CHAPTER VI

SUMMARY AND CONCLUSIONS
The particulate hexokinase of ox heart prepared according to the procedure of Crane and Sols was solubilized with a yield of about 50 per cent by treatment with pancreatic elastase followed by repeated freezing and thawing. The solubilized enzyme remained in the supernatant fraction when centrifuged at 100,000 x g for 1 to 2 hours.

The soluble enzyme was purified 500-fold by treatment with salmine sulfate, fractionation with calcium phosphate gel and ammonium sulfate and chromatography on DEAE-cellulose and Sephadex G-200. The maximum specific activity obtained was about 58 μmoles of glucose phosphorylated per milligram of enzyme at 30°. The yield of enzyme was, however, only 10 per cent.

The addition of sucrose (0.5 to 1 M) in the presence of phosphate (0.1 to 0.2 M) and TE (0.005 to 0.01 M) was required to stabilize the enzyme when its specific activity was more than 10 units/mg. Lower concentrations of sucrose were relatively ineffective. The final purified enzyme was, however, very unstable at 0° or -20° and lost most of its activity within a few days. In the presence of 8 mg of serum albumin per ml the purified enzyme could be stored for several months at -20° without loss of activity.

The ultraviolet absorption of the purified enzyme was that of a typical protein.

Purified particulate hexokinase showed no mobility towards the anode at pH 8.5 or 7 in 5-7% acrylamide gel.
Soluble ox heart hexokinase showed the presence of two isoenzymes on acrylamide gel electrophoresis.

The molecular weight of the solubilized enzyme as determined by Sephadex G-200 chromatography was approximately 90,000.

The pH optimum of the enzyme was unaltered between 5.8 and 8.

The increase in activity of the enzyme between 20° and 30° was approximately 2. At higher temperatures there was inactivation of the enzyme.

Hexokinase was active with Mg$^{2+}$ or Mn$^{2+}$ but not with Ca$^{2+}$ and Zn$^{2+}$.

The enzyme was active only with ATP and not with GTP, UTP, CTP or ADP.

The relative activities with glucose, fructose and mannose were 1, 1.8 and 0.7 respectively. There was no activity with L-sorbose, D-sorbitol, rhamnose, cellobiose, lactose or mannitol.

$K_m$ for Mg$^{2+}$ was 3 mM at 10 mM ATP.

$K_m$ for mannose was 0.038 mM and for fructose 2 mM at 5 mM ATP.

$K_m$ values for glucose were 0.052, 0.062, 0.062, 0.100 and 0.112 mM at 10, 5, 2, 1.2 and 0.8 mM ATP respectively.

$K_m$ values for ATP were 0.5, 0.78, 0.96, 1.10, 1.12 mM at 15, 3, 1.5, 1 and 0.5 mM glucose respectively.

Double reciprocal plots of activity vs substrates at fixed concentrations suggested a possible "ping-pong" mechanism but further work will be needed to elucidate the mechanism.
The enzyme was inhibited by 5-thio-D-glucose competitively with respect to mannose. Ki for 5-thio-D-glucose was 1.4 mM.

The enzyme was inhibited by ADP non-competitively with respect to ATP at a Mg:ATP ratio of 2:1.

Glucose-6-P inhibited heart hexokinase and this inhibition was reversed partially by Pi. Ki values for glucose-6-P were 0.11, 0.16, 0.2 mM at 5, 10, 30 mM Pi respectively. Ki values for glucose-6-P at 1.5, 5 and 10 mM ATP were 0.22, 0.17 and 0.15 mM respectively.

Insulin, bovine growth hormone, adrenalin and prednisolone and serotonin had no effect on hexokinase activity either directly or in the presence of 0.15 mM glucose-6-P with or without 10 mM Pi.

NaF, ammonium sulfate, KCl, 3-phosphoglyceric acid, phosphoenol pyruvate and TE had no effect on enzyme activity. The activity and stability of the enzyme were not influenced by treatment with 1 mM DFP.

The enzyme was inhibited 10 and 20% at 1 and 4 mM iodoacetate respectively. pCMB inhibited the enzyme 50% at 0.001 mM.