SUMMARY & CONCLUSIONS

Guanidines are ubiquitous compounds found in invertebrates and vertebrates. These compounds are normally present in low concentration in human blood and urine. Guanidines at low concentration act as good pharmacological agents and used in the treatment of infantile and juvenile atrophy and to correct electrophysiological defects in several myopathies.

In the present investigation the role of guanidines in phosphagen synthesis has been elucidated since these compounds are known to be immediate precursors for energy rich phosphates, which have significant role in the regulation of muscle metabolism. For the present study Guanidine hydrochloride was selected due to rapidity of its entry into the tissues and glycocyamine was chosen since it is a direct precursor for phosphagen synthesis.
Wistar strain male albino rats (150±5 gr) were maintained under laboratory conditions and fed with standard rat feed and water ad libitum. The rats were divided into four groups of six each. The third and fourth groups were given intraperitoneal injections of guanidine hydrochloride (2.0 milli moles/kg/day) for seven consecutive days after due standardization. The first and second batches received isovolumetric dose of saline alone for the same period. In a second set of four groups of rats, third and fourth group received 0.02 milli moles of guanidinoacetic acid/kg/day for a period of one week. First and second groups were given isovolumetric dose of saline for the same period. The second and fourth groups of rats of two sets were subjected to continuous swimming to induce fatigue. Required tissues were excised in cold. The tissues were weighed, homogenized in required media and biochemical analyses were carried out.

Creatine and creatinine levels were decreased in liver and kidney of the guanidine treated rat after exhaustion. Creatine and creatinine levels were increased in the muscles during guanidine treatment due to the transport of the same from the liver, kidney and serum or its formation from the administered guanidino compound. The increased creatine levels in the exhausted muscle suggest enhanced utilization of phosphocreatine to rephosphorylate ADP to reimburse depleted ATP levels during sustained muscular activity. Decreased creatinine levels in the liver, kidney and the serum of exhausted rat suggest reduced nonenzymatic depletion of creatine to creatinine and its retention due to altered glomerular filtration rate. Guanidine treatment also depressed the glycine amidinotransferase activity in the kidney due to nephrotoxic nature of the test compound, and accumulation of high ornithine and creatine and depletion of ATP.

Creatine phosphokinase activity was increased in muscle indicating rapid utilization of rich phosphates for prolonged contractile work. Creatine phosphate levels were low in the tissues and serum during exhaustion due to rapid utilization. Guanidine treatment significantly elevated the phosphocreatine levels in the tissues as guanidines are precursors and are utilized for enhanced formation of phosphagens.

Glucose and glycogen levels were depleted during guanidine stress and arduous physical work. It is apparent that the animal needs extensive pools of energy fuels during strenuous physical work, hence the greater metabolic utilization of glycogen possibly to meet
Higher energy demands to mitigate guanidine toxicity and contractile stress. The severe contractile stress considerably increased ammonia content and due to this altered ammonia production the muscle needs higher level of glycogen to counter hyperammonemia. The guanidine administration might have decreased the rate of synthesis of glycogen leading to depleted glycogen level. Decreased synthesis of energy fuels, increased demand for energy to mitigate the effect of fatigue toxins and test compound might have led to glycogen depletion in the tissues of guanidine treated rat under strenuous physical work.

Low glucose level in the tissues of experimental animals suggest its utilization in EMP pathway to mitigate energy crisis during contractile stress and guanidine toxicity. Failure of release of Ca$^{2+}$ was a causative factor for the diminished glucose level in exhausted rat. The rate of suppressed glucose synthesis was reported during uremic conditions.

Phosphorylase activities (both 'active' and 'total') were found to decrease in both the experimental conditions. During intensive physical work the phosphorylase activity diminishes with an associated halt in glycogen degradation which probably results in the decreased energy supply. Altered permeability properties of muscle membranes were reported during exhaustive work which might be responsible for the diminished phosphorylase activities. Guanidino compounds, as uremic toxins were reported to inhibit the phosphorylase system. Accumulation of organic acids during exhaustive work and guanidine hydrochloride as a potent protein denaturant depressed phosphorylases in the present study in the guanidine treated exhausted rat.

The administered guanidine was found to interfere with succinate-fumarate interconversions by inhibiting succinate dehydrogenase activity of mitochondria during intensive physical work. Guanidine hydrochloride treatment causes hyperammonemia in the tissues of rat which depletes TCA cycle intermediates. Low NAD dependent lactate dehydrogenase activity, reduced formation of pyruvate, decreased mobilization of metabolites into citric acid cycle are the possible reasons for the depressed level of succinate dehydrogenase activity in the tissues of rat after guanidine treatment and strenuous physical work.

Malate dehydrogenase activity in the present investigation was low in all the tissues studied during exhaustive work. The malate dehydrogenase activity was reduced during
ular fatigue. Higher depletion of pyruvate, its substrate, cofactor and oxygen deficiency 
consequent insuffcient energy metabolism during intensive physical work were attributed 
decreased malate dehydrogenase activity during extensive physical work. Accumulation 
ic compounds, such as methylguanidine and guanidinosuccinic acid decrease reox state 
hereby malate dehydrogenase activity in the guanidine treated rat.

Hyperammonemia and hyperlacticaciduria are probably the apt causes for the 
tished tissue glutamate dehydrogenase activity during extensive physical work and 
idine treatment. Inactivation of enzyme proper, low availability or substrate, elevated 
ic acids, disturbed cellular activities, lowered sulhydryl groups, depressed 
amidination reactions are responsible for decreased enzyme activity of the tissues under 
immental conditions.

The energy synthesizing capacity of the tissues may be assessed through 
chrome-C-oxidase activity, which reveals the operational efficiency of electron transport 
em. Guanidine treatment and fatigue create hypoxic conditions leading to disturbances in 
cellular atmosphere which inturn prevent the reoxidation of cytochromes by inhibiting 
chrome-C-oxidase. Low activity of cytochrome-C-oxidase in guanidine administered 
austed rat reflect reduced oxygen availability and depressed energy synthesizing capacity 
the tissues. Inner mitochondrial structure was found to alter during guanidine 
inistration leading to low operation of electron transport system during strenuous work 
lanidine stress.

Myosine ATPase is an important enzyme in energy transduction reactions in muscles. 
the present investigation myosin ATPase activity was decreased in the muscles during 
austive work and guanidine treatment. Enhanced creatine phosphokinase activity and its 
ncompetitive inhibitory influence on myosin ATPase activity, non availability of the 
strate are responsible for diminished myosin ATPase activity in the muscle.

Dissociation of ATPases by guanidine hydrochloride might reduce energy transduction 
contractile unit. Hence the diminished myosin ATPase activity was observed in the muscle 
der experimental conditions.
Sulfhydryl groups are highly reactive moieties with important functions in many biological processes. The sulfhydryl groups are known to have protective effect against free radical toxicity and chemically reactive metabolite induced toxicity. In the present investigation the total and protein bound sulfhydryl groups were lowered in exhausted rat but free sulfhydryl group content was elevated. Elevated levels of free sulfhydryl groups seem to be an adaptive mechanism to protect and maintain normal cellular functions. Altered membrane permeability properties, oxidative stress, dissociated enzyme moieties, rapid oxidation of sulfhydryl groups, lowered thiol groups, reduced synthesis of glutathione (GSH), disruption of cell integrity, higher affinity of guanidine hydrochloride towards sulfhydryl groups, inhibited calcium uptake, might deplete total and protein-bound sulfhydryl groups in the guanidine treated exhausted rat. The alterations suggest that the oxido reductase system was severely disturbed in the tissues under guanidine and contractile stress.

Since creatine phosphate levels were found to increase in the muscles after guanidine treatment alteration in the contractile efficiency has been studied by recording twitch cycles using kymograph. The onset of fatigue in control and experimental muscles was also recorded.

The twitch duration time was prolonged in experimental muscles suggesting that muscles need more time to complete twitch cycle in the presence of guanidines as compared to controls.

The half-contraction time and half relaxation time showed continuous increase with the increase in guanidine hydrochloride and guanidinoacetic acid concentration in the medium. It also indicated more efficient pattern of contraction as the amplitude was also found to increase. The HRT/HCT also showed an increase with glycocyamine in the medium suggesting the effect of administered guanidine on relaxation phase than on the contraction phase. The increased amplitude also indicates improved muscle function as was evinced by delay in the onset of fatigue.

The time required for the onset of fatigue in the muscle was more in rats treated with guanidino compounds both under in vivo and in vitro conditions suggesting that the working capacity is increased in the muscles in the presence of guanidino compound.
Increased twitch properties and delayed onset of fatigue in the experimental muscles could be viewed that guanidino compounds have some beneficial role to play in improving endurance capacity of muscles by shifting the contractile properties towards slow type.

Glycocyamine has greater modulatory influence on contractile properties of muscles as evinced by enhanced twitch kinetic characteristics over guanidine hydrochloride.

To sum up the present findings suggest that guanidines have significant modulatory impact on creatine metabolism and phosphagen levels. The kymographic recordings indicated that guanidino compounds have some beneficial role to play in improving endurance capacity of the muscles by shifting contractile properties to slow type as reflected by delayed fatigue time, prolonged half-relaxation time and twitch duration. Though the endurance capacity was increased the energy synthesis from citric acid cycle operation was not geared up as the enzymes of oxidative phosphorylation were inhibited. Improved phosphagen levels and endurance capacity of working muscles in response to guanidine treatment need further confirmatory evidence to understand their specific role in the regulation of contractile properties of muscle.