STUDIES ON THE CULTURAL CHARACTERISTICS OF PASTEURELLA TULARENSE

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There has been relatively little work published on the cultural characteristics of Pasteurella tularense. Previous articles have been concerned mainly with the description of media favorable to its growth. McCoy and Chapin (1912) first isolated the organism on coagulated egg medium and Francis in 1922 reported that the addition of cystine to blood or serum agar stimulated the growth of the organism. Francis, using glucose, mannose, levulose and glycerol which were added to cystine agar slants containing brom-thymol-blue as an indicator found that the three or four strains tested by him fermented these carbohydrates. Shaw (1930) however, reports that two human strains isolated by him show no fermentation of glucose.

In view of these somewhat meager reports and in connection with a study of the fermentation of the Pasteurella group as a whole the following studies were undertaken.

The organisms used were collected from various sources. The origin of the different strains is as follows: isolated from human cases, seven; rabbits, four; ground squirrels, one; wood ticks, three; field mice, one; fox, two; grouse, one; muskrat, one; quail, one; twenty-one in all.

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2 Personal communication.
3 My thanks are extended to Dr. R. G. Green of the University of Minnesota and to Dr. Edward R. Francis of the National Institute of Health for their kindness in supplying a number of these strains.
FERMENTATION REACTIONS

The basic medium was meat-extract broth, 2 per cent peptone and 1.5 per cent agar to which cystine hydrochloride was added to make 0.01 per cent. The reaction was adjusted to pH 7.4, and enough phenol-red indicator was added to give a distinct salmon pink shade. The carbohydrates were added to make a final concentration of 1 per cent. The medium was then sterilized in the autoclave and before tubing 5 per cent of horse serum was added, and the tubes slanted. Arabinose, xylose, rhamnose; glucose, galactose, mannose, levulose; lactose, maltose, sucrose; melizitose, raffinose; dextrin; mannitol, glycerol, sorbitol, dulcitol; salicin were the carbohydrates used. The media were inoculated with the 21 strains of Pasteurella tularense, incubated at 37°C and observed daily. In all cases good growth occurred on the slant. Glucose and glycerol were fermented by all the strains, mannose was fermented by all but two; levulose by all but five; maltose by all but one. The other carbohydrates were not fermented. Francis who used three or four strains of Pasteurella tularense found that all of them fermented glucose, mannose, levulose and glycerol. He apparently did not use maltose. It is apparent that our results agree closely with those of Francis except for the few strains which were negative in mannose and levulose. These negative strains were carefully checked but always failed to give fermentation although they grew well. It will be noted that all of our strains fermented glucose as did those of Francis whereas Shaw reports that his two human strains did not.

An interesting aspect of these fermentation tests was the increase in alkalinity in all media in which the carbohydrate was not fermented. The color of the phenol red in these tubes showed a deep pink after the fourth day and reached a reaction of approximately pH 8.0 to 8.4 after the seventh day. The tubes showing fermentation reached a pH of 6.4 to 6.6 on the seventh to ninth day and then slowly decreased in acidity until at the seventeenth to the twenty-third day they also showed a reaction of pH 8.0 to 8.4.

Bond and Downs in a study of the Pasteurella group used the
above carbohydrates but were not able to group the organisms according to their fermentation reaction nor to find that the fermentation reactions of P. tularense showed any close resemblance to those of the other members of the group.

HYDROGEN SULPHIDE PRODUCTION BY PASTEURELLA TULARENSE

Because of the stimulative effect of cystine on the growth of P. tularense it was thought probable that it might utilize this sulphur compound with the production of hydrogen sulphide. For this study a basic slant medium of semi-solid, glucose, 2 per cent Bacto-peptone, meat-extract agar containing cystine or cystine hydrochloride was used. Ferrous sulphate was added as an indicator in some series, in others a strip of moistened lead acetate paper was introduced into the tube after seven days incubation and the tubes tightly stoppered. Sixteen strains were tested, all of which gave positive tests for hydrogen sulphide. A series of tests was then made to find out if the cystine was the source of the hydrogen sulphide. For these tests the basic medium was glucose meat-extract agar to which was added the following:

1. Basic medium plus 5 per cent rabbit blood
2. Basic medium plus 2 per cent peptone
3. Basic medium plus blood and peptone
4. Basic medium plus sodium thiosulphate
5. Basic medium plus sodium thiosulphate plus blood
6. Basic medium plus sodium thiosulphate plus peptone
7. Basic medium plus sodium thiosulphate plus peptone and blood

There was no hydrogen sulphide production on any of these media although there was fairly good growth. These results would suggest that cystine was the source of the hydrogen sulphide. Although there is cystine present both in the peptone and blood the use of additional cystine stimulates growth and the added cystine is apparently largely broken down into \( \text{H}_2\text{S} \). Almy and James (1926) using Proteus vulgaris inoculated into 100 cc. of a peptone medium to which 0.01 gram of cystine was added recovered the theoretical amount of \( \text{H}_2\text{S} \) to be expected from the breakdown of 0.01 gram of cystine. It is a common experience to be able to demonstrate \( \text{H}_2\text{S} \) production by Escher-
ichia coli in a medium to which cystine is added, whereas in the ordinary peptone medium it is not detectable with lead acetate paper or a ferrous sulphate indicator. Almy and James (1926), Tanner (1917), Sasaki and Otsuka (1912) have all shown cystine to be readily available as a source of H₂S while Burrows (1934) Scheff and Scheff (1934) and others have shown that cystine has a stimulative effect on growth. Cultures of P. tularense in a cystine medium evidently show both of these effects. It is evident that P. tularense can not utilize sodium thiosulphate as a source of hydrogen sulphide as many organisms do. Treece⁴ has shown this substance to be an excellent source of hydrogen sulphide in the coli and Salmonella groups. Tarr (1933) has shown that whereas Proteus vulgaris produces hydrogen sulphide from sodium thiosulphate, Erythrobacillus prodigiosus can not form it from this source. Both organisms however produce hydrogen sulphide from cystine.

GROWTH ON MEDIA CONTAINING VARIOUS CHEMICAL COMPOUNDS

Because of the marked stimulative effect of cystine on the growth of P. tularense it was decided to try out a series of sulphur compounds to see if any of them could be used as a substitute for cystine. Francis (1923) reports on a series of amino acids, among them cystine and tryptophane, and also on inorganic sulphur compounds which were added to agar used for the inoculation of P. tularense. He found that only cystine or cystine hydrochloride supported growth. The following sulphur compounds failed to support growth: sodium sulphite, sodium hydrogen sulphite, sodium thiosulphate, ammonium sulphate, magnesium sulphate, potassium sulphate, resublimed and precipitated sulphur. The possible inhibitive effect of these substances was not tested for.

In our series of tests the same compounds were used and in addition: urea, thio-urea, ferric ammonium citrate, ferrous sulphate and sodium nitrate and nitrite. These were added to make a final concentration of 0.1 per cent to each of two series of tubes. In one series cystine was added. Five per cent horse serum and

⁴ E. L. Treece, personal communication.
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1 per cent glucose was added to all of the media. There was slight growth on all of the media containing no cystine but there was nothing to indicate that any of the substances used had a stimulative effect similar to that of cystine. The tubes containing the sulphur compounds and other salts plus cystine showed the same abundant growth seen in the control tubes of glucose, cystine serum agar. These compounds therefore showed no inhibitive effect on the growth of P. tularense.

GROWTH ON SYNTHETIC AND SEMISYNTHETIC MEDIA

A study of the cultural characteristics of P. tularense is rather difficult because of its growth requirements. It will not grow on liquid media and it seems to require blood or serum, in addition to cystine to give abundant growth. Scott and Brandly (1933) have reported the use of a synthetic medium which seems to give abundant growth with a large number of different species of bacteria. Accordingly it was considered desirable to see if P. tularense would grow on such a relatively simple medium. No growth was obtained on this medium. However, when 5 per cent horse serum or rabbit blood was added a fair but not an abundant growth occurred.

The further addition of glucose to this medium had a marked stimulative effect on growth. The synthetic medium with the addition of cystine but without serum or blood showed slight but unmistakable growth. This medium containing cystine and blood or serum seemed to be as favorable for growth as the usual meat extract agar. However, it is in this case scarcely more simple than the usual medium.

SUMMARY AND CONCLUSIONS

1. Twenty-one strains of Pasteurella tularense were found to ferment glucose and glycerol. Most of the strains also fermented mannose, levulose and maltose. All strains failed to ferment galactose, lactose, sucrose; raffinose, melizitose, arabinose, rhamsone, xylose; mannitol, dulcitol, sorbitol; salicin.

2. P. tularense produces an alkaline reaction in media not containing a utilizable carbohydrate; but, in the presence
of a utilizable carbohydrate it produces an initial acidity followed by an alkaline reversion.

3. *P. tularense* produces hydrogen sulphide in media containing cystine.

4. *P. tularense* is apparently unable to produce hydrogen sulphide in detectable amounts from media containing peptone and blood or serum.

5. *P. tularense* does not produce H$_2$S in detectable amounts from sodium thiosulphate.

6. The addition of a number of sulphur-containing compounds failed to stimulate the growth of *P. tularense* but in the presence of these compounds there was no inhibition of the usual stimulative effect of cystine.

7. Scott and Brandly’s synthetic medium with the addition of blood or serum supports the growth of *P. tularense*.

REFERENCES

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