5. SUMMARY
1) Immunodiffusion studies showed that the haemolymph of *Micrococcus luteus* induced third instar larvae of *Musca domestica* formed distinct precipitin line against bacterial antigens obtained by treating bacteria with 50 mM EDTA and 1% SDS. The *M. luteus* induced immune serum of *M. domestica* larvae produced precipitin lines with antigens of *Escherichia coli* and *Bacillus thuringiensis* as well. Haemolymph obtained from non-induced larvae did not form the precipitin line.

2) Immune serum of *M. domestica* larvae was shown to be sensitive to trypsin treatment and thermostable. Appropriate experiments suggested that the antibacterial substance of immune haemolymph could bind with bacterial cell wall component.

3) Immunoelectrophoretic studies using bacterial antigens and immune serum of *M. domestica* larvae revealed a single precipitin arc towards the anodic end of the immunoelectropherogram.

4) When control and immunized haemolymph were subjected to SDS-PAGE approximately 41 subunit proteins, with molecular weight ranging from 14-120 KD were resolved. Densitometer scan of control and 12h inoculated haemolymph showed an increase in peak height and area of low molecular weight protein subunits ranging from 15-20 KD.

5) 2 µl of cell free immune serum when placed in wells punched on thin agar seeded with *M. luteus*, produced a bacteria free zone around the well, indicating antibacterial activity of immunized haemolymph. Such activity was absent in control or in haemolymph obtained from non-immunized larvae. Aseptic injury to larvae can also induce antibacterial activity. Antibacterial activity is concentration dependent and use of serially diluted immune haemolymph results inhibition zones that are proportionately lesser in diameter.
6) Haemolymph of induced pupae exhibited antibacterial activity a day after vaccination. Immune haemolymph when subjected to heat treatment at 100°C for 5 min in ammonium acetate-acetic acid buffer pH 3.6 retained the antibacterial activity, indicating that substance is acid and thermostable.

7) Antibacterial activity could be demonstrated in liquid cultures of *M. luteus* by including the immune haemolymph as a part of LB medium, whereas normal smooth curve could be obtained in medium devoid of immune haemolymph. There was an extensive lysis of the bacteria in cultures that included immune haemolymph as revealed by OD measurements. *E. coli, Pseudomonas aeruginosa* and *B. thuringiensis* cultures were also sensitive to *M. luteus* induced immune haemolymph, but *S. aureus* appeared to be resistant.

8) Assays to estimate the bactericidal activity in immune haemolymph in terms of viable count of colony forming units showed that the bactericidal substance reduced the viable cells from $4 \times 10^6$ to around 90 colony forming units. Bacteria kept in immune haemolymph containing phosphate buffer, when assayed for viability did not show any reduction in number, suggesting that in non-growing conditions, the antibacterial substance did not cause the lysis of bacteria.

9) The immunized haemolymph when subjected to acidic PAGE followed by antibacterial assay showed two zones of inhibition on the gel; a relatively small zone close to the origin indicating the involvement of high molecular weight slow moving attacin-like fraction and another a larger zone caused by a fast moving, relatively low molecular weight cecropin-like fraction.
10) Induction kinetic studies suggested that the antibacterial activity appeared in the haemolymph as early as 3h after inoculation and gradually increased between 5h and 18h as revealed by larger diameter of inhibition zone. By 24h the activity reached a maximum and persisted until two days after pupation.

11) The purification regime of the cationic, thermostable and low molecular weight multicomponent antibacterial substances present in the immune haemolymph included cation exchange chromatography on CM-cellulose followed by heat treatment, gel permeation chromatography on Sephadex G-50 column and further purification in CM-cellulose and hydroxyapatite cation exchange columns.

12) The eluate from CM-cellulose column containing thermostable proteins of immune haemolymph resolved itself into 3 peaks when passed through a Sephadex G-50 column. The fractions of all the 3 peaks exhibited antibacterial activity, although the fractions of third peak exhibited a weak activity. Peak II, termed muscinII, that exhibited high antibacterial activity was further purified in cation exchange columns.

13) The purification of MII, resulted in two fractions, MIIA and MIIB. MIIB, because of its strong antibacterial activity was further purified on the hydroxyapatite column and a single homogenous peak was obtained.

14) At the end of these purification procedures approximately 0.09 mg of pure MIIB with a biological activity of 31,000 units/mg was obtained and there was a 34,000 fold increase in the specific activity of final purified product.
15) MIIB is thermostable, sensitive to trypsin treatment and has a molecular weight of about 7690 daltons as determined by Sephadex G-50 gel filtration chromatography. Phase contrast microscopy revealed that there was a pronounced reduction in number of bacteria treated with MIIB.

16) MIIB has a broad antibacterial spectrum and is active against a number of Gram positive and Gram negative bacteria. *M. luteus, P. aeruginosa, B. thuringiensis* and *B. subtilis* are highly sensitive to MIIB and *Staphylococcus aureus, Enterobacter cloacae* and *E. coli* from human source appear to be least sensitive. *E. coli* K12 strain, *B. megaterium* and *B. polymyxa* exhibited intermediate sensitivity. These results are based on minimal inhibitory concentrations required to lyse 50% of bacteria in culture.