SUMMARY

The alkaline protease activity in the gut of *Spodoptera litura* was found to increase with the development of larvae and decreased with the onset of pupation. The pattern of changes in protein concentration in the gut was parallel to the protease activity. During day 7 to day 9, the larvae became more voracious feeder which presumably accounts for the enhanced protein concentration in the gut. The protein concentration falls after day 9 as the larvae gradually become pupae and give up food consumption.

The change in protease activity during starvation of the larvae showed an abrupt increase at 4 hours which decline consistently on further starvation. There was a slight decrease in protein concentration of gut content at 4 hours with a slower decline on further starvation.

Incubation of crude enzyme solution at 37 ± 1°C for 55 hours resulted in a sharp decrease in protein concentration until 22 hours without any further significant change until 55 hours. The enzyme activity remained almost unaltered till 22 hours but decreased subsequently till 55 hours. A 42% loss in enzyme activity was observed when the crude enzyme solution was dialysed at 37°C, whereas only 10% loss in activity was observed at 4°C.
To purify the alkaline proteases, the fifth instar larvae of *Spodoptera litura* were dissected and their intestines collected in an ice cold beaker. The contents were squashed out by means of a glass rod in 0.1M Tris buffer, pH 8.0, to get the crude enzyme solution. Since the crude extract contains dark brown pigments and phenols, it was removed by acetone fractionation. The acetone fraction was passed through Sephadex G-75 followed by exchange chromatography on DEAE-Sephadex A-50. The alkaline proteases appeared in three well defined peaks which were homogeneous as judged by polyacrylamide gel electrophoresis. The percentage of recovery for protease I - III were 18.7, 19.4 and 13.6 with 9, 7 and 8 fold purification. Protease I - III exhibited molecular weight values of 17000, 21000 and 53000 by gel filtration on Sephadex G-200 and 18000, 23000 and 50000 by sodium dodecylsulfate polyacrylamide gel electrophoresis, respectively. The non-appearance of more than one band on SDS polyacrylamide gel electrophoresis of all the three proteases rules out the possibility of native protein III to exist in dimeric or trimeric form. The Stoke's radii for protease I - III were calculated as 1.89 nm, 2.16 nm and 3.08 nm respectively. The values of diffusion constant for the three proteases were respectively $11.79 \times 10^{-2} \, \text{cm}^2/\text{Sec}$, $10.32 \times 10^{-7} \, \text{cm}^2/\text{Sec}$ and $7.24 \times 10^{-7} \, \text{cm}^2/\text{Sec}$. These observations suggested that protease I and II are more compact and globular than protease III.

The optimum pH for the crude extract was 11.0, with a shoulder between pH 8.0 and 9.0. Optimal pH for protease I - III
using casein as substrate were 11.0, 10.5 and 9.0 respectively.
The temperature optima for protease I - III obtained by 20 minutes
reaction at pH 8.0 were 60°C, 55°C and 50°C respectively. The
effect of pH on the stability of enzyme showed that protease
I and II are relatively more stable than protease III at
extremes of acidic or alkaline pH. There was no remarkable
difference in terms of thermal inactivation of all the proteases.
The inactivation began at 45°C, and at 60°C, almost all the
proteases lost fully activity. Lineweaver-Burk plots were
obtained with each of the proteases using casein as substrate.
The Km values obtained were $5.7 \times 10^{-6}$M, $2.9 \times 10^{-6}$M and
$2.1 \times 10^{-6}$M respectively for protease I, II, and III. All the
proteases hydrolysed BAPA, BAEE but not HTEE suggesting the
trypsin-like nature. Protease I is most active against casein
as well as BAPA as a substrate. Though, protease III was more
active than protease II against casein, the situation was
reverse for BAPA as substrate.

In an attempt to understand about the nature of the
enzymes, the effect of various inhibitors on protease activity
was studied. Metal chelating agent, EDTA, did not show any
inhibitory effect on the proteolytic activity of each enzyme.
The activity of each proteases remained unaltered after the
treatment with the thiol specific reagents, iodoacetic acid,
N-ethylmaleimide, PCMB, $\beta$-mercaptoethanol and cysteine hydro-
chloride. Like trypsin and chymotrypsin all the proteases were
completely inhibited by N-bromosuccinimide. PMSF which is known
to react specially with functional serine residue, inhibited all the proteases. TLCK was inhibitory to all the proteases indicating the participation of histidine in the active site. The chymotrypsin specific inhibitor, TPCK had no inhibitory effect on any of the enzymes. Each of the proteases were inhibited by SBTI and LBTI but ovomucoid only inhibited the protease I and II.

These proteases required no metal ions for their full activity. Ca^{++}, Co^{++}, Mg^{++} and Mn^{++} had almost no influence on the activity whereas the heavy metal ions like Zn^{++} and Cu^{++} caused strong inhibition. However, Hg^{++} caused complete inhibition of all the three proteases.

No evidence could be found for the existence of any of these proteases as inactive precursors.