

Improved hepatic function and survival with adenosine triphosphate-magnesium chloride after hepatic ischemia

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The present study was undertaken to determine the effect of infusion of adenosine triphosphate-magnesium chloride ($ATP\text{-}MgCl_2$) after 60 or 90 minutes of total hepatic ischemia, since previous work had shown a protective effect of the administration of this complex in postischemic acute renal failure and shock. Following the release of the hepatic vascular occlusion, $ATP\text{-}MgCl_2$, ATP alone, or $MgCl_2$ alone (0.25 ml, 12.5 μ moles each) was given intravenously to treated animals, and saline (0.25 ml) was given to the controls. Survival was measured over a period of 5 days. The survival rate after 60 and 90 minutes of ischemia was 87.5% and 69.2% in the $ATP\text{-}MgCl_2$ -treated animals, 43.8% and 23.1% in the control group, respectively. When either ATP or $MgCl_2$ alone was given after 60 minutes of hepatic ischemia, the survival rate was 20% and 30%, respectively. In another group of animals, serum enzymes and hepatic ATP levels were measured 1 hour following the release of 60 minutes of ischemia. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels were greatly increased following ischemia, and the levels of these enzymes were significantly lowered with $ATP\text{-}MgCl_2$ treatment. Hepatic cellular ATP levels and energy charge were significantly decreased during occlusion; however, ATP levels were increased and the energy charge returned to normal following $ATP\text{-}MgCl_2$ treatment. Thus increased survival and improved hepatic function after ischemia was associated with elevated cellular ATP levels following $ATP\text{-}MgCl_2$ administration. While the precise mechanism of action of $ATP\text{-}MgCl_2$ remains unknown, these observations may have important implications for future use in organ preservation, management of postischemic acute hepatic failure, and multiple systems failure.

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DESPITE ITS IMPORTANCE for liver transplantation²⁶ and the problems of hepatic²² and multiple organ failure,^{2, 14} 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.^{5, 6, 21, 23} The cause or causes of these abnormalities are not established, although hepatic ischemia has been implicated. Previously we have shown that hepatic mitochondrial function as well as

cell membrane transport of Na^+ and K^+ is depressed during early hemorrhagic shock,^{3, 4} 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. Studies on ischemic necrosis of hepatocytes have suggested that the onset of cell death may be the result of either the progressive accumulation of lactic acid or the marked decrease of available high energy phosphate compounds.¹⁵

Previous work from our laboratory has shown that adenosine triphosphate (ATP) levels in the liver decrease during shock⁷ and that infusion of adenosine triphosphate-magnesium chloride ($ATP\text{-}MgCl_2$) at the end of the shock period restored cellular ATP levels⁸ and proved beneficial in the treatment of shock.⁹ We have shown also that ATP uptake by anoxic or hypoxic organs is greater than in

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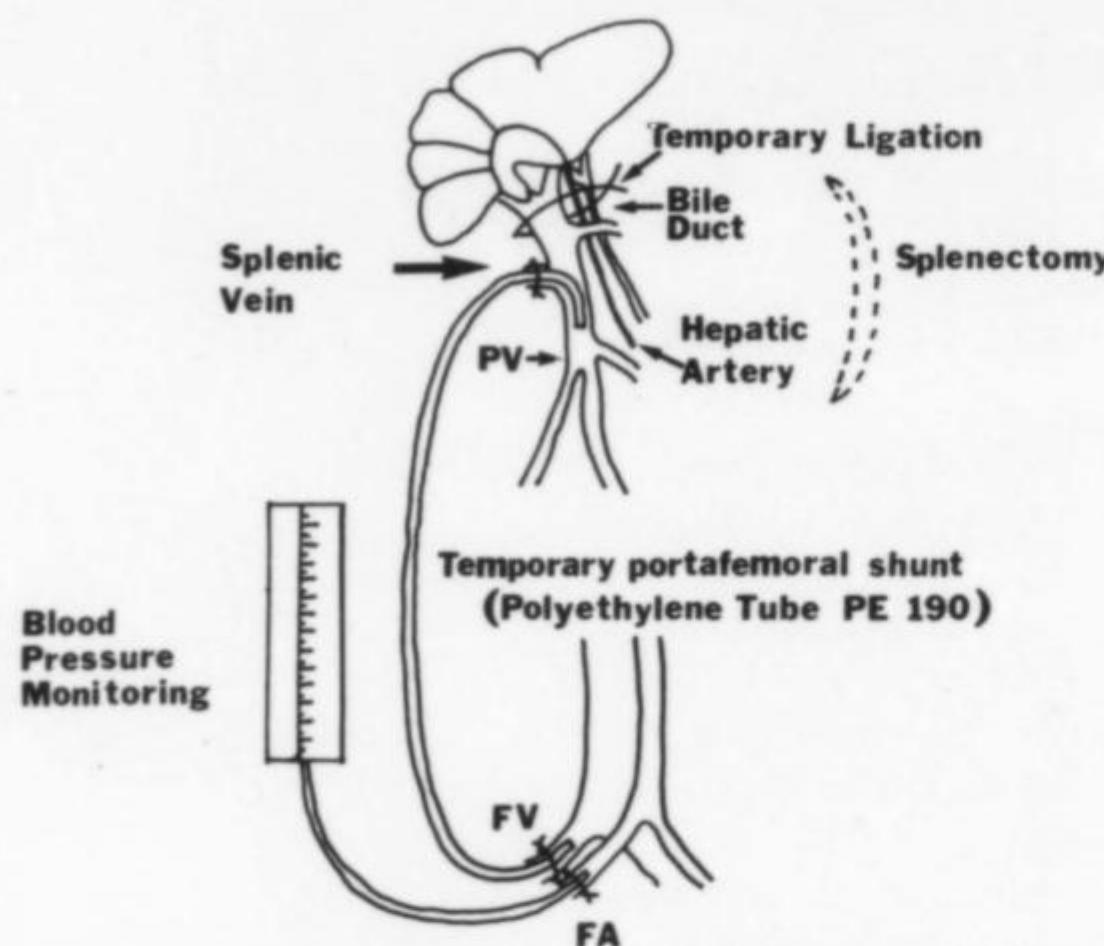


Fig. 1. Model used to produce total hepatic ischemia. A tourniquet (using No. 3-0 silk) was placed around the hepatic artery, portal vein, and the common bile duct. Portal blood was shunted from the splenich to the femoral vein with the use of a PE-190 tubing.

control organs.¹¹ Moreover, recent studies from our laboratories have shown that ATP-MgCl₂ accelerated the recovery of postischemic acute renal failure.²⁴ Since infusion of ATP-MgCl₂ accelerated the recovery of postischemic acute renal failure and proved beneficial in the treatment of shock, the present study was undertaken to determine whether infusion of ATP-MgCl₂ after 60 or 90 minutes of hepatic ischemia would have any beneficial effect on the recovery of hepatic function. The results to be presented indicate that infusion of ATP-MgCl₂ to animals following hepatic ischemia did improve hepatic function and survival of animals. To the best of our knowledge, this is the first demonstration that an agent has proved successful in the treatment of postischemic hepatic failure.

METHODS

Hepatic ischemic procedure. Male albino Holtzman rats, weighing 300 to 350 gm, were fasted for 16 hours prior to the experiment but were allowed water *ad libitum*. The rats were anesthetized lightly with ether, and the abdominal cavity was opened through a midline incision. Splenectomy was performed, following which a temporary extracorporeal splenofemoral shunt was established between the splenic vein and the right femoral vein using a PE-190 polyethylene tubing (Fig. 1). In order to produce total hepatic ischemia, the portal vein as well as the hepatic artery and the bile duct were

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. Blood pressure was monitored via a catheter inserted into the right femoral artery using a precalibrated 1.75 m length of PE-50 tubing. After heparinization (10 mg/kg of body weight), hepatic ischemia was produced for 60, 90, or 120 minutes. During the ischemia period, 0.7 ml of saline was given intravenously at 20 minute intervals for volume replacement. At the end of the ischemic period, the tourniquet around the portal vein, hepatic artery, and the bile duct was removed in order to reestablish 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). Following the administration of one of the above compounds and protamine (10 mg/kg), the catheters were removed and the rats then were allowed food and water *ad libitum*.

Criteria for survival. An animal was considered to survive if he lived at least 5 days following the termination of the experiment. Many animals were allowed to survive for longer periods, of up to 14 days, and showed no ill effects. Autopsy was performed on animals which died following hepatic ischemia as well as those which were put to death. If there were any complications, such as a large hematoma, around the portal vein (two untreated and three treated rats) or intestinal obstruction due to adhesion (one untreated rat), those rats were excluded from the study.

Serum enzyme measurements. Another set of animals was prepared in exactly the same manner as outlined above and was used for blood enzyme measurements. One hour after the hepatic ischemia and infusion of one of the above compounds, blood samples for enzyme study were removed via a catheter inserted into the descending aorta. The serum was separated, and the serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels were measured by the ultraviolet determination method using Sigma kit. Alkaline phosphatase in the serum was measured using Sigma quantitative kinetic determination kit.

Hepatic adenine nucleotide measurements. One hour after 60 minutes of hepatic ischemia and treatment, small pieces of liver were removed using precooled stainless steel tongs which were immersed

Table I. Effect of adenosine triphosphate-magnesium chloride (ATP-MgCl₂) administration following hepatic ischemia

<i>Ischemia time (min)</i>	<i>Treatment*</i>	<i>No. of survivors</i>	<i>No. of deaths</i>	<i>Survival rate (%)</i>
60	Saline (nontreated)	7	9	43.8
60	ATP-MgCl ₂	14	2	87.5†
60	ATP alone	2	8	20
60	MgCl ₂ alone	3	7	30
90	Saline (nontreated)	3	10	23.1
90	ATP-MgCl ₂	9	4	69.2‡
120	Saline (nontreated)	0	10	0
120	ATP-MgCl ₂	0	11	0

*Nontreated animals were studied concurrently with the treatment group on the same day. ATP-MgCl₂ (0.25 ml, 12.5 μmoles each) was given intravenously at the end of the hepatic ischemia. In another series of experiments, ATP and MgCl₂ (0.25 ml, 12.5 μmoles each) were given separately. Nontreated animals received 0.25 ml of saline. Survival was measured over a period of 5 days. For details, see "Methods" section.

†p < 0.01 compared to their nontreated controls.

‡p < 0.02 compared to their nontreated controls.

in liquid N₂. The samples then were homogenized in a solution containing trichloroacetic acid (5%) and HC1 (0.05M) and centrifuged. The supernatant was extracted four times with water-saturated ether and then was neutralized with 1M Tris base. ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were assayed spectrophotometrically using the method of Lowry et al.¹⁸ The energy charge (ATP + ½ADP)/(ATP + ADP + AMP), which is a good indicator of cellular energy status, was calculated from the observed adenine nucleotide values.

Hepatic blood flow measurement during ischemia. The completeness of hepatic ischemia was determined in four animals. Following the occlusion of the portal vein, the hepatic artery, and the bile duct, a catheter was inserted into the aortic arch via the abdominal aorta and Sr⁸⁵ microspheres (average diameter, 15 microns) were injected into the aortic arch. Five minutes following the injection of the microspheres, liver samples were removed and placed in the gamma counter for radioactivity measurements.

Statistical analysis. The chi-square test was used to analyze the difference between survival rates. Student's t test was used to analyze the data for statistical significance of difference between means of enzyme and nucleotide values. Differences with p

Table II. Serum GOT, GPT, and alkaline phosphatase levels after hepatic ischemia (international units per liter)*

	<i>GOT</i>	<i>GPT</i>	<i>Alkaline phosphatase</i>
Sham operation group	37 ± 2.4	12 ± 1.9	33 ± 1.9
Nontreated group	1,976 ± 176.1	1,141 ± 110.5	46 ± 2.3
ATP-MgCl ₂ -treated group	729 ± 95.8†	520 ± 127.1†	45 ± 5.1

Legend: GOT, glutamic oxaloacetic transaminase. GPT, glutamic pyruvic transaminase. ATP-MgCl₂, adenosine triphosphate-magnesium chloride.

*One hour following 60 minutes of hepatic ischemia or sham operation, blood samples were taken from the descending aorta and serum was separated. GOT, GPT, and alkaline phosphatase measurements were made, as described in the "Methods." Values are mean ± SEM of 10 animals in each group.

†p < 0.001 compared to nontreated group and sham operation group.

values of less than 0.05 were considered to be significant.

RESULTS

Blood flow to the liver during ischemia. Labelled microspheres were injected into the aortic arch of animals following the ligation of the hepatic artery, the portal vein, and the common bile duct. Radioactivity was not detected in the liver, indicating that there was no blood getting to the liver when the above vessels were occluded.

Survival rates. By increasing the ischemic period from 60 to 90 minutes, the survival rate decreased in the nontreated group (Table I). Following 120 minutes of hepatic ischemia, there were no survivors. The survival rate after 60 minutes of hepatic ischemia was 87.5% with ATP-MgCl₂ treatment, as compared with 43.8% in the nontreated group (p < 0.01). However, if ATP or MgCl₂ was given alone following 60 minutes of ischemia, the survival rate was 20% and 30%, respectively, which was not statistically different from the nontreated group. Thus, there was no beneficial effect on survival when ATP or MgCl₂ was given separately. The beneficial effect of the nucleotide was specific therefore to ATP in combination with MgCl₂. The beneficial effect of ATP-MgCl₂ was apparent, even when this complex was given 90 minutes following hepatic ischemia. The survival rate in the 90 minutes of hepatic ischemia following ATP-MgCl₂ treatment series was 69.2%, as compared with 23.1% in the nontreated group (p < 0.02). However, following 120 minutes

Table III. Hepatic cellular adenine nucleotides and energy charge after hepatic ischemia (micromoles per gram)*

	<i>ATP</i>	<i>ADP</i>	<i>AMP</i>	<i>Energy charge</i>
Sham operation group	2.47 ± 0.08	0.73 ± 0.10	0.14 ± 0.04	0.85 ± 0.01
Nontreated group	1.20 ± 0.11	0.62 ± 0.11	0.26 ± 0.09	0.71 ± 0.04
ATP-MgCl ₂ -treated group	1.87 ± 0.10	0.60 ± 0.16	0.14 ± 0.04	0.84 ± 0.03
Comparison between nontreated vs. treated	p < 0.001	NS	NS	p < 0.05
Comparison between sham vs. treated	p < 0.001	NS	NS	NS

Legend: ATP, adenosine triphosphate. ADP, adenosine diphosphate. AMP, adenosine monophosphate. NS, not significant.

*One hour following 60 minutes of hepatic ischemia with or without ATP-MgCl₂ treatment or sham operation, small pieces of liver were removed and frozen in liquid N₂. A protein-free extract of the tissues was made and adenine nucleotides were measured enzymatically. For details, see "Methods." Values are mean ± SEM of eight animals in each group. The energy charge was calculated as: energy charge = (ATP + ½ ADP)/(ATP + ADP + AMP).

of hepatic ischemia, ATP-MgCl₂ infusion did not improve the survival rate and all animals died.

Serum enzyme levels. SGOT and SGPT levels in the nontreated animals after 60 minutes of hepatic ischemia were 1,976 ± 176 and 1,141 ± 111 IU/liter ± SEM, respectively (Table II). In the ATP-MgCl₂-treated series, these values were 729 ± 96 and 520 ± 127 IU/liter, respectively (p < 0.001 for both enzymes). The levels of SGOT and SGPT in the sham-operated animals were 37 ± 2 and 12 ± 2 IU/liter, respectively. There was no difference in the alkaline phosphatase levels among the sham-operated group, the nontreated group, and the hepatic ischemic group treated with ATP-MgCl₂. Preliminary experiments have indicated that the alkaline phosphatase levels were normal, even at 24 hours following 60 minutes of ischemia. Since the alkaline phosphatase levels were not affected in the various group, it suggests that no significant damage to the biliary tract system was produced with our experimental procedure.

Hepatic adenine nucleotide levels and energy charge. Following 60 minutes of ischemia, hepatic ATP levels were zero in four rats studied. However, 1 hour following 60 minutes of ischemia (Table III), the hepatic ATP levels (micromoles per gram) were found to be 1.20 ± 0.11, and this value increased to 1.87 ± 0.10 following ATP-MgCl₂ treatment (p < 0.001). Although there was significant increases in hepatic ATP levels following ATP-MgCl₂ treatment, this value still was lower than the value observed in the sham-operated animals (2.47 ± 0.08) (p < 0.001). There was no significant difference in hepatic ADP and AMP levels in various groups of animals studied. The hepatic energy charge in the nontreated group decreased significantly following hepatic ischemia; however, this

value returned to normal following ATP-MgCl₂ treatment.

DISCUSSION

Since allowing a minimum flow of blood to the liver through small collateral vessels during vascular occlusion by itself will improve the survival rate of animals,¹⁵ and in order to provide a uniform insult, it is important that the experimental preparation used is producing complete hepatic ischemia. In the present study the bile duct also was occluded along with the portal vein and hepatic artery. The reason for this was that small blood vessels along the bile duct were present in approximately 50% of the rats studied. Occlusion of these vessels, therefore, assured complete hepatic ischemia. The possibility of collateral vessels to the liver was examined, and they were present in approximately 4% of the rats studied. In such animals those collaterals also were ligated. Our findings are in agreement with the results of Mays,²⁰ who also found that there were virtually no collaterals to the liver in lower animals.

Since certain animals, such as monkeys, can tolerate portal occlusion without a portacaval shunt,¹³ we initially ligated the hepatic artery and portal vein in the rat without establishing such a shunt. However, after 30 minutes of occlusion, all 28 rats which were studied in this way died because of intestinal hemorrhage and pooling of blood in the splanchnic area. Similar results have been observed by Van der Meer, Van der Kely, and Valdenburg.²⁷ For this reason a temporary portacaval shunt was established in all rats used in this study.

It has been suggested by Battersby et al.¹ that the hepatic veins also should be occluded along with the portal vein and the hepatic artery in order to produce total hepatic ischemia in the pig. However,

Farkouh et al.¹⁵ have shown that they could produce total hepatic ischemia by occlusion of only the hepatic artery and the portal vein in the dog. Since we did not occlude the hepatic veins in the present study, it was essential therefore to determine whether there was any blood flow to the liver during portal vein, hepatic artery, and common bile duct occlusion. Our studies using microspheres confirmed that there was no detectable blood flow to the liver following the occlusion of the above vessels. However, following the release of the vascular occlusion, the color of the liver changed from maroon to brownish red, indicating that blood flow was re-established at least on the surface of the liver.

In the present study, splenectomy was performed on all animals, since it was necessary to ligate the splenic vein for establishing a portacaval shunt. Moreover, splenectomy was performed since the spleen is the second largest part of the reticuloendothelial system (RES) and it could play an important role on the survival of animals following hepatic ischemia.

The results presented in Table I indicate that the survival rate of animals decreased as the ischemic period was prolonged. Since none of the animals survived following 120 minutes of hepatic ischemia, irreversible cellular damage occurred sometime between 90 and 120 minutes of ischemia. Thus if hepatic ischemia is required for periods of longer than 90 minutes, some method of hepatic preservation is essential.

The studies of Farkouh et al.¹⁵ have shown that in dogs survival rate after 60 minutes of hepatic ischemia was 40% and 0%, respectively, after 90 to 110 minutes of hepatic ischemia. In the above studies the critical point of hepatic ischemia in the dog was somewhere between 70 and 80 minutes.¹⁵ On the other hand, the studies of MacKenzie et al.¹⁹ have shown that the survival rate after 75 minutes of hepatic ischemia was 60% in dogs. The difference in the critical time of hepatic ischemia in our studies and those of Farkouh et al.¹⁵ may be due to species differences.

The results presented in this paper clearly indicate that infusion of ATP-MgCl₂ following 60 or 90 minutes of total hepatic ischemia proved beneficial for the survival of animals. While the precise mechanism by which this agent improved hepatic function and survival after hepatic ischemia cannot be determined from the present studies, several factors seem important. Postischemic acute hepatic failure is characterized by increased hepatic vascular

resistance and decreased hepatic sinusoidal flow. Both ATP and Mg²⁺ are known to have marked and potent vasodilating effects.¹⁶ However, in our studies of hemorrhagic shock, the effectiveness of this complex (ATP-MgCl₂) could not be attributed completely to its vasoactive effects, since the infusion of ATP alone, MgCl₂ alone, ADP- or AMP-MgCl₂ (which are more potent vasodilators) had no beneficial effect.⁹ Moreover, the infusion of MgCl₂ or ATP separately was ineffective in restoring hepatic function and survival in the present study. Thus the beneficial effect of ATP-MgCl₂ on hepatic function and survival of animals following hepatic ischemia does not seem to be due primarily to vasodilatation.

Measurement of enzyme levels 1 hour following hepatic ischemia indicated that there was a dramatic increase in the SGOT and SGPT levels. However, following ATP-MgCl₂ treatment, the levels of these enzymes were at least 50% lower, suggesting that the function of the hepatocytes following hepatic ischemia and ATP-MgCl₂ treatment was much better. Despite the fact that there was a significant decrease in the level of the above enzyme following ATP-MgCl₂ treatment, these levels still were considerably higher than in controls. However, because these measurements were made 1 hour following ischemia, it is quite likely that the above enzyme levels returned to normal within a reasonable period of time following ATP-MgCl₂ treatment, since the majority of the ATP-MgCl₂-treated rats survived hepatic ischemia.

The results presented in Table III indicate that hepatic ATP levels were increased following ATP-MgCl₂ treatment. However, these levels still were lower than in controls. The fact that the energy charge was normal in such livers would suggest that a new equilibrium in the energy production and utilization was achieved in the hepatic cells following hepatic ischemia and ATP-MgCl₂ treatment. Since there was a significant increase in hepatic ATP levels following ATP-MgCl₂ treatment, it would suggest that parenterally administered ATP was able to cross the liver cell membrane and partially restore the hepatic ATP levels. Previously we have demonstrated that ATP can cross the cell membrane,¹⁰ and that under the altered cellular environment, such as is found in ischemia, more ATP is taken up by the cells.¹¹ Another possibility is that ATP-MgCl₂ treatment provided for more rapid recycling of ATP levels by some mechanism.

It has been suggested that the restoration of RES

function¹⁵ and bile production¹⁷ is extremely important in the survival of animals following an insult. Moreover, it has been suggested that the microsomal membrane dysfunction is related to the onset of irreversible cellular damage and cell death during hepatic ischemia.¹² Although we have not measured RES function, bile production, or microsomal membrane function in the present study, it would appear that the above parameters must have been affected beneficially by ATP-MgCl₂ following hepatic ischemia, since the majority of these animals survived.

The results presented in this paper indicate that administration of ATP-MgCl₂ following 60 or 90 minutes of hepatic ischemia significantly increased survival rate. ATP-MgCl₂-treated rats showed less severe hepatocyte damage and elevated cellular ATP levels than nontreated rats. Thus 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 and management of post ischemic acute hepatic failure and multiple systems failure.

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DISCUSSION

Dr. Benjamin F. Rush, Jr., (Newark N. J.). I would like to congratulate Dr. Chaudry for adding another fine

argument to the case that he and Dr. Baue have so often espoused in favor of ATP treatment of ischemic shock.

[Slide] I apologize for this rather simple diagram, but we have a long tradition in this Society that one cannot discuss a paper without a slide, so I thought I would put this one up. The unhappy cell in the center of the diagram has the problem that has been involving those of us who have been working in the field for many years: how to prop up these sick cells, which has been attempted in a number of different ways. I am convinced that perfusion is an important part of the picture of resuscitating the cell, but there is more than vascular dilatation going on here. Several years ago, Dr. Blackwood from our laboratory reported on the effectiveness of creatine phosphate in ischemic shock, and this is a precursor in vivo of ATP, another bit of evidence in favor of ATP treatment to improve metabolism in shocked animals.

One particular point that I am interested in is your final slide, regarding the mechanism through which ATP may function. While it is simple to assume that ATP supplies new available energy to the cell, it is certain that the total amount of energy needed by the body is many times greater than the dose given.

We all know that Dr. Schumer has been trying to show that cells that have been ischemic for too long a period seem blocked in the anaerobic phase. ATP is one of the things that turns off glycolysis and turns on the Krebs cycle. Glycogen is another substance which gives this signal, and alanine has been effective as well.

My question would be this: are you really convinced that the ATP you give is a source of new energy, or are you saying that it supplies an enzymatic signal which restores aerobic metabolism?

Chairman Bushwald. Did you get the question? [Laughter] Would you like to state your question once again?

Dr. Rush. Are you simply turning a switch, or are you actually supplying energy to the cell?

Dr. Terblanche (Cape Town, South Africa). I would like to ask Dr. Chaudry a question. Dr. Battersby from Australia, when working in our laboratory some years ago, investigated 30 minutes of normothermic hepatic ischemia in the pig. He found that, unless the vena cava also was occluded, significant retrograde blood flow occurred into the liver and complete hepatic ischaemia was not achieved. (Br J Surg 61:27, 1974). As the vena cava could not be occluded in your model, did you feel you had complete hepatic ischemia? I enjoyed this interesting paper.

Dr. Folkert O. Belzer (Madison, Wis.). I enjoyed this paper, and I believe that the authors have shown very nicely that magnesium is extremely important. However, I am very skeptical that exogenous ATP ever gets into the cell. First, it is a large molecule. Second, there are very active extracellular ATPase enzymes which will break down extracellular ATP very rapidly.

My question to you is have you tried to do these

experiments by just adding adenosine and magnesium? I believe that the exogenous ATP is broken down very rapidly to adenosine and that your additional effect is best explained by high levels of extracellular adenosine which will permeate the cell.

Dr. Donald Dean Trunkey (San Francisco, Calif.). In regard to the 60 minute ischemia time, when you used ATP alone or magnesium chloride alone, you actually showed a decrease in survival. I can understand that possibility with magnesium chloride, but have you speculated as to why, with ATP alone, mortality increased, rather than stayed at the control level? Second, with regard to the speculation that this works inside the cell, previous work has shown the mitochondria is probably not the target organelle. Is this providing cyclic nucleotide substrate for the cell, or just maintaining cellular divalent cations. Is this action on the microtubular assembly? What can you tell us in that regard?

Dr. Kenneth G. Swan (Newark, N. J.). I would like to challenge your first conclusion, namely, that you modified survival of rats who sustained hepatic ischemia with the use of ATP magnesium chloride beyond 60 minutes to include the 90 minute observation period, in which you stated that the difference between treated and untreated rats was significantly different at the 5% level ($p < 0.05$). Since you provided the raw data leading to these determinations in your abstract, which at the 90 minute level included six of 10 survivals in the treated group vs. three of 12 survivals in the untreated group, I took the liberty of statistically assessing the probability of significance for the 90 minute observation period. According to my determination, chi-square, including Yates' correction for small numbers, equals 1.5058, which correlates with a p value of 0.2198. Utilizing the same data and applying Fischer's exact test, I arrived at a p value of 0.9029. In view of the fact that, utilizing standard statistical analyses of your data, I arrive at probabilities of significance of approximately 10% and 20%, my question is what statistical analyses did you use to arrive at a biological significance with a confidence level of greater than 95%?

Chairman Buchwald. He used a pocket calculator. [Laughter] I would like to ask one brief question. Your slide showed a controlled time of 44%, ATP and magnesium chloride of 88%, ATP alone of 20%; thus it would seem that ATP alone was detrimental. The ATP and magnesium chloride, by a doubling, was beneficial. Could you comment on that please?

Dr. Irshad H. Chaudry (closing). With reference to Dr. Rush's question, I quite agree that the amount of ATP we are giving to animals is probably not enough to replenish the cellular ATP levels. I believe that the mechanism by which ATP-MgCl₂ proves beneficial in the treatment of postischemic hepatic failure is rather complex. I suspect—and this is simply a hypothesis at this stage—that what happens following ATP-MgCl₂ administration is a combination of different things. It could improve the microcirculation, provide energy directly to cells in which

ATP levels are lowered, have a "priming" effect on the resynthesis of cellular ATP levels, or a combination of the above.

A number of investigators have shown that hypoxanthine and other precursors for the synthesis of ATP are lost during ischemia or low-flow states and therefore the resynthesis of ATP becomes a problem. Perhaps administered ATP acts by "priming" the resynthesis of ATP. It also is possible that administered ATP may be sending some signals somewhere in the cell to trigger the resynthesis of ATP.

The second question was did we have complete hepatic ischemia with our experimental model? The answer to that question is "yes." We occluded every vessel to the liver, including any collateral circulation, but did not occlude the hepatic veins. To double check whether or not we had total hepatic ischemia, we infused radioactive microspheres following the occlusion of the portal vein, hepatic artery, and the common bile duct, and we could not detect any radioactivity in the liver. Thus we concluded that there was no appreciable amount of blood flow to the liver with our experimental procedure. Since we could not detect any radioactivity in the liver, we feel certain that we were producing total hepatic ischemia.

The question of whether or not ATP gets into cells has been asked frequently. On the basis of our experimental observations, as well as on work from a number of other investigators, we feel strongly that ATP indeed can cross or enter the cell membrane.

Two years ago we presented at this meeting evidence that ATP uptake by tissues during low-flow or ischemic conditions is enhanced. The basis for that conclusion was as follows: We incubated tissues from animals in shock with ¹⁴C-labeled ATP and, following incubation, we homogenized the tissues. We then subjected the medium as well as the tissue extract to electrophoresis in order to separate and to measure the various individual nucleotides. Our results indicated that the radioactivity present in the tissue exceeded the radioactivity present in the extracellular space, and therefore we concluded that the excessive radioactivity was present intracellularly.

We also have studied ATP synthesis using ADP as a substrate and adenosine as a substrate. However, the amount of ATP which is found intracellularly when we use ATP as a substrate is significantly more as compared to use of ADP as a substrate, and the amount of ATP formed when adenosine was used as a substrate is only about 2%, as compared with use of ATP as a substrate. Thus, on the basis of those observations, we feel that the cell membrane is permeable to ATP and to a lesser extent to ADP.

The next question was whether it was adenosine rather than ATP which may be acting beneficially in the treatment of postischemic hepatic failure. We have not studied whether or not adenosine has any beneficial effect on the reversal of postischemic hepatic failure; however, from our studies on hemorrhagic shock, in which we infused various breakdown products of ATP including adenosine, none of them had any salutary effects. Moreover, in order to synthesize ATP from adenosine, it takes a significant amount of time. I do not think that the cells which already are depleted of ATP stores can withstand further lack of energy or slow regeneration of ATP. Thus our approach was why not provide ATP directly to the cells.

The next question was why ATP alone decreases survival rate as compared to ATP-MgCl₂. I would like to point out that there was no statistical difference in the survival rates between the nontreated group and the group which received ATP alone; however, ATP-MgCl₂-treated series showed a significant improvement in survival. The reason why ATP alone did not prove beneficial could be due to the fact that ATP is a biologically complexing agent. It acts like EDTA. Because of its complexing nature, ATP could complex with vascular calcium and magnesium and produce a totally different hemodynamic effect. Such undesirable effects are eliminated by giving MgCl₂ along with ATP. Moreover, the presence of MgCl₂ inhibits the deamination and dephosphorylation of ATP, thereby providing higher ATP levels to the target tissues.

The next question was what is the exact site of action of ATP-MgCl₂? This answer is that we do not know. It could be improving the microcirculation, it could be decreasing tissue damage in the centrilobular region, or it could be priming the energy system for the resynthesis of ATP.

The statistical method we used to evaluate our results of various treatment protocols was the chi-square method, and our analysis showed a significant increase in the survival of animals following ATP-MgCl₂ treatment in 60 as well as 90 minutes of hepatic ischemia series.

I believe that the Chairman's question was why did ATP alone have adverse effects on survival as compared with ATP-MgCl₂? I would like to mention that we have done only 10 animals in the ATP-alone series, and I believe that the results with ATP alone were not significantly different compared to the nontreated series.

The important point, however, is that we did not get any increased survival with ATP alone and the reason is that administration of ATP alone and ATP-MgCl₂ produces different hemodynamic effects.

I would like to thank all the discussants for their valuable comments.