Chapter IV

Conclusion

In this experiment, tetanus toxoid microcapsules were prepared. Lecithin acted as the microcapsules wall and carboxymethyl chitin acted as the stabilizer. The size of microcapsules were varied in diameters. They could be separated by three speeds of centrifugation 2,000, 5,000, and 12,000 rpm. Then TTMA, TTMB and TTMC were obtained respectively.

The results from physical testing with particle size analysis were; the mean diameter was 5.07 micrometers in TTMA , 3.70 micrometers in TTMB and 2.94 micrometers in TTMC . The median was 4.2 micrometers in TTMA,3.25 micrometers in TTMB, and 2.89 micrometers in TTMC. The mode was in range 2.1-5.0 micrometers with 40 % frequency in TTMA, 50.49 % frequency in TTMB, and 53.32 % frequency in TTMC. According to these results, the largest microcapsules was TTMA, the second was TTMB and the smallest was TTMC .

As in potency testing of adsorbed tetanus toxoid and tetanus toxoid microcapsules, the results were that; TT could survived the mice during day 7-90 ;TTMA could that during day 30-180 but; TTMB could that during day 15 to 180 ; and TTMC could that during day 15-120. Although TTMA and TTMB could survive the mice until 180 days but the effeciency is difference . TTMB is the most effective in conparison with TTMA and TTMC.

According to the determination in antibody levels in mice which immunized with TT, TTMA, TTMB, TTMC, and the mixture preparations;

TT+TTMA, TT+TTMB, and TT+TTMC. the results were corresponded to the potency testing. Adsorbed tetanus toxoid had the shortest onset , the antibody was first found at day 3 and increased to the protective levels at day 30 . The high antibody level was persisted until day 75. TTMA had a longer onset than TT, first found the antibody response at day 15, and increased to the protective levels at day 75 and persisted until day 120. In TTMB, the antibody response was first found at day 7, increased to the protective levels at day 30 and persisted until day 180 . And in TTMC, the antibody response was first found at day 3, then increased to the protective levels at day 30 and persisted until day 60.

Therefore, in in attempt to produce the most effective preparation, the mixture of TT and TTMA, TT and TTMB , TT and TTMC at ratio 1:1 were prepared. It was found that TT+TTMB was the best one; the onset was the shortest (the antibody was first found at day 30). The duration was the longest , it persisted more than 180 days.The second one was TT+TTMA and the third one was TT+TTMC.

It was noticed that the microcapsules in the mixture of adsorbed tetanus toxoid and tetanus toxoid microcapsules could potentiate the immune response of tetanus toxoid that caused the antibody level in the mixture of adsorbed tetanus toxoid and tetanus toxoid microcapsules was dominantly higher than adsorbed tetanus toxoid alone.

In antibody or immunoglobulin production, immunoglobulin M (IgM) was the main immunoglobulin produced early in the primary response of tetanus toxoid immunization. Next to Ig M , immunoglobulin G (Ig G) was following produced. Ig M levels tend to decline earlier than Ig G. When adsorbed tetanus toxoid in the microcapsules diffused to circulation,

it acted as the second antigen. So a rapid antibody response to higher level than the primary immune response was produced. This is attribute to the antigen sensitive "memory cells" after the first contact with non-encapsulated tetanus toxoid. In this period amount of Ig M produced is similar to that after the first contact with adsorbed tetanus toxoid alone and a much larger amount of Ig G is produced and the level tend to persist much longer than in the primary response.

In the consideration of the correlation between the quantity of survived mice in potency testing and the antibody level in serum, it was found that they had a great correlation. Such as , TT+TTMA, the antibody level was at the higest during day 30-120 and most of the mice were survived during day 30-120.

Therefore the antibody levels or titers that could protected the mice should increase to 2.0 unit/ml in the early period and decrease to not lower than 1.1 or 1.2 unit/ml. If the level was lower than this, the mice could not save furthermore.

Other than this research, there are many problems for further study. Such as, in the precipitation of traces of lecithin, 1000 rpm centrifugation may be chosen instead of 2000 rpm centrifugation. According to this, the size of microcapsules obtained from 5000 rpm may larger and the immunizing activity may be longer. And the very small amount of the precipitates obtained from 12000 rpm centrifugation that could protect the mice in a pattern similar to non-encapsulated tetanus toxoid may need not to collect.