1. A nuclease activity degrading native and denatured DNA and having a pH optimum of 10.5 was identified in the midgut contents of the larvae of the insect Anagasta kuhniella. Denatured DNA was hydrolysed at almost double the rate of native DNA. Further studies with crude extract indicated that the enzyme does not require the presence of a divalent metal ion for its activity but was stimulated by Mg^{++}. It was however strongly inhibited by EDTA suggesting the presence of a tightly bound metal. The nuclease was highly stable and retained 70% of its activity after incubation for three hours at 37°C.

2. The enzyme was purified about 160 fold by Heparin-agarose affinity chromatography. The purified enzyme was homogeneous and showed a single protein band on native polyacrylamide gel electrophoresis. The band also possessed nuclease activity and hydrolysed both DNA and RNA. Attempts to electrophorese the enzyme on polyacrylamide gels under denaturing conditions (containing SDS) were unsuccessful. This was considered to be due to the glycoprotein nature

of the enzyme, which may contain upto 50% carbohydrate.

3. The purified enzyme also showed an optimum pH of 10.5 with a sharp decline in activity at pH 11.0. The rate of hydrolysis of denatured DNA was again found to be significantly greater than native DNA. An absolute requirement for an added divalent cation was not found. However, the addition of Mg^{++} and Mn^{++} resulted in 72% and 35% stimulation respectively. The lack of an absolute requirement for an added divalent cation and inhibition by EDTA again suggested the possibility of a tightly bound metal ion to the enzyme. The enzyme does not seem to require an -SH group for its activity since sulfhydryl binding reagents like N-ethylmaleimide and p-chloromercuribenzoate and -SH donors such as mercaptoethanol and glutathione were without effect.

4. From emulsion chromatography on Sephadex G-150, the molecular weight and the stokes radius of the nuclease was calculated to be 18,200 and 2.1 nm respectively.

5. The Km value for the enzyme as determined in terms of acid soluble DNA nucleotide formed by the hydrolysis of both single stranded and double stranded DNA was of the order of 4×10^{-6} M. The same value with DNA as substrate was calculated to be 5×10^{-6} M.

6. The optimum temperature of the nuclease with both native and denatured DNA was found to be 55°C. Considerably greater hydrolysis of denatured DNA relative to native DNA was seen at the optimum temperature at pH 10.5. It was demonstrated that the partial destabilization of the secondary structure of duplex DNA at pH 10.5 may contribute significantly to the relatively higher rate of hydrolysis of denatured DNA. This was carried out by comparing the enzymatic hydrolysis of native and covalently cross-linked DNA at pH 10.5 and 8.5 and by studying the effect of sodium chloride on the hydrolysis of native and denatured DNA at 45°C and 60°C.

7. The study of the products of nucleolytic hydrolysis of denatured DNA on Sephadex G-100 showed that oligonucleotides are produced during the course of

reaction and that they are progressively reduced in size until the entire DNA is rendered acid soluble. The chromatographic profile obtained after various periods of digestion was consistent with an endonucleolytic mode of action.

8. The proteolytic enzyme trypsin, does not hydrolyse the larval nuclease. In contrast the pancreatic DNase-I was completely inactivated. Presumably the large carbohydrate content of the enzyme protects it from proteolysis by shielding the sites susceptible to trypsin.

9. The glycoprotein nature of the larval nuclease was concluded by several criteria; (a) the purified homogeneous enzyme shows a 30% carbohydrate content based on glucose as standard ; (b) the elution profile of the purified enzyme from a Sepharose column shows that the enzyme activity, enzyme protein and carbohydrate are eluted as a single peak and at the same positions; (c) the larval nuclease binds to the lectin Concanavalin-A; (d) it is refractory to the action of trypsin and protein - carbohydrate conjugates are known to be resistant to proteolysis.