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List of abbreviations

| | |
|---------------------|--|
| BoNT | Botulinum neurotoxin |
| CNS | Central Nervous System |
| ELISA | Enzyme Linked Immunosorbent Assay |
| FPLC | Fast Protein Liquid Chromatography |
| GLn-Lys bond | Glycine – Lysine bond |
| HPLC | High performance Liquid Chromatography |
| kDa | Kilo Dalton |
| MLD | Minimal Lethal Dose |
| TeNT | Tetanus Neurotoxin |
| TIG | Human anti-tetanus immunoglobulin |
| VAMP | Vesicle associated membrane protein |



6. Summary

Recent studies on toxins and serum of *Clostridium tetani*

Tetanus disease is one of the most dramatic and globally prevalent disease of humans and vertebrate animals. The global fatality rate of tetanus has been estimated as 30-50%. Fortunately, it is successfully controlled through immunization with tetanus toxoid. Hyperimmune serum obtained from sheep or horse confers effective protection in unimmunized animals and humans.

The results of the experiments included in the present study showed that:

- 1- Tetanus toxin was produced in a modified **Mueller and Miller (1954)**, media and gave a titer of 70 Lf/ml.

- 2- Toxin was purified from culture filtrate by ammonium sulphate and produce an Lf content of 2000 Lf/ml, protein content of 16.4 mg/ml and 2.624 mg protein Nitrogen per ml.

- 3- Purified tetanus toxin was further purified by chromatography on sephadex G-100 and yield one peak.

- 4- purified toxin was digested by papain enzyme and then applied to chromatography on sephadex G-100 to separate the yield of digestion.



5- Fragment C was inoculated in mice to assess its toxicity and was atoxic even in large amounts.

6- Antitetanic serum was fractionated to yield IgG by two method; ammonium sulphate and caprylic acid.

7- Caprylic acid was used in different concentrations and the best concentration was 5% which produce good yield and good purity.

8- Caprylic acid was used in different pH values and the best pH was 5.8

9- F(ab)₂ was produced by pepsin alone and by pepsin and caprylic acid and resulted in that the best pH was 3.25 and that pepsin alone give better yield and better purity.

10- F(ab) was produced by limited papain digestion for different time and was found that 6 hours give a good balance between yield and purity.

11- IgG, F(ab)₂ or F(ab) were compared for their protective capacity against 4 MLD of tetanus toxin and it was found that F(ab) give the best protection.

From the above mentioned study we conclude the following:-

- Tetanus toxin can be digested with papain enzyme and chromatographed to yield fragment C.
- Fragment C with a molecular weight of approximately 48 kDa is atoxic when injected in mice .



-IgG is best produced from antitetanic horse serum by caprylic acid at 5% concentration and at pH 5.8.

- Pepsin enzyme produce F(ab)₂ in good yield ;270 IU at pH 3.25.

- Twenty five units of F(ab) give 100% protection to mice injected with 4 MLD of tetanus toxin, while IgG and F(ab)₂ produce 75% protection at the same dose.