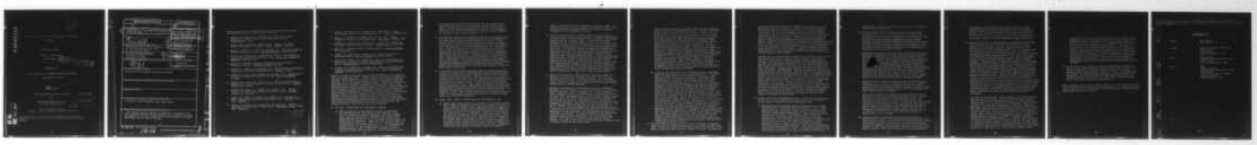
AD-A047 442 WASHINGTON UNIV ST LOUIS MO SCHOOL OF MEDICINE F/G 6/16
ALTERATIONS IN TISSUE METABOLISM (THE LUNG) WITH INJURY AND SHO--ETC(U)
JUL 75 A E BAUE DADA17-69-C-9165
UNCLASSIFIED NL

| OF |
AD
A047442



END
DATE
FILMED
1-78
DDC

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

Annual Summary Report

ARTHUR E. BAUE, M.D.

July 15, 1975

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Washington, D. C. 20314

DA-17-69-C-9165

Washington University School of Medicine

and

The Jewish Hospital of St. Louis
St. Louis, Missouri 63110

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: SIX
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): ALTERATIONS IN TISSUE METABOLISM (THE LUNG) WITH INJURY AND SHOCK.

5. TYPE OF REPORT & PERIOD COVERED: Annual Summary Report. no. 6, July 1, 1974-June 30, 1975

6. PERFORMING ORG. REPORT NUMBER: 1 Jul 74-30 Jun 75

7. AUTHOR(s): Arthur E. Baue, M.D.

8. CONTRACT OR GRANT NUMBER(s): DADA17-69-C-9165

9. PERFORMING ORGANIZATION NAME AND ADDRESS: Washington University School of Medicine and The Jewish Hospital of St. Louis, St. Louis, Missouri 63110

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 62110A 3A 162110A821 065

11. CONTROLLING OFFICE NAME AND ADDRESS: U.S. Army Medical Research and Development Command, Washington, D.C. 20314

12. REPORT DATE: July 1975

13. NUMBER OF PAGES: Twelve

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): 11 p.

15. SECURITY CLASS. (of this report): Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for Public Release; Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Alterations, Tissue, Metabolism, Lung, Injury, Shock

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

370 150

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Recent progress can best be summarized by citing the publications from our laboratory supported by the previous year's contract.

a. Chaudry, I.H., Sayeed, M.M., and Baue, A.E.: Reversal of Insulin Resistance by In Vivo Infusion of ATP in Experimental Shock. Surgical Forum, In Press.

b. Chaudry, I.H., Sayeed, M.M., and Baue, A.E.: Evidence for Enhanced Uptake of ATP by Muscle of Animals in Shock. Surgery. In Press.

c. Meyers, J.; Lembeck, L., O'Kane, H., and Baue, A.E.: Changes in Functional Residual Capacity of the lung. Archives of Surgery, 110:576-583, 1975.

d. Chaudry, I.H. and Baue, A.E: The Effect of Insulin on Glucose Uptake in Soleus Muscle during Severe Hemorrhagic Shock. Can. J. of Physiology and Pharmacol., 53:67-73, 1975

e. Baue, A.E.: Multiple, Progessive or Sequential Sustems Failure—A Syndrome of the '70's. Archives of Surgery, In Press.

f. Sayeed, M.M., Chaudry, I.H., and Baue, A.E.: Na^+-K^+ Transport and Adenosine Nucleotides in the Lung in Hemorrhagic Shock. Surgery, In Press.

g. Sayeed, M.M., Senior, R.M., Chaudry, I.H., and Baue, A.E.: Characteristics of Sodium and Potassium Transport in the Lung. Am. J. of Physiology. In Press.

h. Chaudry, I.H., Adzick, N.S., and Baue, A.E.: Effect of Hydrocortisone a. Dexamethasone on Xylose Transport by Isolated Rat Soleus Muscle. Biochim. Biophys. Acta, In Press.

i. Chaudry, I.H., Planer, G.J., Sayeed, M.M., and Baue, A.E.: Enhanced Uptake of ^{14}C-ATP by Liver and Kidney of Animals in Shock. Fed. Proc., 34:379, 1975. (Abstract).

j. Sayeed, M.M., Adler, R., D'Agrosa, L.S., Chaudry, I.H., and Baue, A.E.: Hepatic Transmembrane Potentials in Hemorrhagic Shock. Fed. Proc. 34:379, 1975. (Abstract)

k. Chaudry, I.H., Planer, G.J., Sayeed, M.M., and Baue, A.E.: Effect of Osmolarity on Glucose Uptake in Soleus Muscle. Biophysical Journal, 15:312, 1975. (Abstract).

l. Chaudry, I.H., Sayeed, M.M., and Baue, A.E.: Degradation of Adenine Nucleotides by Soleus Muscle in Hemorrhagic Shock. Surgery 77:180-185, 1975.

m. Sayeed, M.M., Wurth, M.A., Chaudry, I.H., and Baue, A.E.: Cation Transport in the Liver in Hemorrhagic Shock. Circ. Shock, 1:195-207, 1974.

n. Sayeed, M.M., Chaudry, I.H., and Baue, A.E.: Active Na-K Transport and ATP Levels in Lung and Liver during Shock. Surgical Forum, 25:5-7, 1974.

o. Baue, A.E.: The Energy Crisis in Surgical Patients. Archives of Surgery, 109:349-350, 1974.

p. Chaudry, I.H., Sayeed, M.M., and Baue, A.E.: Insulin Resistance in Experimental Shock. Archives of Surgery, 109:412-415, 1974.

q. Adzick, N.S., Fishman, L.J., Sayeed, M.M., Baue, A.E., and Chaudry, I.H.: The Effects of Steroids on Insulin Stimulated Xylose Transport in Soleus Muscle. The Physiologist, 17:170, 1974 (Abstract).

r. Chaudry, I.H., Sayeed, M.M., and Baue, A.E.: Effect of Hemorrhagic Shock on Tissue Adenine Nucleotides in Conscious Rats. Can. J. of Phys. and Pharmacol., 52:131-137, 1974.

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° (chilling), followed by 60 minutes at 37°C (rewarming). Fresh tissue cation contents (mean ± SE) in mmoles/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 (mmoles/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 1mM 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 triphospate, adenosine disphosphate, 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 circulatory 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-$MgCl_2$) 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 $MgCl_2$. 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, AMPdeaminase) 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 phenomonon 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)-$MgCl_2$ 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 <u>in vitro</u> 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-HCO_3 buffer containing 10 mM glucose, 5 mM ($8\text{-}^{14}C$) ATP, 5 mM ($8\text{-}^{14}C$) ADP, or 0.5 mM ($8\text{-}^{14}C$) adenosine, and 5 mM $MgCl_2$ for 1 hr under an atmosphere of 95% O_2 - 5% CO_2. 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 ^{14}C-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-$MgCl_2$ 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-$MgCl_2$ 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-HCO_3 buffer containing 10 mM glucose, 5 mM (8-^{14}C) ATP (0.45 μC/μmole) and 5 mM $MgCl_2$ in 95% O_2-5% CO_2 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 ^{14}C-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 ^{14}C-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-$MgCl_2$ 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-$MgCl_2$ 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 μ, 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-$MgCl_2$) 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-$MgCl_2$ on tissue insulin resistance in shock. The results indicate that insulin resistance can be overcome by the infusion of ATP-$MgCl_2$ 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-$MgCl_2$ (0.25 ml, 25 mM/kg) 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-$MgCl_2$ infusion and the two soleus muscles from each animal were incubated for 1 hour at 37°C in Krebs-HCO_3 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-$MgCl_2$ 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-$MgCl_2$ 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-$MgCl_2$ 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 (0.1unit/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 unit/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 uU-200 mU/ml and 10^{-4}M 10^{-2} respectively. Incubations were carried out in a metabolic shaker for 30 minutes at 37°; shaking rate 110 cycles/min; atmosphere 95% O_2-5% CO_2. The muscles were then rinsed, blotted, frozen and homogenized in $Ba(OH)_2$-$ZnSO_4$ amd the supernatant was analyzed for xylose. The results indicate that in control as well as in muscles from ADX animals, 100 mU/ml insulin was required for maximal xylose transport. Hydrocortisone (10^{-4}M), Dexamethasone (10^{-4}M) or Hydrocortisone-21 Na succinate (10^{-2}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/ml insulin. In this study, steroids failed to inhibit insulin-stimulated transport at concentrations higher than known blood steroid levels during shock (10^{-5}-10^{-6}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 714, containing 10 mM glucose and varying amounts of sorbitol to give the required osmolarity. The results, expressed in μmoles/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^{-4} 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

DISTRIBUTION LIST

4 copies	HQDA (SGRD-AJ) Washington DC 20314
12 copies	Defense Documentation Center (DDC) ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences Office of the Secretary of Defense 6917 Arlington Road Bethesda, Maryland 20014