THE PROTECTIVE VALUE OF AN AUTOGENOUS BACTERIN AND TOXOID AGAINST EXPERIMENTAL CORYNEBACTERIUM PYOGENES INFECTION IN MICE

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SUMMARY

Bacteria and toxin from a broth culture of a porcine strain of Corynebacterium pyogenes were lethal to white Swiss mice within 24th of inoculation. The pyogenic factor was shown to be a component of the bacterial cells and not of the toxin. Mouse-protection tests using a formalised bacterin and formalized and heat-inactivated toxoid revealed that only the formalized toxoid conferred protection to experimental challenge. It is suggested that this toxoid may be of value in protecting animals against the natural infection.

INTRODUCTION

The role of Corynebacterium pyogenes in livestock diseases has been reviewed by several workers including McCracken and McCaughey (1973), Ueda et al. (1972), Smith et al. (1971), Suzuki et al. (1973), Smith (1966), Smith and Reynolds (1911) and Collier (1956). Several attempts have been made to control infections with this organism, particularly those occurring as outbreaks. Such methods include antibiotic therapy, Surgery, segregation and slaughter of badly affected animals and improved hygiene.

Corner et al. (1965), found the use of an autogenous bacterin consisting of a killed suspension of hemolytin streptococci 75%, and staphylococci 25%, and a commercial bacterin* consisting of pyogenic streptococci 50%, C. pyogenes 30%, Staphylococcus aureus 10% and Escherichia coli 10% in the control of swine abscesses caused by these organisms was of value. Oladosu and Felade (1976), investigated an outbreak of multiple abscesses involving a hard of 350 Duroc pigs at Ikorodu Farm Settlement, Lagos State of Nigeria involving C. pyogenes. This paper presents the findings of a preliminary attempt to assess the protective value of a C. pyogenes bacterin, heat-inactivated toxoid and formalized toxoid prepared from this porcine strain in experimentally infected white Swiss mice.

MATERIALS AND METHODS

Preparation of Toxoid

A 24th broth culture of the C. pyogenes strain was centrifuged and the supernatant recentrifuged and checked for sterility by plating on blood agar. An aliquot of this supernatant was washed twice in 0.85% saline and was heated at 65°C for 1hr. (heat-inactivated toxoid). To the remainder, an equal volume of 0.3% formalin in phosphate buffered saline, pH 7.4, was added (formalized toxoid).

Preparation of Bacterin

The bacterial deposit from above was washed twice in 0.85% saline and a viable count performed by the method of miles and Misra (1938) giving a result of 1.4 x 10^7 bacteria per ml. The bacterial suspension was then treated with an equal volume of 0.3% formalin in phosphate buffered saline, pH 7.4 (bacterin). The bacterin was checked for sterility.

To determine the challenge dose, graded doses of bacteria and toxin 0.1, 0.2, 0.3, 0.4, and 0.5ml were inoculated intraperitoneally into two groups of mice; that mice died within 24th. However, ten-fold dilutions of 0.1ml amount of both toxin and bacteria produced no death of mice. It was therefore decided to use 0.1 ml as challenge done for toxin and bacterial suspension throughout the investigation.

Five groups of mice weighing between 10.15g were immunized intraperitoneally as follows:-

*N Mixed Bacterin (Bovine) Formula No. 2
Pitman-Moore Co., Indianapolis Ind.

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Group A: 6 mice each immunized with 0.1 ml of bacterin.
Group B: 6 mice each immunized with 0.1ml of formolized toxoid.
Group C: 6 mice each immunized with 0.1ml of heat-inactivated toxoid.
Group D: 6 mice (unimmunized)
Group E: 6 mice (unimmunized).

The mice were kept under observation until the 11th day when they were challenged by the intraperitoneal route. Three mice in groups A, B, C, and D each received 0.1 ml of the bacterial suspension, and the remaining three in these groups each received same amount of toxin. All the six mice in group E were left as the unchallenged control. Dead mice in the respective groups were examined for post-mortem lesions at intervals. Surviving mice were later challenged on the 20th day with 0.2ml of bacterial suspension and toxin as shown in Table 1. On the 40th day survivors were killed with chloroform and examined for post-mortem lesions.

RESULTS

All unimmunized mice in Group D died within 24th of inoculation. Post-mortem examination revealed pulmonary, hepatic and splenic congestion and hemorrhages, intercostal acclamosis, and hypertrophy of the liver and adrenal glands. The observations on the immunized mice are summarized in Table 1.

Post-mortem findings on the 21st Day in the toxin-challenged mice were mainly those of tomaemia and in the bacteria-challenged group, multiple abscesses were seen in the lung, kidneys and peritoneum, with marked congestion of

<table>
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<th>Group</th>
<th>Day 0 Vaccination No.</th>
<th>Day 1 (One) Survival</th>
<th>Day 11</th>
<th>Day 12 Survival*</th>
<th>Day 19 Survival</th>
<th>Day 20</th>
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Day 12 Survival, Day 26 Survival, Day 30 Survival, Day 40 Survival

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CHALLENGE (FIRST DOSE)

CHALLENGE (DOUBLE DOSE)

* Group A  
  a = bacteria immunized challenged with bacteria
  b = bacteria immunized challenged with toxin
  c = Total survival

Groups B, C
  a = Toxoid immunized challenged with toxin
  b = Toxoid immunised challenged with bacteria.
the liver and spleen. All surviving mice killed on the 40th day had no significant postmortem findings with the exception of one mouse in Group A which had peritoneal abscesses yielding a pure culture of *C. pyogenes*.

**DISCUSSION**

Both the bacterial suspension and the centrifuged toxin were highly lethal for mice. The fact that abscesses were observed in the bacteria-challenged groups of mice only, indicated that the pyogenic factor was associated with the bacterial cells and not the toxin. Similar observations were made by Lovell, Foggie and Pearson (1950).

Based on the number of surviving mice on the 40th day, the formolized toxoid conferred a greater degree of immunity than the bacterin. The heat-inactivated toxoid was of no value in protecting mice against experimental challenge with either bacteria or toxin and, whereas the bacterin protected mice against toxin challenged, the formolized toxoid was relatively ineffective in protecting mice challenged with the bacterial suspension. An attempt is being made to assess the efficacy of the formolized toxoid in Duroc breeds of pigs.

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**REFERENCES**


