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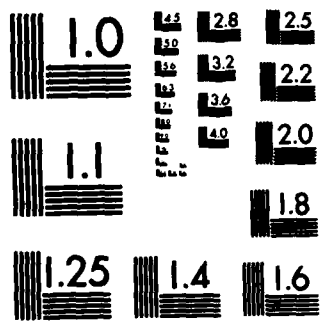
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The Use of ATP-MgCl₂ In The Treatment of Injury and Shock

Annual Report

Arthur E. Baue, M.D.
Irshad H. Chaudry, Ph.D.

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<p>In normal as well as hypovolemic awake dogs, cardiac output can be increased significantly by infusion of ATP-MgCl₂ intravenously at rates of 0.5-2.5mg/kg/min. The higher doses of ATP-MgCl₂ may have detrimental hemodynamic effects, however, such effects are immediately reversible by ceasing ATP-MgCl₂ infusion. Moreover, the deleterious hemodynamic effects of very high doses of ATP-MgCl₂ can be markedly ameliorated by atropine. Infusion of ATP-MgCl₂ did not affect hepatic or renal function, myocardium</p>			

or blood chemistry shortly after infusion or even after a prolonged period of time. Infusion of even massive doses of ATP-MgCl₂ have no ill effects on the survival of dogs. ATP-MgCl₂ can be prepared sterile and pyrogen free and the solution is stable at room temperature for at least 6 months. The response of the primates to ATP-MgCl₂ infusion was somewhat similar to that of dogs. Reinfusion or "priming" the system with ATP-MgCl₂ can increase cardiac output without exacerbation of hypotension in normal or hypovolemic primates. There were no long-term adverse neurological effects of ATP-MgCl₂ infusion in primates. We feel that we now have met all the requirements for obtaining approval from the Food and Drug Administration for ATP-MgCl₂ Phase I clinical studies.

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SUMMARY

The purpose of our studies was to determine how ATP-MgCl₂ might be used, what potential problems with ATP-MgCl₂ might be and develop all the necessary background information in order to initiate clinical trials. Our studies have shown that in normal alert dogs, cardiac output can be increased significantly by infusion of ATP-MgCl₂ intravenously at rates of 0.5-2.5mg/kg/min. Although higher doses of ATP-MgCl₂ may have detrimental hemodynamic effects, such effects are immediately reversible by ceasing ATP-MgCl₂ infusion. Moreover, the deleterious hemodynamic effects (bradycardia and decreased cardiac output) of very high doses of ATP-MgCl₂ can be markedly ameliorated by the administration of atropine. The results also indicate that the hemodynamic effects observed are caused by ATP-MgCl₂ and are not dependent upon the presence of vanadate. ATP-MgCl₂ infusion can produce profound peripheral vasodilatory effects while actually increasing cardiac output even in awake hypovolemic dogs. This suggests that ATP-MgCl₂ may prove beneficial in improving tissue perfusion in low output states. Infusion of ATP-MgCl₂ did not adversely affect platelet counts, white blood counts and differential or serum magnesium levels. Moreover, infusion of ATP-MgCl₂ did not adversely affect hepatic or renal function or the myocardium either shortly after infusion or even after a prolonged period of time. Infusion of even massive doses of ATP-MgCl₂ have no ill effects on the survival of animals. ATP-MgCl₂ can be prepared sterile and pyrogen free and the solution is stable at room temperatures for prolonged periods of time if stored in sealed ampules.

The response of the primates to ATP-MgCl₂ infusion was similar to that of dogs. However, the absolute response in cardiac output were different in the two species. Whereas; continuous infusion of ATP-MgCl₂ in dogs increased cardiac output, reinfusion or "priming" the system with ATP-MgCl₂ was required in order to increase cardiac output in primates. Nonetheless, the fact remains that after "priming" the system with ATP-MgCl₂, cardiac output can be increased up to 55% in conscious normovolemic as well as hypovolemic primates. Moreover, improvement of cardiac output can be obtained with ATP-MgCl₂ without exacerbation of hypotension in hypovolemic primates. The results also suggest that Cynomolgus monkeys tend to be extremely tolerant to hemorrhage and that in order to produce a reproducible model of hemorrhagic shock in these monkeys, the catheters should be inserted in the central lines rather than the peripheral lines for monitoring peripheral blood pressure and bleeding.

The neurological examination of conscious normal primates during ATP-MgCl₂ infusion was also carried out. Infusion of low doses of ATP-MgCl₂ for 7 minutes showed no change in EEG activity. Infusion of high doses of ATP-MgCl₂, however, decreased the voltage and slow waves appeared symmetrically as the mean arterial blood pressure began to fall to a level of 45mmHg. When the infusion was stopped, the EEG pattern remained slowed for sometime, however, the activity picked up when the mean arterial blood pressure reached 90mmHg. At the end of the experiment, the animals were alert and the EEG was back to baseline normal Beta-activity. The behavioral changes noted during the infusion of high doses of ATP-MgCl₂ were consistent with that of suppression of level of consciousness and drowsiness when the mean arterial blood

pressure was within the range of 20-55mmHg. The animals, however, remained symmetrical when stimulated with a normal grasp and pupils remained reactive. The levels of responsiveness increased with the return of blood pressure to a mean of 90mmHg and this was concomitant with the return to a normal EEG baseline activity.

We feel that we now have met all the requirements for obtaining approval from the Food and Drug Administration for ATP-MgCl₂ Phase I clinical studies.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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Body of Report

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

1. Kreis, D.J., Chaudry, I.H., Schleck, S. and Baue, A.E.: Red blood cell sodium, potassium and ATP levels during hemorrhagic shock. *J. Surg. Res.* 31:225-231, 1981.
2. Chaudry, I.H. and Baue, A.E.: Overview of hemorrhagic shock. In: Cell Injury and Shock, Anoxia and Ischemia (ed. Cowley, R.A. and Trump, B.F.) Williams and Wilkins, Baltimore, pp 203-219, 1982.
3. Chaudry, I.H., Clemens, M.G. and Baue, A.E.: Alterations in cell function with ischemia and shock and their correction. *Arch. Surg.* 116:1309-1317, 1981.
4. Baue, A.E., Clemens, M.G. and Chaudry, I.H.: Hemodynamic effects of ATP-MgCl₂ infusion in conscious, normovolemic (NV) and hypovolemic (HV) dogs. *Fed. Proc.* 41(#4):1131, 1982.
5. Clemens, M.G., McDermott, P.H., Chaudry, I.H. and Baue, A.E.: Hemodynamic effects of ATP-MgCl₂ infusion in conscious, normovolemic and hypovolemic primates. *Circ. Shock* 9:185, 1982.
6. Chaudry, I.H.: Preparation of ATP-MgCl₂ and precautions for its use in the study and treatment of shock and ischemia. *Am. J. Physiol.* 242:R604-605, 1982.

The following manuscripts have been submitted for publication:

1. Klay, J.W., Chaudry, I.H., Geha, A.S. and Baue, A.E.: Effect of adenosine triphosphate-magnesium chloride infusion. I. Cardiovascular effects in normal, anesthetized dogs. *Am. J. Physiol.* - Submitted.
2. Klay, J.W., Chaudry, I.H., Geha, A.S. and Baue, A.E.: Effect of adenosine triphosphate-magnesium chloride infusion. II. Cardiovascular effects in acute hypovolemia. *Am. J. Physiol.* - Submitted.

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

1. Chaudry, I.H. and Baue, A.E.: Energy problems during shock and their correction with adenosine triphosphate-magnesium chloride. In: The Role of Chemical Mediators in the Hemodynamic, Pulmonary and Metabolic Failure in the Acutely-Ill and Injured Patients. McConn R. (ed), Raven Press, New York, pp 361-376, 1982.
2. Chaudry, I.H., Schleck, S., Kovacs, K.F. and Baue, A.E.: Effect of prolonged starvation on reticuloendothelial function and survival following trauma. *J. Trauma* 21:604-611, 1981.

A couple of papers are being prepared for submission for publication but are not cited now because they have not been completed. We have also 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 the Fifth Annual Shock Society Meeting in Vermont, the Annual Federation Meeting in New Orleans, Louisiana, and various other lectures at regional and local programs on shock and circulatory failure.

The principal findings of the last year will now be summarized.

1. Hemodynamic Responses to ATP-MgCl₂ Infusion Via the Right Atrium in Normal Alert Dogs.

We have now extended our observations on conscious alert dogs to include infusion of higher doses of ATP-MgCl₂. Infusion of ATP-MgCl₂ at rates of up to 2.5mg/kg/min consistently produces vasodilation, increased pulse pressure, increased cardiac output and a tachycardia. At these doses, myocardial contractility as assessed by left ventricular dP/dT showed little if any, change. At higher doses, however, a bradycardia and variable decrease in dP/dT was observed. At an infusion rate of 5.0mg/kg/min, cardiac output was still increased by ATP-MgCl₂ infusion. Infusion of 10mg/kg/min, however, consistently produced a fall in cardiac output along with bradycardia and decreased contractility which was rapidly reversed on termination of infusion. At all concentrations of ATP-MgCl₂ above threshold, pulse pressure was markedly increased apparently as a result of decreased peripheral resistance. These results indicate that infusion of ATP-MgCl₂ at rates of 0.5-2.5mg/kg/min is effective in increasing cardiac output along with marked peripheral vasodilation. Thus, while higher doses may have detrimental hemodynamic effects, these effects are immediately reversible by ceasing ATP-MgCl₂ infusion.

2. Hemodynamic (Dose-Response) Effect of ATP-MgCl₂ Infusion in Hypovolemic Awake Dogs.

The hemodynamic responses of alert hypovolemic dogs (bled to mean arterial pressure of about 80mmHg) to ATP-MgCl₂ were similar to those of normal dogs. Infusion of ATP-MgCl₂ at rates below 2.5mg/kg/min resulted in peripheral vasodilation, increase in pulse pressure and increase in cardiac output. At higher doses, the peripheral vasodilatory response was still observed, but cardiac output tended to fall. The increase in cardiac output in the hypovolemic dogs was, however, not as marked as that seen in normal dogs (about 35% peak increase in hypovolemic dogs compared to greater than 100% increase in some normovolemic dogs). Furthermore, while the normovolemic dogs responded with a tachycardia at lower doses of ATP-MgCl₂, the hypovolemic dogs showed a bradycardia with all effective doses of ATP-MgCl₂. The observations that ATP-MgCl₂ infusion can produce profound peripheral vasodilatory effects while actually increasing cardiac output even in hypovolemic dogs suggest that ATP-MgCl₂ may prove beneficial in improving tissue perfusion in low output states.

3. Effect of Vanadate Contamination of ATP From Equine Muscle.

The ATP that we have used for all experiments demonstrating the salutary effects of ATP-MgCl₂ has been Sigma's equine muscle ATP. This has been considered to be the highest purity ATP available. Recent reports, however, have demonstrated that a major contaminant of equine muscle ATP is vanadate. The vanadate ion has subsequently been shown to have a variety of physiological effects, including inhibition of Na⁺-K⁺ ATPase. To test the possibility that the hemodynamic effects seen during ATP-MgCl₂ infusion are dependent upon the presence of vanadate, several experiments were performed using Sigma's new vanadium-free ATP from equine muscle. The hemodynamic responses to the vanadium-free ATP were indistinguishable from the responses to the vanadium-containing ATP. These results clearly demonstrate that the hemodynamic effects observed are caused by ATP-MgCl₂ and are not dependent upon the presence of vanadate.

4. Effect of ATP-MgCl₂ Infusion on Hepatic and Renal Function.

We have conducted extensive studies dealing with the effects of ATP-MgCl₂ infusion on serum GOT, GPT, alkaline phosphatase and creatinine levels before and 5 days following ATP-MgCl₂ infusion. Our results indicated that there was no significant effect of ATP-MgCl₂ on the levels of any of the above parameters. Thus, we can conclude that infusion of ATP-MgCl₂ did not adversely affect hepatic or renal function either shortly after infusion or even after a prolonged period of time.

5. Effect of ATP-MgCl₂ Infusion on CPK and LDH Enzymes.

We have also studied the effects of ATP-MgCl₂ infusion on CPK and CPK isoenzymes as well as LDH and its isoenzymes following infusion of ATP-MgCl₂. There was no significant effect of ATP-MgCl₂ on the level of any of the above enzymes indicating that there was no deleterious effects of ATP-MgCl₂ infusion on the myocardium as well.

6. Effect of ATP-MgCl₂ on Serum Magnesium Levels.

Serum magnesium levels were also measured with and without ATP-MgCl₂ infusion in a number of dogs. The results indicated that following infusion of ATP-MgCl₂, the serum magnesium levels increased approximately 25%. However, 5 days following ATP-MgCl₂ infusion, the levels restore to normal. It is not surprising that the serum magnesium levels increase following infusion of ATP-MgCl₂ since MgCl₂ along with ATP was infused. Samples for magnesium levels were not taken between days 1 and 5 post ATP-MgCl₂ infusion. It is, however, possible that magnesium levels return to normal prior to day 5. The return towards normal magnesium levels 5 days following ATP-MgCl₂ infusion, however, indicates that the short term increase in serum magnesium levels is not of any major concern.

7. Effect of ATP-MgCl₂ on Platelet Counts, White Blood Counts and Differential.

We have conducted additional studies on WBC and differential, platelets, hematocrit and hemoglobin measurements prior to ATP-MgCl₂

infusion and at various intervals during and 5 days following infusion of ATP-MgCl₂. There was no significant effect of ATP-MgCl₂ on any of the above parameters. WBC counts were slightly elevated, however, this was due to incisions made for catheters rather than to ATP-MgCl₂ infusion. In addition, WBC counts returned to normal within 5 days post-ATP-MgCl₂ infusion.

8. Safety of Massive ATP-MgCl₂ Infusion in Dogs.

We have now carried out additional studies in which we infused ATP-MgCl₂ in awake normovolemic and hypovolemic dogs at a concentration which is 3 times the dose of the required efficacious concentration of ATP-MgCl₂. Our results indicated that none of the animals died as a result of this either immediately or even after 3 months (at which time the observations were terminated). These experiments therefore clearly indicate that infusion of even massive doses of ATP-MgCl₂ have no ill effect on the survival of animals. In addition, the animals did not exhibit any altered behavior during or following ATP-MgCl₂ infusion.

9. The Effect of Atropine on ATP-MgCl₂ Induced Bradycardia.

One of the major problems associated with administration of very large doses of ATP-MgCl₂ is a paradoxical bradycardia in the face of severe hypotension. Since these doses also produced increased salivation, we administered atropine to three dogs to test whether the bradycardia was produced by increased parasympathetic activity. While ATP-MgCl₂ infusion at a rate of 2.5mg/kg/min consistently decreased heart rate by as much as 34% in the control state, heart rate increased in response to 2.5 or 5.0mg/kg/min after the administration of atropine. Thus, blockade of parasympathetic transmission reversed the heart rate response to one associated with concomitant increases in cardiac output. These results indicate that the deleterious hemodynamic effects of very high doses of ATP-MgCl₂ can be markedly ameliorated by the administration of atropine.

10. Stability of ATP-MgCl₂ Complex.

Our results indicated that if ATP-MgCl₂ solutions were stored at room temperature, there was some bacterial growth within a month of storage and the ATP contents of the solution decreased by approximately 25%. There was no significant decrease in ATP contents of the solutions if such solutions were stored frozen at -70°C. In view of this, additional studies were conducted to determine whether bacterial growth and therefore the breakdown of ATP could be prevented during storage of ATP-MgCl₂ solutions at room temperatures. The following solutions were prepared:

1. ATP in sterile water and pH adjusted to 7.4
2. ATP in phosphate buffer (pH 7.4)
3. ATP-MgCl₂ in sterile water and pH adjusted to 7.4
4. ATP-MgCl₂ in phosphate buffer (pH 7.4)

The sodium hydroxide solution used to adjust the pH was filtered through millipore filter prior to use. The ATP or ATP-MgCl₂ solution was passed through 0.22micron millipore filter prior to storage in microfuge tubes. Half of the microfuge tubes were stored at room temperature while the other half were stored at -70°C.

There was no bacterial growth even after 6 months of storage of ATP-MgCl₂ at room temperature. After 6 months of storage, there was a slight decrease in the concentration of ATP solution which was stored at room temperature but was not found to be significantly different from the calculated concentration of ATP-MgCl₂. Overall the results showed that more than 95% of ATP was detected enzymatically if the ATP-MgCl₂ solution was kept at room temperature or frozen for a period of at least 6 months. The presence or absence of phosphate buffer did not appear to make any difference in terms of the stability of ATP.

The above results lead us to conclude that no significant decrease in ATP contents of the ATP-MgCl₂ solution occur for a period of six months if such solutions are kept sealed at room temperature. It should be mentioned, however, that although ATP concentration as determined enzymatically was essentially unchanged by keeping them at room temperature, it is not known at present whether storing ATP-MgCl₂ solutions at room temperature for prolonged periods of time affects the biological activity of ATP complexed with MgCl₂.

11. Pyrogenicity and Sterility Studies

We have used different batches of ATP to test whether the ATP-MgCl₂ solution which we have been using contains any pyrogens. These studies were carried out by injecting ATP-MgCl₂ solutions in rabbits and recording any change in their body temperature. There was no significant increase in body temperature of rabbits following ATP-MgCl₂ administration suggesting the lack of pyrogens in the batches of ATP that we used. Thus, up to now we have now tested three different batches of ATP and have not been able to find any pyrogens in them.

We have also conducted sterility studies on two additional batches of ATP and our results indicate that the ATP-MgCl₂ solutions were indeed sterile in the manner in which they were prepared. Thus, we can conclude that ATP and MgCl₂ solution could be prepared sterile and the batches of ATP that we used did not contain any pyrogens. Hence, ATP-MgCl₂ solution meets the requirement for administration in humans.

12. Hemodynamic Response to ATP-MgCl₂ Infusion in Normovolemic Primates.

We experienced considerable delay in conducting hemodynamic studies in alert primates because of the quarantine regulations (two months) in our primate research facility. The delay was also caused because of the need for extensive chair training of primates following release from quarantine. Chair training was essential since this allowed reliable study of alert animals. We have, however, been able to conduct four studies so far and have obtained some very promising data. In many ways, the response of the primates to ATP-MgCl₂ infusion is similar to that of the dogs in previous studies. There are, however, significant differences between the two species. In normovolemic primates we have been unable to show any cardiovascular effects with an ATP-MgCl₂ dose of 0.1mg/kg/min via the right atrium. At doses of 0.5mg/kg/min and higher they appear to be much more sensitive to the effects of ATP-MgCl₂ infusion than do the dogs. This is especially evident with response to the ATP-MgCl₂-induced bradycardia that we have previously observed in dogs. This effect is evident at a dose of 0.5mg/kg/min (about 10% decrease in heart rate) and is profound at 2.5mg/kg/min (about

50% decrease). As a result of this paradoxical bradycardia, there is no significant increase in cardiac output at either 0.5 or 1.0mg/kg/min and at 2.5mg/kg/min cardiac output falls. Interestingly, if the mid-range doses (0.5 and 1.0mg/kg/min) are repeated after the monkey has been "primed" with a high-range dose (2.5mg/kg/min) significant increases in cardiac output are observed (up to 55% increase). This is not the result of reversing the heart rate response since there was no significant difference in heart rate response before vs after the high-range dose. At the present time the mechanism of this phenomenon is unclear. Nonetheless, the fact remains that by reinfusion of ATP-MgCl₂, cardiac output can be increased up to 55% even in conscious normovolemic primates.

13. Hemodynamic Response to ATP-MgCl₂ Infusion in Hypovolemic Primates.

We have conducted four experiments in which we infused ATP-MgCl₂ during hypovolemic conditions. Since the same monkeys were previously being used in normovolemic experiments, it was possible to make some interesting comparisons between the two conditions in the same animal. The most notable difference was the hypovolemic (mean arterial blood pressure 75-80mmHg) results in a greatly increased sensitivity to the effects of ATP-MgCl₂ infusion via the right atrium. While the monkeys tolerated infusion of ATP-MgCl₂ at a rate of 2.5mg/kg/min under normovolemic conditions, infusion of 1.0mg/kg/min ATP-MgCl₂ produced severe hypotension (mean arterial blood pressure 15-20mmHg) when administered under hypovolemic conditions. The animal, however, recovered quite well from this severe hypotension once the infusion was terminated. Subsequently, infusion of 0.1mg/kg/min ATP-MgCl₂, a dose which was previously ineffective, resulted in an increase in cardiac output of 55-60%. This dose, however, did not produce a fall in mean arterial blood pressure. Thus, after "priming" the system with ATP-MgCl₂, improvement of cardiac output can be obtained with ATP-MgCl₂ without exacerbation of hypotension.

14. Hemorrhagic Shock in Primates.

We initially attempted to produce a reproducible hemorrhage shock model of proper severity in primates for survival studies with ATP-MgCl₂ administration. Under nembutal anesthesia, blood was removed at a rate sufficient to reduce mean arterial pressure (MAP) to 40mmHg within 15 minutes and MAP was then maintained at 40mmHg for five hours. This required removal of about 50% of the calculated blood volume which decreased cardiac output to less than 40% of control and central venous pressure to less than 1mmHg. Despite this severe cardiovascular insult, reinfusion of less than 10% of the shed blood was required to maintain a MAP of 40mmHg for five hours. At that time the shed blood was returned and the catheters removed. The primate survived the procedure with no apparent ill effects. These results indicate that a more severe shock model will be needed for the planned survival studies.

The severity of the model was then increased by lowering the mean arterial pressure (MAP) to 30 mmHg within 10 minutes and maintaining at 30 ± 5 mmHg. Maximum bleedout occurred within 1 hour from the onset of hemorrhage. This required the removal of a total volume of blood equal to approximately 3% of the animal's body weight. The primates were maintained at a pressure of 30 ± 5 mmHg for five hours. In addition, we have found that the animals could be humanely maintained without

anesthesia after the bleedout has begun. This lack of anesthesia also increases the severity of the insult. Using this model, we have performed well regulated experiments in two male and two female primates. We did not include the third male primate in the study since we could not bleed that animal rapidly enough to drop his MAP to 30 mmHg within ten minutes from the onset of hemorrhage. In the males, both animals used and included in the study required reinfusion of 30-40% of their shed blood to maintain a MAP of 30 mmHg for five hours and were lethargic the day following the experiment. On the second day, one primate steadily improved and ultimately survived while the other became more lethargic and expired approximately 48 hours after the experiment. Thus, in the male primates this appeared initially to be a suitable model to study the effects of ATP-MgCl₂ on survival after shock. The females, however, appear to be more tolerant to hemorrhage. In contrast to the males, in both experiments with females, less than 10% of the total shed blood needed to be returned in order to maintain an MAP for 30 mmHg for five hours. Both animals survived with no apparent ill effects.

Autopsy was performed on the primates which died. The findings indicated that there was massive filariasis involving perirenal left lymphatics and connective tissue which was thickened. Exaggeration of hepatic lobular architecture was also observed. Renal cortical changes were also present. These hypovolemia-induced changes were deemed sufficient to cause death. Diffuse microfilarial infestations were also observed and may have increased this animal's susceptibility to hemorrhage. Thus, although the animal died of hemorrhagic shock, it is not known whether this animal was healthy to begin with.

In order to ascertain that we could produce a reproducible hemorrhagic shock model in primates, additional studies were conducted. It has been possible to perform many more experiments than the original number of primates purchased because the procedure has been repeated on survivors after several weeks recovery under the supervision of the veterinary staff in our primate facility. In these five experiments *Cynomolgus* monkeys were fasted for 24 hours prior to the experiment. The primates were then lightly anesthetized and a 20 gauge Jelco catheter was placed in the femoral artery. The animals were bled rapidly within 10 minutes to a mean arterial pressure of 27 ± 2 mmHg and maintained at this level for 5 hours or until 40% of the shed blood had to be returned in order to maintain the above mean arterial pressure. The remaining shed blood was then returned following which the animals were returned to their cages. Survival was measured over a period of 5 days. Of these five primates, only 1 required more than 12% of its shed volume returned within the 5 hours time constraint of the experiment. This one primate required 40% of his shed blood to be returned within 2.5 hours following the initial hemorrhage and died within 24 hours. The four other primates, however, maintained their mean arterial blood pressure for the duration of the experiment with the return of only 4-12% of shed volume and recovered with no apparent ill effects resulting from the hemorrhage procedure.

From these results we are left to conclude that *Cynomolgus* monkeys tend to be extremely tolerant to hemorrhage and thus a reproducible hemorrhagic shock model cannot be established in this species. However, in a recent site visit, Dr. Ryan Neville of the Letterman Army Institute of Research informed us that Dr. Nelson Gurll from Iowa also had problems

producing a reproducible hemorrhagic shock model in Cynomolgus monkeys but that he has now ironed out those problems. We have now contacted Dr. Gurll to seek his advise concerning the Cynomolgus monkeys. He informed us that he had problems producing a reproducible hemorrhagic model in this species and that the problem was due to the small size of the animals which he and we have been using. Dr. Gurll has been successful in producing a reproducible model of hemorrhagic shock in these monkeys by inserting the catheters in the central lines for monitoring arterial blood pressures and bleeding. According to Dr. Gurll, the peripheral vessels in these small animals collapse after hemorrhagic and thus the mean arterial pressure at the peripheral site is an underestimation of the true mean arterial pressure of the animal. We agreed that this certainly is possible and we now plan to introduce catheters into the central line for monitoring the mean arterial blood pressure. Since Dr. Gurll has been successful in producing hemorrhagic shock in Cynomolgus monkeys, we will follow his advice concerning the procedure and we do hope to have a reproducible primate model of hemorrhagic shock in the very near future. We certainly hope to wrap up the hemorrhagic shock studies in primates within the present period of the contract.

15. Effect of ATP-MgCl₂ Infusion on Behavior and EEG in Primates.

Four experiments have been performed so far concerning the effect of ATP-MgCl₂ infusion on behavior and EEG in primates. ATP-MgCl₂ was administered intravenously at varying dosages and the following report summarizes the effects of this drug on the monkeys' neurological condition and his electroencephalogram. The animals, after arousing from ketamine anesthesia and being placed in a restraining chair, were allowed to become alert. Their neurological examination showed no asymmetry of movement of the forelimb and hind limbs. Grasp in each of the limbs was strong and symmetrical. There was fine lateral horizontal nystagmus, probably secondary to ketamine. Pupils were equal and round and reactive to light. The animals maintained themselves in correct posture. There was no facial asymmetry. In the first experiment monitoring took place with a needle electrode placed in the temporal bipolar montage and the second through fourth experiments were performed with a parasagittal montage with two needle electrodes placed at positions CZ and occipitally approximately one centimeter from the midline and symmetrically placed. They were not subjected to trauma during the experiments. Baseline EEG patterns were symmetrical showing good Beta activity and no sharp activity or slowing. There were occasional artifactual waves and a considerable amount of muscle artifacts when the monkeys moved their heads; however, when quiet, the recording was very adequate for monitoring the brain's electrical activity. Infusion of 0.1mg/kg/min ATP-MgCl₂ for seven minutes showed no cardiovascular effects and the electrical activity remained unchanged. The infusion was stopped and again no change in the EEG was noted. With the infusion of 0.5mg/kg/min ATP-MgCl₂, the voltage began to decrease and slow waves appeared symmetrically as a mean arterial blood pressure began to fall to a level of 45mmHg. At that mean arterial blood pressure, there was significant depression in voltage and slow wave static throughout the period of time that the mean arterial blood pressure remained at approximately 40-50mmHg. When the infusion was stopped, the EEG pattern remained slowed and with low voltage, while the mean arterial blood pressure continued to slowly rise to 65mmHg. When the mean arterial blood pressure reached 93mmHg, the activity picked up. There was still some low voltage activity noted. The slowing,

however, had disappeared. There was gradual recovery of the EEG pattern until it returned to normal, approximately 15 minutes later, with a mean arterial blood pressure of 93mmHg. ATP-MgCl₂ at a rate of 1.0mg/kg/min was then infused. Again, cardiovascular effects were noted. The mean arterial blood pressure dropped to 35mmHg and EEG again slowed and the voltage decreased bilaterally. At a mean arterial blood pressure of around 30mmHg, there was a maximal depression of voltage and maximal slowing bilaterally throughout the period of time the mean arterial blood pressure was around 30mmHg. There was significant and marked depression of voltage with the occasional slow activity. Infusion was then stopped and again, as mean arterial blood pressure began to rise, there was a slow concomitant reversal of the EEG to a normal baseline appearance. However, the EEG did not return to normal until the mean arterial blood pressure was noted to be 90mmHg. After things had returned back to normal in both cardiovascular and EEG responses, 2.5mg/kg/min ATP-MgCl₂ was infused. There was a rapid diminishing of the EEG pattern again, and at this time, the mean arterial blood pressure dropped to 25mmHg. There was maximal depression of voltage and slowing again bilaterally and this was the most severe depression of electrical activity seen throughout the experiment. The infusion was then stopped and the arterial blood pressure began to rise along with an increase in the electrical activity. There was suppression through mean arterial pressure of 45, 55, 65 and 70mmHg following which the EEG gradually returned to normal. Again 1.0mg/kg/min ATP-MgCl₂ was infused and at this time the same EEG suppression following depression of blood pressure was noted. This again returned to normal as the mean arterial blood pressure rose to 90mmHg.

At the end of the experiment, the animals were alert and the EEG was back to baseline normal Beta activity. The behavioral changes noted during the experiment were consistently that of suppression of level of consciousness and drowsiness when the mean arterial blood pressure fell within the range of 20-55mmHg. The animal remained symmetrical when stimulated with a normal grasp and pupils remained reactive. The level of responsiveness increased with the return of blood pressure to a mean of 90mmHg and this was concomitant with the return to a normal EEG baseline. All four animals showed the same EEG and behavioral patterns.

16. IND Application to Food and Drug Association.

We have been in touch with Dr. Stewart Ehrreich at the FDA concerning ATP-MgCl₂ for clinical studies. He informed us that he feels that we have all the information needed to fill the IND application. We will be visiting with Dr. Ehrreich within the month of August or September of 1982 and will submit the ATP-MgCl₂ application for approval by the FDA. We do not feel that we will have any problems in the approval from the FDA for using ATP-MgCl₂ in Phase I and Phase II clinical studies and we certainly hope to conduct a fair number if not all of Phase I studies within the present contract period.

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