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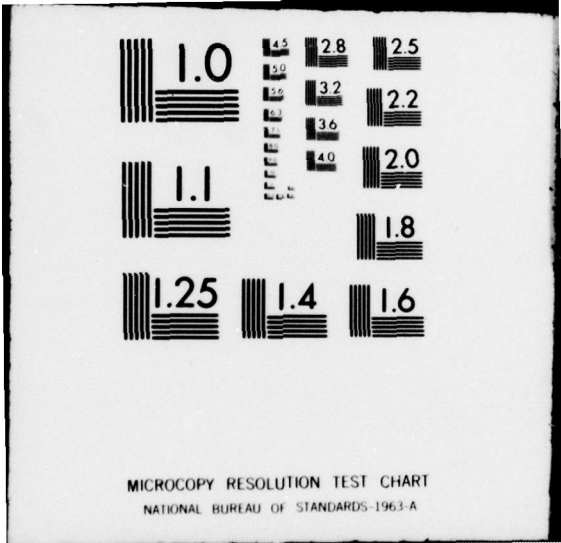
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ALTERATIONS IN TISSUE METABOLISM
WITH INJURY AND SHOCK

Annual Summary Report (A)

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Our studies have shown that infusion of ATP-MgCl ₂ following 60 or 90 minutes of hepatic ischemia improved hepatic function, cellular architecture and improved the survival of animals. To the best of our knowledge, this is the first demonstration that an agent has proved successful in the treatment of post-ischemic hepatic failure. Our results have also shown that infusion of ATP-MgCl ₂ following a severe renal insult significantly improved both			

glomerular and tubular function and preserved cellular structure. Thus, ATP-MgCl₂ appears to enhance recovery from severe acute renal failure. These observations may have important implications for future use in organ preservation and management of post-ischemic acute renal failure. Our results also indicate that the accelerated recovery of post-ischemic acute renal failure by ATP-MgCl₂ infusion could be due to provision of energy to ischemic kidneys thereby decreasing the severity of the necrotic lesion. Studies with sepsis have shown that infusion of ATP-MgCl₂ plus glucose following severe sepsis restored cellular ATP levels and reticuloendothelial system function and improved the survival of animals. Another study indicated that there was no change in arterial prostaglandin levels, however, renal vein samples did show a slight increase in prostaglandin E levels during hemorrhagic shock. In another area of research, we proposed that the activation of glucose transport by the enzyme trypsin takes place via the unmasking of the transport system through its proteolytic action while insulin exerts its metabolic effect through membrane conformation alterations.

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

- A. Hirasawa, H., Chaudry, I.H. and Baue, A.E. "Improved Hepatic Function and Survival with ATP-MgCl₂ After Hepatic Ischemia." *Surgery* 82:655-662, 1978.
- B. Hirasawa, H., Chaudry, I.H. and Baue, A.E. "Beneficial Effect of ATP-MgCl₂-Glucose Administration on Survival Following Sepsis." *Surg. Forum.* 29:11-14, 1978.
- C. Glazier, W.B., Chaudry, I.H., Siegel, N.J., Kashgarian, M., Lytton, B. and Baue, A.E. "Enhanced Recovery from Severe Ischemic Renal Injury with ATP-MgCl₂ Administration After the Insult." *Surg. Forum.* 29: 82-84, 1978.
- D. Lytton, B., Glazier, W.B., Chaudry, I.H. and Baue, A.E. "The Use of ATP-MgCl₂ in the Treatment of Post-Ischemic Renal Failure." *J. Urology* In Press.
- E. Chaudry, I.H., Wichterman, K. and Baue, A.E. "Effect of Sepsis on Tissue Adenine Nucleotide Levels." *Surgery*, In Press.
- F. Wichterman, K.A., Chaudry, I.H. and Baue, A.E. "Studies of Peripheral Glucose Uptake During Sepsis." *Arch. Surg.* In Press.
- G. Hirasawa, H., Chaudry, I.H. and Baue, A.E. "Effect of ATP-MgCl₂ on Liver Ultrastructure Following Hepatic Ischemia." *Fed. Proc.* 37:370, 1978 (Abstract).
- H. Wichterman, K.A., Chaudry, I.H. and Baue, A.E. "Effect of Insulin on Glucose Uptake in Muscle During Sepsis." *Fed. Proc.* 37:456, 1978 (Abstract).
- I. Chaudry, I.H., Hirasawa, H. and Baue, A.E. "Further Evidence of ATP Uptake by Muscle Cells." *Biophys. Soc.* 22nd Annual Meeting, P. 106a, 1978.
- J. Chaudry, I.H., Hirasawa, H. and Baue, A.E. "Impairment of Reticulo-endothelial System (RES) Function with Sepsis and its Improvement with ATP-MgCl₂ and Glucose Administration." *Circ. Soc.* 5:218, 1978 (Abstract).
- K. Chaudry, I.H. and Baue, A.E. "Beneficial Effect of ATP-MgCl₂ Following Various Adverse Circulatory Conditions." *Intern. Conf. on Physiological and Reg. Functions of Adenosine and Adenine Nucleotides.* Banff, Canada, Abstract #2, pg. 8, June, 1978.
- L. Chaudry, I.H. and Baue, A.E. "Transport of ATP into Tissue Cells. Sixth Intern. Biophys. Congress. Kyoto, Japan. pg. 361, 1978.

The following papers which were in press have now been published. They are:

1. Chaudry, I.H., Adzick, N.S. and Baue, A.E. "Glucocorticoid Effect on Gluconeogenesis During Hemorrhagic Shock." *J. Surg. Res.* 24:26-34, 1978.
2. Chaudry, I.H., Sayeed, M.M. and Baue, A.E. "Evidence for the Enhanced Uptake of ATP by Liver and Kidney in Hemorrhagic Shock." *Am. J. Physiol.* 233(3):R83-R88, 1977.
3. Chaudry, I.H. and Baue, A.E. "Depletion and Replenishment of Cellular Cyclic AMP in Hemorrhagic Shock." *Surg. Gynecol. & Obstet.* 145:877-881, 1977.
4. Chaudry, I.H., Wichterman, K. and Baue, A.E. "Alterations in Tissue Adenine Nucleotides During Sepsis." *Physiologist* 20:16, 1977 (Abstract).
5. Baue, A.E. "Subcellular Response to Injury" in J. Najarian and J. Delaney, editors. Critical Surgical Care, pp. 281-292, Miami, Florida: Symposia Specialists, 1977.
6. Baue, A.E. "Sequential or Multiple Systems Failure" in J. Najarian and J. Delaney, editors, Critical Surgical Care, pp. 293-300, Miami, Florida, Symposia Specialists, 1977.
7. Baue, A.E. "Blood Volume in Oligemic Shock" in J.D. Hardy, editor. Rhoads Textbook of Surgery, Principles and Practices, pp. 70-77, Philadelphia, Lippincott, 1977.

Copies of the manuscripts "In Press" or reprints of those already published are included for review. A number of 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 participation and presentation of our work at Mid-Hudson Surgical Society, Fishkill, New York; Ninth Annual Surgical Symposium in Portland, Maine; Spring Meeting of the Southwest Missouri Chapter of the ACS in Springfield, Missouri; being a moderator at the International Symposium on Trauma in Washington, D.C.; and presentation of our work at the Society of University Surgeons in Louisville, Kentucky in February, 1978, Annual Biophysical Society Meeting in Washington, D.C. in March, 1978; Federation of American Society for Experimental Biology in Atlantic City, New Jersey, April, 1978, First Annual Shock Society Meeting, Airlie, Virginia in June, 1978 and International Conference on Physiological and Regulatory Function of Adenosine and Adenine Nucleotides in Banff, Alberta, Canada in June, 1978. In addition, several lectures as visiting professor were presented at the following institutions: Tufts Medical Center, Boston, Massachusetts; Jewish Hospital of St. Louis, St. Louis, Missouri; New York Medical College, Valhalla, New York, Chicago Medical School, Chicago, Illinois; New Rochelle Hospital Medical Center, New Rochelle, New York; and various other lectures on regional and local programs on shock and circulatory failure. The principal findings of the past year will now be summarized.

1. Hepatic Ischemia Studies:

A. Improved Hepatic Function and Survival with ATP-MgCl₂ After Hepatic Ischemia.

Despite its importance for liver transplantation and the problems of hepatic and multiple organ failure, little is known about the prevention or treatment of hepatic injury due to shock or ischemia. In severely injured patients, abnormalities of hepatic function and morphology have been observed frequently. The cause or causes of these abnormalities are not established, although hepatic ischemia has been implicated. Previously we have shown hepatic mitochondrial function as well as cell membrane transport of sodium and potassium are depressed during early hemorrhagic shock, indicating the susceptibility of liver to even small insults. Because of the high metabolic rate, the hepatic cells are vulnerable to the deleterious influence of anoxia; however, the cause of cell death in the ischemic liver is not yet clear.

Previous work from our laboratory has shown that ATP levels in the liver decreased during shock and that infusion of ATP-MgCl₂ at the end of the shock period restored cellular ATP levels and proved beneficial in the treatment of shock. We have shown also that ATP uptake by anoxic or hypoxic organs is greater than in control organs. Moreover, recent studies from our laboratory have shown that ATP-MgCl₂ accelerated the recovery of post-ischemic acute renal failure. Since infusion of ATP-MgCl₂ accelerated the recovery of post-ischemic acute renal failure and proved beneficial in the treatment of shock, the present study was undertaken to determine whether infusion of ATP-MgCl₂ following a period of hepatic ischemia would have any beneficial effects on the recovery of hepatic function.

In order to produce total hepatic ischemia in rats, the portal vein as well as the hepatic artery and the bile duct was occluded by placing a tourniquet around the vessels. Collateral vessels other than the above two blood vessels to the liver were sought, and if found they were ligated. Hepatic ischemia was produced for 60 or 90 minutes. A temporary spleno-femoral venous shunt was established during the occlusion. At the end of the ischemic period, the tourniquet around the portal vein, hepatic artery, and the bile duct was removed in order to re-establish the blood flow to the liver. The abdominal incision then was closed in two layers and the animals received intravenously either: 1) 0.25 ml of saline (controls); 2) 0.25 ml of ATP-MgCl₂ (12.5 μmoles each); or 3) 0.25 ml of ATP or MgCl₂ alone (12.5 μmoles each). Survival was measured over a period of five days. The survival rate in 60 and 90 minutes of hepatic ischemia series was 87.5% and 69.2% in the ATP-MgCl₂ treated group, 43.8% and 23.1% in the control group, respectively. When ATP or MgCl₂ alone was given after 60 minutes ischemia, the survival rate was 20% and 30%, respectively. Thus, treatment of rats with ATP-MgCl₂ but not with ATP or MgCl₂ alone following 60 or 90 minutes hepatic ischemia had a salutary effect on the survival of animals.

In searching for the mechanism of the beneficial effect of ATP-MgCl₂ following hepatic ischemia, we have measured serum enzymes and hepatic ATP levels one hour following the release of 60 minutes of ischemia. SGOT and

SGPT levels (IU/ml, mean \pm S.E.) were 738 ± 113 and 552 ± 152 in the ATP-MgCl₂ treated group, 1981 ± 179 and 1141 ± 110 in the non-treated group, respectively (SGOT and SGPT normal values 38 ± 4 and 12 ± 2). Therefore, SGOT and SGPT levels were significantly lowered with ATP-MgCl₂ treatment ($p < 0.01$). Hepatic cellular ATP levels (μ moles/gm) were 1.87 ± 0.10 and 1.20 ± 0.11 ($p < 0.01$) in the ATP-MgCl₂ treated animals and non-treated rats, respectively (control liver ATP = 2.47 ± 0.08). Thus, increased survival and improved hepatic function after ischemia was associated with elevated cellular ATP levels following ATP-MgCl₂ administration. The beneficial effect of ATP-MgCl₂ following hepatic ischemia could be due to: 1) provision of energy directly to hepatocytes; 2) restoration of hepatocyte function particularly RES function; or 3) restoration of hepatic circulation and prevention of cell swelling. While the precise mechanism of action of ATP-MgCl₂ remains unknown, these observations may have important implications for future use in organ preservation, management of post-ischemic hepatic failure and multiple organ failure. To the best of our knowledge, this is the first demonstration that an agent has proved successful in the treatment of post-ischemic hepatic failure.

B. Liver Ultrastructure with ATP-MgCl₂ After Hepatic Ischemia.

To determine if ATP-MgCl₂ has any beneficial effect on liver ultrastructure, total hepatic ischemia in rats was produced for one hour following which the liver was fixed in situ either: 1) immediately following hepatic ischemia (ischemic group), 2) one hour following hepatic ischemia (non-treated) or 3) one hour following hepatic ischemia and ATP-MgCl₂ infusion (treated). Liver fixation was done by perfusing a mixture of glutaraldehyde-paraformaldehyde (2.4%) via the descending aorta. Electron micrographs of the liver from the ischemic group showed swollen mitochondria, lack of cristae, dilation of cytoplasmic membrane and distended endoplasmic reticulum. Photomicrographs of non-treated group showed similar ultrastructural changes to the ischemic group, however, the severity of the changes was less. Hepatocytes of the treated group showed near normal mitochondria, normal endoplasmic reticulum and cytoplasmic membrane. Our studies have also shown that liver ATP levels of non-treated group were approximately 50% of normal. Treatment resulted in elevated cellular ATP levels. Thus, the beneficial effect of ATP-MgCl₂ following hepatic ischemia could be due to provision of energy to hepatocytes thereby improving the microcirculation. This in turn could reverse the ultrastructural changes produced during ischemia.

2. Renal Ischemia Studies.

A. Enhanced Recovery from Severe Ischemic Renal Failure in Rats with ATP-MgCl₂ Administration After the Insult.

Renal ischemia is a common and important clinical problem which may result from a period of hypotension due to shock or local vascular occlusion. The degree of cellular injury and consequent impairment of renal function depends primarily on the duration of the ischemic insult. Similarly a period of warm ischemia in a donor kidney used for transplantation, prior to long-term preservation by cold perfusion or infusion of cold intracellular

solution, has an adverse effect. In man a warm ischemic period of greater than 30 minutes prior to renal preservation probably results in a significant and permanent degree of impairment of renal function. The injury can be modified by several factors such as cooling of the kidney to decrease oxidative metabolism, or the prior administration of mannitol or furosemide which improve renal hemodynamics and enhanced urine flow. The usual clinical situation, however, often does not allow for any form of pretreatment so that any agent given after the ischemic episode, that would improve or accelerate the recovery of renal function, would be of considerable advantage.

As a result of a clearer understanding of some of the mechanisms involved in ischemic renal failure, a number of agents have been given to experimental animals after a period of renal ischemia to ameliorate the subsequent effects of injury. Renin antagonists have been found to decrease the severity of the ensuing renal failure. Previous work from our laboratory has shown that infusion of ATP-MgCl₂ after 30 minutes of renal ischemia would markedly accelerate the recovery from this renal injury. This study was designed to determine whether the infusion of ATP-MgCl₂ would be successful in ameliorating a more severe renal insult.

Male Sprague Dawley rats (200-300 gm) were subjected to 60 minutes of renal ischemia by placing a clamp across the aorta proximal to the left artery and a sling around the right renal artery. After removing the vascular clamp, one group (n = 10) received no infusion and the other group (n = 12) was infused with 25 μ moles of ATP + 25 μ moles of MgCl₂, IV over ten minutes. Twenty-four hours later, the group which received no infusion had: 1) marked reduced glomerular filtration rate (GFR 144 \pm 45 μ l/min/100 gm, B.W.; control 917 \pm 40), 2) diminished renal blood flow (RBF 2999 \pm 401 μ l/min/100 gm B.W.; control 5095 \pm 270), 3) decreased urinary osmolarity (Uosm 700 \pm 64 mosm/kg; control 1425 \pm 138), and 4) increased fractional sodium excretion (FENa 1.33 \pm 0.36%; control 0.17 \pm 0.04). The animals infused with ATP-MgCl₂ showed marked improvement in 1) GFR 328 \pm 64 (p < 0.05), 2) RBF 3604 \pm 177 (p < 0.05), 3) Uosm 952 \pm 55 (p < 0.05) and 4) FENa 0.57 \pm 0.21 (p < 0.05). These results suggest that ATP-MgCl₂ accelerates renal recovery by: 1) diminishing tubular damage at the cellular level by providing energy essential for vital metabolic pathways, and 2) decreasing renal vascular resistance and preventing further ischemic injury.

In animals given no infusion following renal ischemia, EM studies showed marked vacuolization, mitochondrial destruction and loss of brush borders. Rats infused with ATP-MgCl₂ had fewer ultrastructural changes and better preserved structures. These data indicate that ATP-MgCl₂, when infused after severe renal insult significantly improved both glomerular and tubular function and preserved cellular structure. Thus, ATP-MgCl₂ appears to enhance recovery from severe acute renal injury.

B. The Use of ATP-MgCl₂ in the Treatment of Post-Ischemic Renal Injury.

We have also studied the effects of ATP-MgCl₂ in mini-pigs to determine if the protective effect of ATP-MgCl₂ infusion following ischemic renal injury, observed in rats, could be applied to man. The morphologic and physiologic characteristics of the pig kidney, more closely resembles those of man

than do those of most other experimental animals. Furthermore, consistent and reproducible determinations of renal function can be obtained in these animals, without general anesthesia, by the use of relatively small doses of tranquilizing agents that do not affect renal function.

Male miniature pigs (20-25 kg) were subjected to bilateral renal artery occlusion for 60 minutes. The results indicate that renal blood flow (RBF) was reduced to 65% and glomerular filtration rate (GFR) to 40% of normal in control animals. Administration of ATP-MgCl₂ intravenously immediately after 60 minutes of ischemia resulted in a restoration of RBF to normal and GFR to 74% of normal, 24 hours later. Bilateral renal artery occlusion for 90 minutes resulted in a more severe impairment of renal function which was not improved by the administration of ATP-MgCl₂. ATP-MgCl₂ may exert its effect by improving renal blood flow through inhibition of post-ischemic intrarenal vasoconstriction or possibly by enhancing restoration of intracellular adenine nucleotides. These observations may have important implications for future use in organ preservation and management of post-ischemic acute renal failure

C. Mechanism of Accelerated Recovery of Acute Renal Failure with ATP-MgCl₂ Infusion.

Recent studies from our laboratory have shown that infusion of ATP-MgCl₂ accelerates the recovery of post-ischemic renal failure in rats and minipigs. However, the fate and effects of such administered ATP are not known. To study this, bilateral renal artery occlusion in rats was performed for 30 minutes following which ¹⁴C-ATP (12.5 μmoles, specific activity 1.8 mCi/mole) along with equimolar MgCl₂ was infused intravenously in a total volume of 0.25 ml (treated animals). Thirty minutes following the infusion of ATP-MgCl₂, a sample of blood was collected and small pieces of both kidneys and liver were removed and frozen in liquid N₂. The tissues were homogenized in a TCA-HCl mixture and centrifuged. Tissue extracts and deproteinized plasma samples were subjected to electrophoresis in order to separate and measure labeled nucleotides. The intracellular concentration of ATP was calculated by subtracting the counts present in the plasma from the tissue contents. The results indicated that in normal rats, ATP uptake by liver, left and right kidney was proportionally the same. However, following bilateral renal artery occlusion, ATP uptake by kidneys was enhanced two fold with the corresponding decrease in ATP uptake by liver. These results indicate that ischemic organs selectively take up more of the infused ATP. The fact that there was a decrease in ATP uptake by liver (which was not made ischemic) might suggest that ischemic organs trigger some signal which directs more ATP to be transported into such organs. Thus, the accelerated recovery of post-ischemic acute renal failure by ATP-MgCl₂ infusion could be due to provision of energy to ischemic kidneys thereby decreasing the severity of the necrotic lesion.

D. Accelerated Recovery of Acute Renal Failure by Infusion of Adenine Nucleotides - Magnesium Chloride.

Previous studies have failed to demonstrate that any agent which is given after an acute renal injury can effectively modify the recovery process. We have recently shown that ATP-MgCl₂ infusion following the ischemic

insult will successfully ameliorate the recovery of post-ischemic acute renal failure. In the present study, other adenine nucleotides (AMP and ADP) together with $MgCl_2$ were infused after 30 minutes of bilateral renal artery occlusion. Twenty four hours later: 1) rats that received no infusion, ADP or AMP alone or only $MgCl_2$ had reduced GFR ($355 \pm 40 \mu l/min/100 \text{ gm. B.W.}$ vs. 917 ± 36 control, $p < 0.01$), decreased RBF ($3550 \pm 205 \mu l/min/100 \text{ gm B.W.}$ vs. 9095 ± 271 control, $p < 0.01$) and elevated FE_{Na} ($0.65 \pm 0.07\%$ vs. 0.17 ± 0.04 control, $p < 0.01$); 2) rats given dopamine or phenoxy benzamine maintained low GFR (365 ± 50) despite improved RBF (4078 ± 277); 3) rats infused with either ADP- or AMP- $MgCl_2$ had marked improved GFR (606 ± 46 , $p < 0.01$), increased RBF (4289 ± 229 , $p < 0.01$) and normalized FE_{Na} ($0.28 < 0.07\%$, $p < 0.01$).

These data indicate that adenine nucleotide (ATP, ADP or AMP) together with $MgCl_2$ when infused after an acute renal insult significantly improved both glomerular and tubular function and suggest that these agents may effectively accelerate recovery following acute renal failure.

3. Sepsis - Peritonitis Studies.

A. Beneficial Effect of ATP- $MgCl_2$ -Glucose Administration on Survival Following Sepsis.

Infection remains a serious problem in patients after severe injuries or major life threatening operations. Most such patients who do not survive die ultimately of multiple, sequential or progressive systems failure. It is now clear that a critical ingredient in producing organ failure is sepsis. In fact, remote organ failure is often the first sign of peritonitis or other obscure septic processes. A common cause of pulmonary and renal failure is infection. The reasons why and how a septic process produces organ failure and altered metabolism are not clear. Although appropriate antibiotics, surgical drainage and circulatory support remain the mainstays of treatment, more precise support might be provided if the pathophysiology of sepsis was further clarified.

Since previous studies have shown a salutary effect of ATP- $MgCl_2$ administration on survival of animals following hemorrhagic shock and post-ischemic hepatic failure, the present study was undertaken to determine if ATP- $MgCl_2$ would improve survival of animals with sepsis. Peritonitis in fasted (24 hours) Holtzman rats (300-350 gm) was produced by cecal ligation and puncture. Saline (3 ml/100 gm B.W.) was given subcutaneously at that time. Although all rats were not cultured, blood cultures from eight rats so prepared were all positive for E. Coli, Streptococcus Bovis, Proteus Mirabilis, Enterococcus and Bacteroides Fragilis within six hours. Sixteen hours later, the peritoneal cavity was reopened, the gangrenous cecum was removed, the peritoneal cavity was irrigated with warm saline and the abdomen was closed in layers. Saline was given subcutaneously again at 8 and 24 hours (1.5 and 2.5 ml/100 gm B.W., respectively) after cecal removal. Glucose levels in blood were found to be approximately 30 mg% at the time of cecal removal. (Blood glucose levels in rats fasted for 24 hours were found to be 130 mg%). After measuring blood pressure, animals which were normotensive received intravenously either: a) 0.75 ml of ATP (100 μ moles) - $MgCl_2$ (50 μ moles) and 3 ml of saline, b) ATP- $MgCl_2$ + 2 ml of glucose (50%) + 1 ml of saline (ATP- $MgCl_2$ + glucose), c) 3.75 ml of saline (controls) or d) 2 ml of glucose (50%) + 2 ml saline, and survival was then measured over a period of five days.

The survival rate in rats receiving saline, glucose or ATP-MgCl₂ alone was 45% (9/20), 20% (2/10) and 30% (3/10), respectively. Infusion of ATP-MgCl₂ + glucose, however, resulted in an 80% (16/20) survival ($p < 0.025$ compared to controls). In another group of animals, tissue ATP levels were measured three hours following cecal removal. Hepatic and renal cellular ATP levels ($\mu\text{moles/gm}$) were 1.49 ± 0.08 and 0.73 ± 0.10 ($n = 8$) in the saline-treated group and 2.14 ± 0.18 and 1.27 ± 0.10 ($n = 8$) in the ATP-MgCl₂ + glucose treated animals ($p < 0.005$ for both organs), respectively (sham-operated liver and kidney ATP was 1.99 ± 0.13 and 1.23 ± 0.11 , respectively, $n = 10$). Thus, increased survival following sepsis is associated with restoration of cellular ATP levels which followed ATP-MgCl₂ + glucose administration. The precise mechanism of this synergistic effect of ATP-MgCl₂ combined with glucose on survival is not clear. The septic hypoglycemic animal may require glucose specifically with the added effect that ATP provides. The restoration of hepatic and renal ATP levels following ATP-MgCl₂ + glucose infusion could beneficially affect organ function and therefore the survival of animals. Lower doses of ATP (less than 100 μmoles) with glucose were not effective in other animals in this study. Extirpation of the lesion producing the septic process and metabolic support proved helpful without antibiotic treatment.

B. Reticuloendothelial System (RES) Function During Sepsis and the Effect of ATP-MgCl₂-Glucose Administration on it.

Cellular immunity and non-specific host defenses have important influences on infection and survival following severe injury and major operations. The removal of the bacteria from the blood is normally a function of RES, and the main sites of the RES intravascular activity are the liver and spleen, which comprise about 85% and 10% of the total body activity, respectively. Assessment of the functional integrity of the RES following various pathophysiological conditions therefore seems important. Depression of RES function has been found following hemorrhagic shock, burn injury, hepatic ischemia, traumatic shock, abdominal injury and endotoxin shock. Whether or not RES function is depressed during peritonitis is not known.

We have recently found that IV infusion of ATP-MgCl₂ + glucose (but not glucose alone or ATP-MgCl₂ alone) following sepsis had a salutary effect on the survival of animals. To determine whether the RES function is affected during peritonitis and whether ATP-MgCl₂ + glucose has any effect on it, peritonitis in fasted Holtzman rats was produced by cecal ligation and cecal puncture. Saline (3 cc/100 gm B.W.) was given subcutaneously following cecal ligation and puncture. Sixteen hours following cecal ligation and puncture, the peritoneal cavity was reopened and the gangrenous cecum was removed; the peritoneal cavity was irrigated with warm saline and the abdomen was closed in layers. After measuring blood pressure, animals which were normotensive received IV either 3 ml saline (non-treated rats) or 0.75 ml of ATP-MgCl₂ (100 μmoles of ATP and 50 μmoles of MgCl₂) and 2.25 ml of 50% glucose (treated animals). Two hours following the removal of the cecum, RES function was evaluated by measuring the intravascular clearance of a ¹³¹I-triolein labeled gelatinized test lipid emulsion.

The results indicate that the intravascular half-time ($t/2$) (mean \pm S.E. of 8 animals in each group) in control, non-treated and ATP-MgCl₂ + glucose treated animals was 7.6 ± 0.6 , 12.7 ± 1.8 and 7.1 ± 0.3 minutes, respectively.

Since the $t/2$ in the non-treated animals was approximately doubled ($p < 0.02$) as compared to controls, it indicates that significant depression in RES function occurred during sepsis. Administration of ATP-MgCl₂ + glucose following sepsis resulted in the $t/2$ values similar to sham-operated animals indicating that the impairment of phagocytic activity of the RES was reversed with treatment. The beneficial effect of treatment following sepsis does not appear to be due to hypertonicity since administration of 50% mannitol failed to decrease the $t/2$. While the present studies do not define the specific mechanism of the RE depression during peritonitis, the observation that RE depression was reversed by ATP-MgCl₂-glucose treatment is quite significant. The potential for administration of ATP-MgCl₂-glucose as passive therapy at a time of marked RE depression following sepsis suggests a new therapeutic modality in the treatment of peritonitis.

4. Prostaglandin Levels During Shock.

In the past decade there has been a wealth of information concerning prostaglandins. Prostaglandins have been reported to have numerous effects including extensive vasomotor activity. That prostaglandins might be in part responsible for the irreversibility of prolonged hemorrhagic shock is suggested by the wide range of effects they have even at low concentrations and their wide distribution in tissues. It has been shown that the levels of circulating prostaglandins increase during shock in dogs. The object of our studies, therefore, was to determine whether the increased levels during shock is attributable to increased production or diminished metabolism of prostaglandins. In attempting to study this, we started off by measuring the levels of prostaglandins in plasma to determine if they are indeed altered in rats during hemorrhagic shock. Prostaglandins were measured by the radioimmunoassay procedure. Our results indicate that although there was a tremendous variation even in control values, no increase in the arterial prostaglandin levels were observed which is in contrast to the studies reported using dogs. The renal vein samples, however, did show a slight increase in PGE levels during shock.

Considerable doubt now exists concerning the validity of plasma prostaglandins since recent reports have suggested that the plasma levels of prostaglandin by radioimmunoassay disagree with those by gas chromatography-mass spectrometry by 1-2 order of magnitude. In view of these reports, we have abandoned measuring plasma prostaglandin levels during shock. Pharmacologic manipulation of prostaglandins during shock would be easier and more worthwhile and will therefore be tested.

5. Specificity of Insulin Induced Membrane Conformational Change - A Spin Label Study.

Insulin binding specificity and the nature of its binding induced membrane conformational change have been studied with two covalent spin labels on the isolated plasma membrane (PM) and sarcoplasmic reticulum (SR) membrane from guinea pig skeletal muscle. The effect of trypsin has also been investigated and compared with that of insulin. With high specific activity membrane preparations, there were two types of sites present on both PM and SR in terms of reactivity to N-(1-oxyl-2,2,6,6,-tetramethyl-4 piperidiny)-maleimide (MSL). The highly reactive sites were in a more buried environment while the less reactive sites were more exposed. The addition of insulin to membranes labeled

with low spin labeled concentrations (0.05 mM) causes specific reduction of the spin label signal intensity at the PM buried site but not at the SR membrane. When membranes were labeled at high MSL concentration (0.1 mM for PM and 0.6 mM for SR membranes) both sites were labeled with peripheral sites predominant. The addition of insulin caused a dramatic immobilization of the label on the peripheral site on both the PM and SR membrane. However, when N-(1-oxy-2,2,6,6-tetramethyl-4-piperidiny)-bromoacetamide (BrSL) was used as a labeling agent, insulin produced no change in the environment of the BrSL sites on either PM or SR membranes. Therefore, binding of insulin appears to affect specifically the conformation and environment of the peripheral MSL binding sites. In the case of highly reactive buried sites the insulin effect was specific to PM. This effect differed from trypsin (2 μ g - 2 mg), which caused fragmentation of the membrane proteins as indicated by the release of the spin labeled protein fragments. It is proposed that the activation of glucose transport by trypsin takes place via the unmasking of the transport system through its proteolytic action while insulin exerts its metabolic effect through membrane conformation alterations.

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