ALTERATIONS IN TISSUE METABOLISM (THE LUNG) WITH INJURY
AND SHOCK

Annual Summary Report

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Alterations in tissue metabolism (the lung) with injury and shock.

There has been exciting progress in the past year, particularly in the area of membrane transport and energy metabolism as it relates to the lung and compares with the liver, kidney and with skeletal muscle, with energy replenishment and with insulin resistance.
Recent progress can best be summarized by citing the publications from our laboratory supported by the previous year's contract.


Reprints or copies of the manuscripts of these publications or manuscripts in press are enclosed for review. A number of other papers are being prepared for submission for publication, but are not cited now because they have not been completed. Also, we have participated in a number of programs in which the work supported by this contract has been presented. These include several presentations by the responsible investigator at post-graduate courses at the American College of Surgeons in Miami in 1974, a lecture as a Visiting Professor at the University of Texas Southwestern School of Medicine in Dallas, a post-graduate course sponsored by the University of Miami in January of 1975 in Miami, Florida, a presentation of our work at the Society of University Surgeons meeting in Tucson, Arizona in February of 1975, several presentations to Washington University Alumni Association meeting, a presentation at a post-graduate course sponsored by the United States Navy and by the United States Army Research and Development Command in San Diego in March of 1975 and various other regional and local problems on shock and circulatory failure. The principle findings of the past year will now be summarized.

1) The lung — energy levels and cell membrane transport.

a) Basic demonstration of cell membrane transport in lung tissue. This work has now been completed and is being published and in press in the American Journal of Physiology. Specially prepared lung slices were incubated in an oxygenated Kreb's-Ringer Bicarbonate medium for 90 minutes at 0.5°C (chilling), followed by 60 minutes at 37°C (rewarming). Fresh tissue cation contents (mean ± SE) in mmols/Kg dry weight were: sodium, 431 ± 7; potassium, 416 ± 10. After chilling tissue sodium increased to 757 ± 11 and potassium decreased to 113 ± 6. Upon rewarming there was a net increase in tissue potassium of about 15 (mmols/Kg dry weight) and a net decrease in tissue sodium of about 130. Tissue extrusion of sodium
and reaccumulation of potassium observed at 37°C were abolished when
M ouabain, dinitrophenol or iodoacetamide was added to the incubation
medium. Similar results were obtained when the medium contained no
potassium or when medium Na was replaced by choline. The data indicate
the presence of active Na⁺-K⁺ transport in lung cells somewhat similar
to that found in other mammalian tissue.

b) Na-K transport and adenosine nucleotides in the lung in hemorrhagic shock.
This study was undertaken to determine the effects of hemorrhagic shock
on cellular energy production and utilization in the lung. Energy-
dependent Na⁺-K⁺ transport was measured by quantitating tissue cation
changes during a cold (0.5°C) and a subsequent warm (37°C) incubation of
lung slices from rats in late hemorrhagic shock and from unbled control
rats. Active Na⁺ extrusion and K⁺ reaccumulation by the tissue were
observed upon rewarming of lung slices from shock animals. Whereas K⁺
reaccumulation was not altered with shock, the rate of Na⁺ extrusion was
approximately 40 percent higher. The measurement of the intracellular
water content with cold and warm incubations showed no alterations with
shock. Extracellular water increased with chilling in shock tissue but
not in normal tissue. Lung tissue contents of adenosine triphosphate,
adenosine diphosphate, or adenosine monophosphate were likewise unaltered.
Thus cellular energy utilization or production in the lung was not
damaged by hemorrhagic shock but a tendency toward increased interstitial
water seemed to be present.

c) A comparison of active sodium-potassium transport and ATP levels in the
lung and liver during shock. This study shows that the energy-requiring
transport of sodium and potassium is greatly altered in the liver but not
in the lung with hemorrhagic shock. The lack of change in lung cation
transport could be due in part to direct utilization of atmospheric oxygen
in the alveoli by cells of the lung to maintain cellular energy levels
during the low flow state of shock. Lung ATP levels were maintained at
the control level with shock. Thus, neither the energy-requiring nor the
energy-yielding lung cell processes measured were affected by the circula-
tory alterations of shock in the lung. We have found previously that the
capability of mitochondria from the liver but not from the lung was
decreased by shock. These findings indicate that if the lung is altered by
shock, it is more likely to involve the interstitial tissue of the lung
rather than its cellular components.

2) Energy levels - effect of administered ATP, ATP degradation, etc.

a) Further studies were carried out on the effect of hemorrhagic shock on
tissue adenine nucleotides in conscious rats comparing three tissues:
liver, kidney and skeletal muscle. Hemorrhagic shock was produced in
conscious rats by cannulating the subclavian artery and bleeding the
animals to a mean arterial pressure of 40 mm Hg which was maintained
for 1 (early shock) or 2 h (late shock). Analysis of tissues showed
that there was a significant decrease in ATP and ADP levels in liver
and kidney in early and late shock. Associated with the decrease in
ATP and ADP levels were increases in AMP and Pi levels. In contrast
to the above organs, adenine nucleotides and creatine phosphate levels
of skeletal muscle did not decrease in early shock but a significant
reduction of these compounds was observed in late shock. The decrease
in ATP content was greater in liver and kidney than in skeletal
muscle. The present experiments indicate that there is a decrease in the energy available to tissues during severe hemorrhagic shock. This could be due to decreased biosynthesis, to continuing or increased utilization of the nucleotides, or to both.

b) A study of the degradation of adenine nucleotides was carried out in order to determine if there was an increase in breakdown of nucleotides by various enzyme systems during diminished circulation. Our previous studies have shown a salutary effect of adenosine triphosphate-magnesium chloride (ATP-MgCl₂) administered to animals in shock. The presence of adenine nucleotide converting enzymes on cell surfaces and the ability of nucleotides to act at the cell surface have been recognized also. To investigate the fate of administered or externally applied ATP and to determine whether it would be subjected to increased degradation with shock, the soleus muscles from rats subjected to hemorrhagic shock and from control animals were incubated in the presence of ATP, adenosine diphosphate (ADP), or adenosine monophosphate (AMP) with MgCl₂. Comparable degradation of the added nucleotides was observed with both control muscles and those from bled animals. Adenylate kinase activity was detected to the same extent in the medium after incubation with both groups of muscles, but other enzymes were not, suggesting that the latter enzymes were located on the exterior surface of the muscle cell. Thus with shock there was no increase in the breakdown of the nucleotides by the enzymes on the muscle surface (ATPase, AMP deaminase) or the cellular enzyme, adenylate kinase.

c) Uptake of ATP by tissues. There has been considerable dispute as to whether or not ATP can get into cells when administered or in the extracellular environment on theoretical grounds. One would suspect that ATP could not do this. We have actually measured this phenomenon though and find some qualitative evidence for ATP getting into various tissues which are reported following.

Although it has been shown that infusion of adenosine triphosphate (ATP)-MgCl₂ proved beneficial in the treatment of shock, it is not known whether this effect is due to improvement in the microcirculation or direct provision of energy. In searching for the mechanism of this, we have now examined the in vitro uptake of ATP by soleus muscle of animals in shock. Rats were bled to a mean arterial pressure of 40 mm Hg and so maintained for two hours. Following sacrifice, the two soleus muscles from each animal were removed and incubated in Krebs-HCO₃ buffer containing 10 mM glucose, 5 mM (8-¹³C) ATP, 5 mM (8-¹⁴C) ADP, or 0.5 mM (8-¹⁴C) adenosine, and 5 mM MgCl₂ for 1 hr under an atmosphere of 95% O₂ - 5% CO₂. Following homogenization and centrifugation, samples of the muscle extract and the medium were subjected to electrophoresis to separate the various nucleotides. The concentrations of the several nucleotides in medium and muscle were calculated from the radioactivity observed in each fraction. The uptake of ¹⁴C-ATP by muscles from animals in shock was three times greater than the uptake by control muscles. This leads us to conclude that the beneficial effect of ATP-MgCl₂ to animals in shock could be due to provision of energy directly to tissues in which ATP levels were lowered.
We have previously shown that infusion of ATP-MgCl₂ had a beneficial effect on the survival of animals after shock. However, the fate and effects of such administered ATP are not known. To study this, the uptake of ATP by tissues from animals in shock was measured. Rats were bled to a mean arterial pressure of 40 mm Hg, so maintained for 2 hrs, then sacrificed. Liver and kidney were removed and slices (0.3-0.5 mm thick) were incubated in 1.0 ml of Krebs-HCO₃ buffer containing 10 mM glucose, 5 mM (3⁻¹⁴C) ATP (0.43 μC/μmol), and 5 mM MgCl₂ in 95% O₂-5% CO₂ for 1 hr and then homogenized. An ATP regenerating system (PEP-PK) was added to maintain higher ATP:ADP ratio in the medium during incubation. Tissue and medium samples were subjected to electrophoresis to separate and measure the various nucleotides. Inulin was used to measure the extracellular space which allowed calculation of the intracellular concentration of nucleotides. Intracellular ¹⁴C-ATP values (mean of 8 experiments), expressed in μmoles/g tissue, were: control liver and kidney, 0.55±0.02 and 0.38±0.01 respectively; 'shock' liver and kidney, 1.44±0.03 and 0.82±0.02 respectively. Uptake of ¹⁴C-ATP by liver and kidney slices from animals in shock was at least two times greater than the corresponding uptake in control slices. Thus, the beneficial effect of ATP-MgCl₂ in shock could be due to provision of energy directly to tissues in which ATP levels were lowered.

d) A study is also carried out of the relationship between nicotinamide adenine dinucleotide and survival in hemorrhagic shock. It has been shown that administration of nicotinamide following severe sepsis favorably affects the survival rate in rats. The object of this investigation was to determine if nicotinamide would also have a salutary effect in hemorrhagic shock. In the first group of rats, nicotinamide adenine dinucleotide (NAD), 25-100 μmoles, nicotinamide, 100 μmoles, or nicotinic acid, 100 μmoles, was infused intravenously following which the animals were bled to a mean arterial pressure of 40 mm Hg for 1½ hrs. The remaining shed blood was then returned slowly, the vessels ligated and the animals returned to cages. In the second group of rats, animals were bled to 40 mm Hg for 1½ hrs. NAD, nicotinamide or nicotinic acid was then given intravenously followed by return of the shed blood. Control animals were bled for the same period and given the shed blood and an equal volume of saline. Survival was measured over a period of 12 hrs. Mortality was 100% in control rats and also in the 24 rats receiving NAD, nicotinamide or nicotinic acid prior to shock. In the 50 rats who received NAD, nicotinamide or nicotinic acid following shock, no beneficial effect was observed. Experiments from our laboratory have also shown that during shock tissue NAD levels decrease significantly. Infusion of nicotinamide following shock resulted in restoring NAD levels in liver and kidney, but despite this, the animals failed to survive. These results indicate that infusion of nicotinamide, NAD or nicotinic acid failed to have any salutary effect on the survival of rats in hemorrhagic shock, whereas previous work from our laboratory has clearly shown a beneficial effect of ATP-MgCl₂ for animals in shock.

3) Cell membrane potential and cation transport in the liver. We reported previously that active Na-K transport in liver was impaired in hemorrhagic shock. We have now studied the relationship between resting hepatic transmembrane potentials (HIP) and cation transport with shock. Rats were bled...
to a mean arterial blood pressure of 40 mm Hg, and maintained for 1 hour (intermediate shock) or 2 hours (late shock). Some intermediate shock animals were treated with shed blood plus Ringers lactate and studied 1 hour later. HTP was recorded in situ, in livers of animals in shock with a KCl microelectrode (tip diameter <1 µm, resistance 10-20 MΩ). Cation transport was measured, in vitro, by determination of net Na+ extrusion and K+ accumulation by liver slices. HTP decreased from 40±0.04 mv in control animals to 31±2 in intermediate shock and 19±0.4 in late shock animals. Active K+ accumulation by liver slices in mmoles/(hrxkg dry wt) was: 110±7 in control, 15±4 in intermediate shock and 10±7 in late shock animals. Active Na+ extrusion was 132±24 mmoles/(hrxkg dry wt) in control animals but was not measurable in shock animals. A partial recovery of both the cation transport activity and the HTP was observed in animals treated after intermediate shock. These data further support the failure of the active cation transport in liver in shock.

A review of all our previous work studying cation transport in the liver in hemorrhagic shock was also reported. Cation transport capability was measured in liver slices of rats bled to mean arterial blood pressure of 40 mm Hg. This pressure was maintained for: (a) ½ hour without return of any shed blood (early shock), (b) 1 hour with slow return of 30% of shed blood (intermediate shock) or (c) 2 hours with slow return of 70% of shed blood (late shock). In vitro sodium-potassium transport was inhibited in early, intermediate, and late shock. This was accompanied by a loss of cell volume regulation. Sodium and water contents increased and tissue adenosine nucleotides (ATP, ADP, and AMP) decreased with shock. The decreases in ATP, ATP:ADP ratio, and the energy charge values in intermediate and in late shock probably were not of such a magnitude to indicate complete loss of the in vitro sodium-potassium transport. The impairment of cation transport with shock could thus be related to factor (or factors) other than energy availability. The in vitro cation transport was restored to normal with reinfusion of all shed blood and Ringer's lactate in early shock. This did not occur in intermediate shock. Alterations in sodium-potassium transport would severely impair the capability of the cells to extrude water which would lead to cell swelling. This in turn could contribute to critical loss of interstitial and vascular fluid resulting in decreases in the effective circulatory volume in shock.

4) Hormonal effects on cell membrane processes. Further work was carried out in this area. We have published the work now on effect of insulin on glucose uptake in soleus muscle during hemorrhagic shock.

a) Hemorrhagic shock was produced by bleeding conscious rats to a mean arterial pressure of 40 mm Hg, which was maintained for 2 h. Basal glucose uptake by isolated soleus muscle from normal rats and rats subjected to hemorrhagic shock ('shock' muscles) increased with the increase in medium glucose concentration. Uptake values were similar in both groups of muscles. This indicates that there were no alterations in the basal glucose carrier mechanism during shock. Whereas insulin (0.1 U/ml) stimulated glucose uptake in control muscles under aerobic as well as under anaerobic conditions, it had no stimulatory effect in 'shock' muscles under either environment. Maximal stimulation of glucose uptake in 'shock' muscles was observed at an insulin concentration of 0.2 U/ml. The ability of muscle to bind insulin was not altered during shock. The present experiments indicate that insulin responsiveness to tissues is altered in shock. This
could be due to alterations in the insulin sensitivity of the glucose
carrier mechanism during shock.

b) We have now found that this insulin resistance occurring during
hemorrhagic shock can be reversed by the in vivo infusion of ATP. We
have previously demonstrated alterations in cell membrane function
in shock with insulin resistance and decreased Na⁺-K⁺ transport. We
have also found that adenosine triphosphate-magnesium chloride
(ATP-MgCl₂) favorably influenced energy levels and survival of animals
in shock. The present study was undertaken to determine the effect
of in vivo infusion of ATP-MgCl₂ on tissue insulin resistance in shock.
The results indicate that insulin resistance can be overcome by the
infusion of ATP-MgCl₂ to animals in shock.

Albino Holtzman rats were fasted for 16 hours and lightly anesthetized
with ether. Cannulation of subclavian arteries and jugular vein was
performed on all animals, after which they were allowed to awaken.
They were then bled to a mean arterial pressure of 40 mm Hg which was
maintained for 1½ hours. The animals then received intravenously over
a period of about 15 minutes (a) saline (0.25 ml) followed by their
shed blood ('shock' untreated animals) or (b) ATP-MgCl₂ (0.25 ml,
25 µM each) plus their shed blood ('shock' treated). Control animals
received 0.25 ml saline but were not bled. The animals were sacrificed
30 minutes after ATP-MgCl₂ infusion and the two soleus muscles from
each animal were incubated for 1 hour at 37°C in Krebs-HCO₃ buffer
containing 10 mM glucose with or without insulin (0.1 U/ml). Glucose
uptake was measured by the disappearance of glucose from the medium.
The muscles were analyzed for ATP contents following incubation.

Basal glucose uptake values by control muscles and those from animals
subjected to shock (treated and untreated) were the same. Infusions
of ATP-MgCl₂ had no effect on basal glucose uptake but permitted
insulin to exert its stimulatory effect on muscles from 'shock' animals.
ATP levels decreased in untreated animals; treated animals showed ATP
levels similar to those of controls.

The results presented above indicate that insulin resistance can be
overcome by the infusion of ATP-MgCl₂ to animals in shock. Although
ATP levels in shock tissues increased following treatment, the
relationship between ATP levels and insulin effect has yet to be
established (unpublished observations). It has been shown that cellular
swelling occurs during shock and it is also known that ATP induces
muscle contractions. Moreover, it has recently been shown that ATP
induces membrane conformational changes. Whether the effect of
ATP-MgCl₂ in overcoming tissue insulin resistance with shock is due
to reversal of cell swelling or due to some other metabolic or
membrane effect is not yet known.

c) Insulin resistance in experimental shock was also measured in
adrenalectomized animals. Previously adrenalectomized (ADX) rats were
bled to a mean arterial pressure of 40 mm Hg and maintained for 1½ hours.
Basal glucose uptake by isolated soleus muscle from ADX normal rats
and ADX rats subjected to shock ('shock' muscles) increased with the
increase in medium glucose concentration and uptake was similar in both
groups of muscles. This indicates that shock per se did not produce
any alterations in the basal glucose carrier mechanism. Insulin (0.1 Units/ml) increased uptake in ADX control but not in ADX shock muscles. Maximal stimulation of glucose uptake in shock muscles was observed at an insulin concentration of 0.2 Units/ml insulin. These experiments provide the first direct evidence that the responsiveness of tissues to insulin is altered during shock. This alteration could not be due to increased steroid or epinephrine output during shock.

d) Studies of the effect of glucocorticoids on sugar transport were also made as were the effects on osmolarity. Previous work from our laboratory has shown that tissues from animals subjected to severe hemorrhage were resistant to insulin. Since the blood level of corticosteroids is known to increase during shock, it is possible that the insulin resistance could have been due to the interaction of steroids with insulin. To test this possibility, Holtzman rats (70-90g) were bilaterally adrenalectomized (ADX) 3-4 days prior to the study. Two soleus muscles from each animal were quickly removed and placed in 1.0 ml of medium containing Krebs-HCO\(_3\) buffer (pH 7.4) and xylose (6 mg/ml). Insulin and steroids when used were added to concentrations ranging from 100 \(\mu\)U-200 \(\mu\)U/ml and \(10^{-4}\)M \(10^{-2}\) respectively. Incubations were carried out in a metabolic shaker for 30 minutes at 37°C; shaking rate 110 cycles/min; atmosphere 95% \(O_2\)-5% \(CO_2\). The muscles were then rinsed, blotted, frozen and homogenized in \(Ba(OH)_2\)-\(ZnSO_4\), and the supernatant was analyzed for xylose. The results indicate that in control as well as in muscles from ADX animals, 100 \(\mu\)U/ml insulin was required for maximal xylose transport. Hydrocortisone (\(10^{-4}\)M), Dexamethasone (\(10^{-4}\)M) or Hydrocortisone-21 Na succinate (\(10^{-3}\)M) had no effect on basal transport. In the presence of \(10^{-4}\)M of any of the above steroids insulin-stimulated transport was not affected at any insulin concentration. When Hydrocortisone-21 Na succinate was used at \(10^{-2}\)M, insulin-stimulated transport was decreased with a maximal inhibitory effect in the presence of 100 \(\mu\)U/ml insulin. In this study, steroids failed to inhibit insulin-stimulated transport at concentrations higher than known blood steroid levels during shock (\(10^{-3}-10^{-2}\)M). Thus, it is unlikely that during shock steroids were responsible for the observed tissue insulin resistance.

The effect of hyperosmolarity on glucose uptake was studied in the presence and absence of insulin. Glucose uptake by isolated rat soleus muscle was measured by incubating the muscles for 1 hour at 37°C in a Tris-HCl buffer, pH 7.4, containing 10 mM glucose and varying amounts of sorbitol to give the required osmolarity. The results, expressed in \(\mu\)moles/g/hr, are mean of 8 determinations in each group, and indicate that under basal conditions, glucose uptake increased with the increase in medium osmolarity. However, in the presence of 0.1 U/ml insulin, uptake reached optimum at physiological osmolarity, i.e. at 300 milliosmoles (mOs). Further increase in osmolarity resulted in a progressive decrease in glucose uptake in the presence of insulin, indicating insulin resistance at higher osmolarity. At 400 mOs, glucose uptake in the absence and presence of 0.1 U/ml insulin was the same. For insulin to produce its optimal stimulatory effect at 400 mOs, 0.25 U/ml insulin was required, which is 250 times the concentration of insulin required to produce its maximal effect at physiological osmolarity. Thus, normal osmolarity is needed for optimal insulin effect on glucose uptake.
To determine the effects of glucocorticoids on sugar transport, xylose transport in isolated rat soleus muscle of bilaterally adrenalectomized animals was studied. The results indicate that in vitro addition of $10^{-6}$ M hydrocortisone, dexamethasone or hydrocortisone sodium succinate had no inhibitory effect on basal xylose transport. Increasing the concentration of hydrocortisone sodium succinate to $10^{-2}$ M also failed to show an inhibitory effect on basal transport. In the presence of both low and high medium insulin, the above steroids failed to inhibit insulin-stimulated transport. When the concentration of hydrocortisone sodium succinate was increased to $10^{-2}$ M, insulin-stimulated transport was decreased. The results thus indicate that glucocorticoids at physiological concentrations or even at concentrations observed under pathological conditions do not inhibit basal or insulin-stimulated sugar transport.

5) Several other areas of activity which were reported which come under the purview of this contract include a clinical study carried out by Dr. Jerry Meyers and responsible investigator entitled "Changes in Functional Residual Capacity of the Lung after Operation." A copy of this publication is enclosed. The editorial by the responsible investigator entitled "The Energy Crisis in Surgical Patients" was published and is enclosed. The editorial "Multiple, Progressive or Sequential Systems Failure—A Syndrome of the '70's" which is in press is also enclosed.

Thus, in summary, there has been exciting progress in the past year, particularly in the area of membrane transport and energy metabolism as it relates to the lung and compares with the liver, kidney and with skeletal muscle, with energy replenishment and with insulin resistance.
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