STUDIES ON *PASTEURELLA MULTOCIDA*

III. CAPSULE STAIN OF *PAST. MULTOCIDA*

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In 1897, PREISZ¹⁾ found a "Hülle" in *Bacillus suisepiticus*, which was unable to be stained by dye solution, but GÓZONY²⁾ began to recommend the use of India Ink preparation to demonstrate the "Hülle" of pasteurella organism in 1913. In 1928, HOFFENREICH³⁾ had extracted a capsular substance of polysaccharide from *Past. aviseptica* while he has carrying out immunity experiments. PRIESTLEY⁴⁾ in 1936, demonstrated an envelopelike structure in the culture of a virulent strain. Two years later, REGAMEY⁵⁾ also found the similar structure of pasteurella organism in the leucocytes of tissue smears. Recently, CARTER^{6,7,8)} classified *Past. multocida* into four types (A, B, C, D) while ROBERTS⁹⁾ classified four types I, II, III and IV, all based on the specific properties of capsular substances.

As mentioned above, there is no question that *Past. multocida* possesses a capsule, but several terms such as capsular substance, capsular structure, and envelopelike structure were used to describe this special structure. So I suspect that there is not any proper stain method other than India Ink to demonstrate very distinctly, the existence of the capsule.

In 1934, I reported a modified method of Benians' spirochetes stain¹⁰⁾ to apply for staining the capsule of *Str. equi*.¹¹⁾ As the demonstration of the capsule is not only necessary for differentiating the pasteurella organisms but is also very important for studying the antigenic structures and virulence of this organism, I began to make an experiment on staining the capsule of pasteurella organisms and found that the stain method applied to the organism of strangle was also able to demonstrate the pasteurella organisms. I hereby introduce the results as follows.

STAINING METHOD

Put 1 to 2 loops of 2% Congo red solution on a clean slide glass, into this mix a small amount of the egg agar culture and smear it over a surface about the size of 1.5 to 2.0 cm in diameter. The smear is allowed to dry in the air and is then fixed by 2% HCl alcoholic solution, after the drying of the smear is complete, the preparation is then stained with 1% crystal violet solution and allowed to stand for 1 to 3 minutes in room temperature.

REAGENTS

0.1 gm of Congo red dye powder was dissolved in 5 cc of distilled water to make a 2% solution and then placed in hot water for a short time so that the color could change to dark red, then it was filtrated for use. This solution shows precipitation in long preservation so it is better to prepare a fresh solution when a clear film is wanted. 1% solution is also useful but a 0.5% solution is not ideal.

1 gm of crystal violet was dissolved in the 100 cc of distilled water to make a 1% solution. In order to avoid the formation of crystals in the stain film, it should be filtered

before use. Other dyes, such as gentian violet, methyl violet and Wright's stain were also useful. Instead of 2% HCl alcoholic solution, 1% and 0.5% solutions were also useful but the Congo red could not be fixed if passed through the flame.

RESULT

By this method, the cell of the bacteria were stained purple and the capsule pink, however the film revealed blue, green and purple colors. The capsule was found larger in a thin layer on Congo red.

When using a loop of distilled water suspension of the culture, to mix with 1 loop of Congo red solution, the capsule stained very distinctly; although the capsule remained transparent or unstained. The cell was not stained after being fixed by a 2% HCl alcoholic solution, but it could be stained by crystal violet solution. Instead of crystal violet, Wright's stain stained the cell of the organisms more deeply.

For the purpose of the demonstration of the capsule, it is best to use egg agar culture. Contrary to this, the organism grown on blood or common agar had a small and thin capsule or none at all.

Those organisms grown on the egg agar, containing 2% NaCl, did not show their capsule. This stain method is not applicable for staining broth culture.

After preparing a suspension of culture in saline, and boiling it on the flame, the capsule of pasteurilla was lost very quickly, although it was not affected in the same suspension which had been treated with N/20 NaOH and 2% HCl alcoholic solution at room temperature. Untreated suspension lost about half of their capsule after being placed at room temperature over night.

CONCLUSION

The results of applying the formerly reported capsule stain method for staining pasteurilla organisms are as follows:

(1) *Past. multocida* grown on egg agar is mixed with 2% Congo red solution on a glass slide and it is then smeared over the surface, about the size of 1 to 2 cm in diameter, then dried and fixed with 2% HCl alcoholic solution. Then let it dry without washing, and stain with 1% crystal violet solution, after which allow it to stand for 1 to 3 minutes at room temperature. By this method, the cell of the bacteria was stained purple and the capsule pink, however the film revealed blue, green and purple color.

(2) A suspension made from egg agar culture loses the capsules after being boiled on the flame.

(3) It was hard to stain those organisms grown on the egg agar containing 2% NaCl.

(4) It was hard to stain the capsule of the organisms grown in broth culture.

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Pasteurella multocida に関する研究III. *Past. multocida* のカプセル染色

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余は *Past. multocida* の莢膜染色につき研究を行った結果次の所見を得た。

1) *Past. multocida* の鶏卵寒天 (Egg agar) 培養を 2% コンゴレッド水溶液と共に載物硝子上にて混和し直径 1.5-2.0 cm の円形大に塗抹、乾燥後 2% 塩酸アルコール溶液を滴下固定し充分乾燥せしめた後、クリスタル紫、メチル紫、ゲンチャナ紫等の 1% 水溶液またはライト氏液を載せ室温で 1-3 分

間染色する時はカプセルはピンク色に菌体は紫黒色に染まる。

2) 2% 食塩を加へた鶏卵寒天に培養した *Past. multocida* のカプセル染色は困難である。

3) 細菌の浮游液を火焰で煮沸すれば、1-2 分でカプセルが消失する。

4) ブイヨン培養からのカプセル染色は困難である。

EXPLANATION OF PLATE

Figs. 1-4 show transparent capsule of *Past. multocida*. Bacteria were taken from distilled water suspension of egg agar culture, and stained by 1% crystal violet solution.

Fig. 1

Fig. 2

Fig. 3

Fig. 4