaking down tissue proteins. These organisms produce gas and foul odor and the process appears to be quite unlike that described in this paper. MacCallum and Hastings (8) described a type of proteolytic streptococcus which they named *M. zymogenes*. This organism was obtained from a case of acute endocarditis. It liquefied gelatin, digested coagulated blood serum and peptonized milk. The proteolytic enzymes were found to be active in sterile filtrates. This organism differed from the typical *S. pyogenes* in being practically always arranged in pairs, in its extremely small size and in being able to survive for 3 or 4 months at room temperature upon agar slants in spite of their dried condition. The appearance upon blood agar was not described.

The digestion of tissues, as described in this paper, by ordinary types of hemolytic streptococci seems to be a new observation. The value of such a readily demonstrable characteristic, when exhibited by a considerable percentage of strains lies in its possible use as a means of classification or identification. The tendency in the past has been to rely almost wholly upon the appearance upon blood agar and fermentation reactions. It is only comparatively lately that the use of other cultural characters has become extensive. Among these characters may be mentioned the hydrolysis of sodium hippurate (9) and growth at various surface tensions (10, 11). In view of the unsatisfactory situation existing in connection with identification and classification of the streptococci, it would seem desirable to make more extensive use of such simple tests as hydrolysis of casein, digestion of animal tissues, etc., for these purposes.

SUMMARY AND CONCLUSIONS.

1. An extracellular, proteolytic enzyme has been observed in more than 30 strains of beta type, aerobic and facultative hemolytic streptococci.

2. The enzyme is readily demonstrable in sterile filtrates of cooked meat cultures.

3. No gas or foul odor is produced.

4. It is partially inactivated by exposure to about 60°C. for 45 minutes or longer.

5. The enzyme manifests itself in cooked meat cultures after about 48 hours incubation at 37°C. The sterile filtrate from a 10 day old culture acts within 18 hours.

6. From 50 to 75 per cent of the meat in a tube of cooked meat medium may be digested in about 3 weeks at room temperature after 5 days initial growth at 37°C.

7. No correlation is found, in the cases studied, between hemolysis and proteolysis.

8. Streptococci not digesting beef tissue will not digest human tissue, and those which do digest beef tissue also digest human tissue. This conclusion applies only to the nine strains studied.

9. Ability to digest animal tissues does not necessarily imply ability to digest casein, coagulated beef serum or gelatin.

10. The disappearance of the meat from cooked meat cultures of hemolytic streptococci is quantitatively roughly paralleled by increase of formol-titrable substances in the fluid portion of the medium.

11. The enzyme resembles trypsin in its action. Streptococci from a variety of sources, bovine, human and otherwise have shown varying degrees of proteolytic activity.

12. The name histase is proposed for this enzyme.

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