

# Beneficial effects of adenosine triphosphate-MgCl<sub>2</sub> administered intravenously to rabbits subjected to haemorrhagic shock

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## Summary

The beneficial effects of adenosine triphosphate (ATP)-MgCl<sub>2</sub> administered as a bolus following fluid infusion or in combination with the infusion fluid were investigated in rabbits subjected to severe but reversible haemorrhagic shock.

ATP-MgCl<sub>2</sub> treatment led to a significant improvement of the metabolic functions of lung and liver tissue. Kidney tissue showed the same tendency, but the improvement did not reach significant levels. The release of lysosomal enzymes *in vivo* was retarded after treatment but not stopped. The mean arterial pressure was kept at a relatively constant level when ATP-MgCl<sub>2</sub> was infused slowly. Administration as a bolus resulted in an immediate dramatic drop in pressure, followed by recovery and then a gradual decrease to levels which appeared to be incompatible with survival.

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cyclic adenosine monophosphate, nicotinamide, tricarboxylic acid cycle intermediates, inosine, adenosine, creatine phosphate, allopurinol, glucose-insulin-potassium and other substrates.<sup>5</sup> Infusion of ATP-MgCl<sub>2</sub> proved beneficial after haemorrhagic shock,<sup>6</sup> post-ischaemic hepatic failure<sup>6</sup> and acute renal failure.<sup>7</sup> Further evidence showed that ATP had a marked protective effect against the deleterious action of phospholipases on cell membranes<sup>8</sup> and that the microcirculation after shock and ischaemia is significantly improved,<sup>5</sup> together with most subcellular functions.

In spite of all the favourable evidence,<sup>4</sup> a high dose of ATP administered intravenously is itself a potent shock-inducing agent, causing respiratory failure and death in animals. Infusion of low doses to hypovolaemic rats did not improve survival or enhance tissue ATP levels,<sup>9</sup> and in shocked, sham-operated and nephrectomized rats the administered radiolabelled ATP in heart, lung and kidney tissue was present almost entirely as non-phosphorylated catabolites.<sup>9</sup> This finding is in direct contrast with claims that ATP-MgCl<sub>2</sub> restored intracellular ATP levels to normal with subsequent reduced mortality rates.<sup>10</sup> Whatever the mechanism of ATP-MgCl<sub>2</sub>, the overwhelming evidence supports its beneficial effects in shock.<sup>11</sup>

In view of the controversy regarding the *in vivo* effects of ATP-MgCl<sub>2</sub> in shock, we planned a series of experiments to investigate its effects on some metabolic variables in our well-standardized rabbit shock model<sup>12</sup> and to compare them with the beneficial results we obtained with hydrocortisone.<sup>1</sup>

## Material and methods

The experimental protocol has been described previously.<sup>13</sup> New Zealand White rabbits weighing 1,6 ± 0,2 kg were used to study the possible beneficial effects of ATP-MgCl<sub>2</sub> in severe haemorrhagic shock.

Rabbits were anaesthetized with intravenous 2,5% thiopentone sodium (30 mg/kg body weight) (Intral Sodium; Maybaker) and transferred to a constant-temperature operating table (38°C). Tracheotomy was performed and a cannula inserted into the trachea to ensure free breathing. A catheter connected to a three-way stopcock was secured in the carotid artery. One opening of the stopcock was attached to a polyethylene tube filled with heparinized saline (Pularin; Evans Medical) 15 U/ml and attached to a mercury manometer or to a pressure transducer of a polygraph (Harvard) to monitor blood pressure. A 20 ml syringe was fitted to the other opening for the withdrawal or replacement of blood. The operation lasted 10 minutes and after a further 10-minute stabilizing period, the control mean arterial pressure was recorded.

The external jugular vein was dissected, cannulated and connected to an infusion bottle. Hypovolaemia was then induced by withdrawing blood over a 10-minute period until the mean arterial pressure reached 30 mmHg (zero time). Small volumes of blood were taken or added during the experiment to maintain an arterial pressure of 30 ± 5 mmHg for a period of 1 hour.

One hour after zero time an infusion consisting of 50% plasma (from blood withdrawn) plus 50% saline, equal in volume to the blood withdrawn, was infused through the jugular vein over 5

Critical evaluation of the effects of adjunct therapy in haemorrhagic shock is extremely controversial. Factors contributing to this controversy are numerous, i.e. the diversity of variables measured, the different animal species used, and differences in the shock models employed as well as the degree of shock at the time of therapy. In our experience<sup>1</sup> any therapeutic regimen, no matter how good it may be, will have no beneficial effects if administered to an animal in an irreversible state of shock. However, Crowell *et al.*<sup>2</sup> claimed that treatment of dogs in irreversible haemorrhagic shock with a combination of adequate oxygen and allopurinol plus hypoxanthine resulted in a significantly increased survival rate. Earlier reports<sup>3</sup> suggested improved survival with parenteral adenosine triphosphate (ATP), and Chaudry *et al.*<sup>4</sup> reported that tissue ATP levels after intravenous, intraperitoneal or intramuscular administration of ATP-MgCl<sub>2</sub> to shocked animals were restored to normal and that this was accompanied by striking improvements in survival.

It is generally agreed that the energy status of the cell is the most important factor in survival in haemorrhagic shock. Attempts have been made to improve energy generation in the cell under adverse conditions with therapeutic agents such as

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minutes. ATP-MgCl<sub>2</sub> was then given either as a bolus (0,5 µmol/kg) or as a constant infusion (5 µmol/kg/h) (10 mM in 0,9% NaCl, pH 7,4, at a rate of 0,5 ml/h). In a third group of animals, hydrocortisone (Solu-Cortef; Upjohn) 50 mg/kg was given together with ATP-MgCl<sub>2</sub> 0,5 µmol/kg as a bolus. After adjunct treatment and infusion, the animals were allowed to survive for another 2 hours. Arterial blood samples were taken after stabilization of the blood pressure, i.e. at zero time and 1 hour, 1 hour and 10 minutes and 3 hours thereafter for the determination of (i) haematoctrit; (ii) total and differential counts; and (iii) β-glucuronidase activity in the blood.<sup>14</sup>

On termination of the hypovolaemic period, the rabbits were exsanguinated and their lungs were perfused *in situ* with 50 ml cold phosphate-buffered saline (PBS) via the pulmonary artery. The lungs, liver and kidneys were excised and transferred to cold PBS until further processing.

Control rabbits of approximately the same weight and age as the experimental animals were anaesthetized in the same way, but hypovolaemia was not induced and no infusion or therapy was given. They were killed by exsanguination and their organs removed as described.

Tissue blocks from the excised organs of control and experimental animals were selected and sliced (0,7 mm) with a McIlwain tissue chopper (only renal cortical tissue was used) for the *in vitro* determination of their capacities to (i) utilize oxygen; and (ii) oxidize glucose as substrate.

### Determination of metabolic capacity of tissue<sup>12</sup>

Triplicate samples of slices of lung (200 mg), liver (200 mg), and kidney (100 mg) from control and experimental rabbits were weighed accurately. The samples were incubated separately in metabolic flasks containing 4 ml Krebs-Ringer bicarbonate, pH 7,4, and saturated with 95% oxygen and 5% carbon dioxide. As substrate 5,5 mM cold glucose with approximately 0,20 µCi

<sup>1-14</sup>C-glucose (56,8 mCi/mmol) or 6-<sup>14</sup>C-glucose (56,1 mCi/mmol) (New England Nuclear) was used. Incubation was performed in a shaking water-bath (90 cycles/min) at 37,5°C for 60 minutes. The <sup>14</sup>CO<sub>2</sub> was trapped in 0,2 ml Carbosorb II (Packard Instrument Co.) added into the centre well of the flask after termination of the incubation period. The CO<sub>2</sub> trapped in the medium was liberated by adding 2,0 ml 6% perchloric acid to the medium in the flasks, which were then incubated for another hour. The Carbosorb was then transferred quantitatively to glass scintillation vials each containing 10 ml Instagel (Packard), and the radioactivity was measured in a Beckman liquid scintillation counter until a counting error of 1% was obtained. DNA determinations were done according to the method of Burton.<sup>14</sup> Results were expressed as disintegrations per minute (dpm) per milligram of DNA.<sup>12</sup>

### Oxygen consumption

Triplicate tissue samples from control and experimental organs were weighed respectively for the determination of oxygen consumption by the direct Warburg method<sup>15</sup> over 1 hour in a Krebs-Ringer phosphate medium with air as the gas phase. Results were expressed as microlitres of O<sub>2</sub> per milligram of wet tissue per hour.

All experiments were repeated at least six times and standard methods were used to compute the mean and the standard error of the mean, using Student's *t*-test.

### Results

Six groups of animals were used to investigate the effect of different forms of adjunct therapy on the capacities of lung, liver and kidney tissues to metabolize glucose and to utilize oxygen *in vitro* (Table I). The groups were (i) normal control rabbits; (ii) rabbits shocked for 1 hour by severe haemorrhage; (iii) rabbits

TABLE I. EFFECT OF INTRAVENOUS ATP-MgCl<sub>2</sub> ON THE METABOLIC CAPACITY OF LUNG, LIVER AND KIDNEY TISSUES TO OXIDIZE 1-AND 6-<sup>14</sup>C-GLUCOSE AND TO UTILIZE OXYGEN

	Lung				Liver				Kidney			
	<sup>14</sup> CO <sub>2</sub> production from glucose (dpm/mg DNA)		O <sub>2</sub> utilized (µl/mg wet tissue/h)		<sup>14</sup> CO <sub>2</sub> production from glucose (dpm/mg DNA)		O <sub>2</sub> utilized (µl/mg wet tissue/h)		<sup>14</sup> CO <sub>2</sub> production from glucose (dpm/mg DNA)		O <sub>2</sub> utilized (µl/mg wet tissue/h)	
	6- <sup>14</sup> CO <sub>2</sub>	1- <sup>14</sup> CO <sub>2</sub>	6- <sup>14</sup> CO <sub>2</sub>	1- <sup>14</sup> CO <sub>2</sub>	3 964 ± 504	0,51 ± 0,05	10 808 ± 5 581	13 092 ± 1 014	1,13 ± 0,12	1- <sup>14</sup> CO <sub>2</sub>	1- <sup>14</sup> CO <sub>2</sub>	1,13 ± 0,12
Control	1 319 ± 233	6 476 ± 425	1,02 ± 0,12	1 451 ± 233	3 964 ± 504	0,51 ± 0,05	10 808 ± 5 581	13 092 ± 1 014	1,13 ± 0,12			
Shock for 1 h	999 ± 127	4 135 ± 1 256	0,67 ± 0,15	1 087 ± 399	3 077 ± 547	0,45 ± 0,11	7 160 ± 1 704	10 597 ± 173	0,94 ± 0,29			
(P < 0,05)	(P < 0,001)	(P < 0,01)	(P < 0,05)	(P > 0,05)	(P < 0,05)	(P > 0,05)	(P < 0,001)	(P < 0,001)	(P > 0,05)			
Shock for 1 h, fluid for 2 h	1 444 ± 157	5 271 ± 611	0,84 ± 0,09	1 131 ± 200	3 197 ± 453	0,57 ± 0,05	8 049 ± 580	10 952 ± 2 268	0,93 ± 0,14			
(P < 0,05)	(P < 0,05)	(P < 0,05)	(P > 0,05)	(P > 0,05)	(P < 0,05)	(P < 0,05)	(P > 0,05)	(P > 0,05)	(P > 0,05)			
Shock for 1 h, fluid for 2 h, ATP-MgCl <sub>2</sub> bolus	1 654 ± 281	6 010 ± 128	0,84 ± 0,14	1 503 ± 323	5 582 ± 1 297	0,58 ± 0,06	8 776 ± 892	13 824 ± 2 965	0,96 ± 0,05			
(P < 0,001)	(P < 0,05)	(P < 0,05)	(P < 0,05)	(P < 0,05)	(P < 0,005)	(P < 0,05)	(P < 0,05)	(P < 0,05)	(P < 0,05)			
Shock for 1 h + infusion for 2 h containing ATP	1 349 ± 151	6 329 ± 712	0,77 ± 0,08	2 038 ± 1 038	4 873 ± 1 262	0,58 ± 0,09	7 951 ± 1 129	10 446 ± 971	0,88 ± 0,08			
(P < 0,001)	(P < 0,005)	(P < 0,05)	(P > 0,05)	(P < 0,05)	(P < 0,005)	(P < 0,05)	(P > 0,05)	(P < 0,05)	(P > 0,05)			
Shock for 1 h, ATP-MgCl <sub>2</sub> + hydrocortisone bolus, fluid for 2 h	2 019 ± 446	6 770 ± 1 364	0,86 ± 0,06	1 488 ± 597	4 804 ± 1 039	0,58 ± 0,04	7 237 ± 1 627	9 054 ± 2 075	0,88 ± 0,05			
(P < 0,001)	(P < 0,005)	(P < 0,05)	(P > 0,05)	(P < 0,05)	(P < 0,005)	(P < 0,05)	(P > 0,05)	(P < 0,05)	(P > 0,05)			

The values for 1 h shock were compared with the control values to evaluate the significance of the decrease in metabolic capacity.

All the other values of adjunct treatment were compared with the values obtained after 1 h shock.

P < 0,05 = significant; P < 0,01 and P < 0,001 = highly significant; ATP-MgCl<sub>2</sub> (bolus) = 0,5 µmol/kg; ATP-MgCl<sub>2</sub> infused at 5,0 µmol/kg/h; cocktail consisted of 0,5 µmol ATP-MgCl<sub>2</sub> + hydrocortisone 50 mg/kg.

shocked for 1 hour followed by fluid replacement; (iv) rabbits shocked for 1 hour followed by fluid replacement and then an ATP-MgCl<sub>2</sub> bolus; (v) rabbits shocked for 1 hour followed by fluid replacement and a constant ATP-MgCl<sub>2</sub> infusion for 2 hours; and (vi) rabbits shocked for 1 hour followed by fluid replacement and an ATP-MgCl<sub>2</sub> plus hydrocortisone bolus. After shock and treatment the animals were allowed to survive for 2 hours before the experiment was terminated.

When comparing the metabolic rates of lung, liver and kidney tissues from normal controls as measured by  $6\text{-}^{14}\text{C}$ -glucose oxidation and oxygen utilization rates, kidney tissue had the highest rate followed by liver and then lung. When  $1\text{-}^{14}\text{C}$ -glucose was used as substrate the oxidation rates followed the sequence kidney > lung > liver.

The *in vitro* oxygen uptake and glucose oxidation rates of liver, lung and kidney tissue were significantly reduced after the rabbits had been exposed to severe haemorrhagic shock (blood pressure  $30 \pm 5 \text{ mmHg}$ ) for 1 hour.<sup>12</sup> Animals exposed to haemorrhagic shock of this degree survived only for 2 hours, unless fluid therapy was given.

Fluid therapy by an infusion volume (50% saline + 50% plasma) equal to the volume of blood removed from the circulation significantly improved the capacities of lung tissue to oxidize  $1\text{-}$  and  $6\text{-}^{14}\text{C}$ -glucose and to utilize oxygen, although these functions were not restored to normal. A very similar but insignificant trend was found with kidney and liver tissues. Administration of ATP-MgCl<sub>2</sub>, either as a bolus following a fluid infusion or by an infusion containing ATP-MgCl<sub>2</sub>, further improved the metabolic capacities of lung and liver tissue to levels significantly above those obtained with fluid alone. A bolus of ATP-MgCl<sub>2</sub> plus hydrocortisone after fluid infusion had a more marked effect on lung tissue ( $P < 0,005$ ) than infusion of ATP-MgCl<sub>2</sub> alone. The response of liver tissue to this form of treatment was less favourable than that of lung tissue.

The metabolic rate as well as other variables measured in kidney tissue did not benefit significantly by any form of treatment. However, a favourable tendency was observed with treatment, as was also found for liver and lung tissue from the same animal under similar experimental conditions (Fig. 1).

On evaluation of the haematocrit values (Table II) a marked decrease was found following haemorrhagic shock due to haemodilution as a result of resorption of fluid from the tissues. This decrease was further accentuated after infusion with fluid because

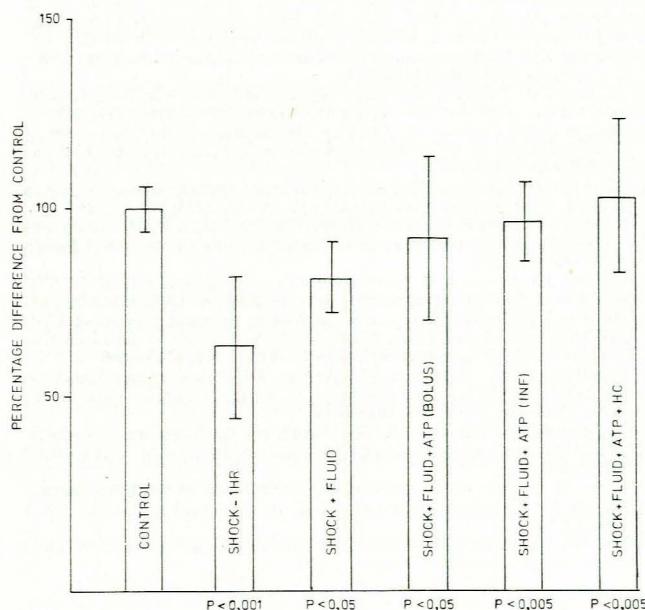


Fig. 1.  $^{14}\text{CO}_2$  production by lung tissue slices from control and severely shocked rabbits followed by different treatment regimens (for statistical analysis shock for 1 h was compared with control values and all the other groups were compared with shock for 1 h).

TABLE II. EFFECT OF ATP-MgCl<sub>2</sub> TREATMENT ON HAEMATOCRIT VALUES AND LYSOSOMAL ENZYME CONCENTRATION IN THE BLOOD OF SHOCKED RABBITS

	Haematocrit	$\beta$ -glucuronidase (mol/l serum/min)*
Control	$39,9 \pm 2,0$	$129,6 \pm 6,9$
Shock for 1 h	$27,1 \pm 2,5$	$725,5 \pm 297,2$ ( $P < 0,001$ )
Shock for 1 h, fluid for 2 h	$21,9 \pm 2,0$	$887,0 \pm 355,5$ ( $P > 0,05$ )
Shock for 1 h, fluid for 2 h, ATP-MgCl <sub>2</sub> bolus	$20,6 \pm 2,5$	$1489,6 \pm 838,9$ ( $P > 0,05$ )
Shock for 1 h, ATP-MgCl <sub>2</sub> infusion for 2 h	$18,7 \pm 3,9$	$1433,0 \pm 646,0$ ( $P > 0,05$ )
Shock for 1 h, ATP-MgCl <sub>2</sub> + hydrocortisone bolus, fluid for 2 h	$23,3 \pm 2,4$	$1308,5 \pm 545,0$ ( $P > 0,01$ )

\* $\beta$ -glucuronidase activity was corrected for haemodilution.

For statistical analysis shock for 1 h was compared with control values and all the other groups were compared with shock for 1 h.

$P < 0,05$  = significant;  $P < 0,01$  and  $P < 0,001$  = highly significant.

of volume expansion. None of the forms of adjunct therapy significantly altered haematocrit values.

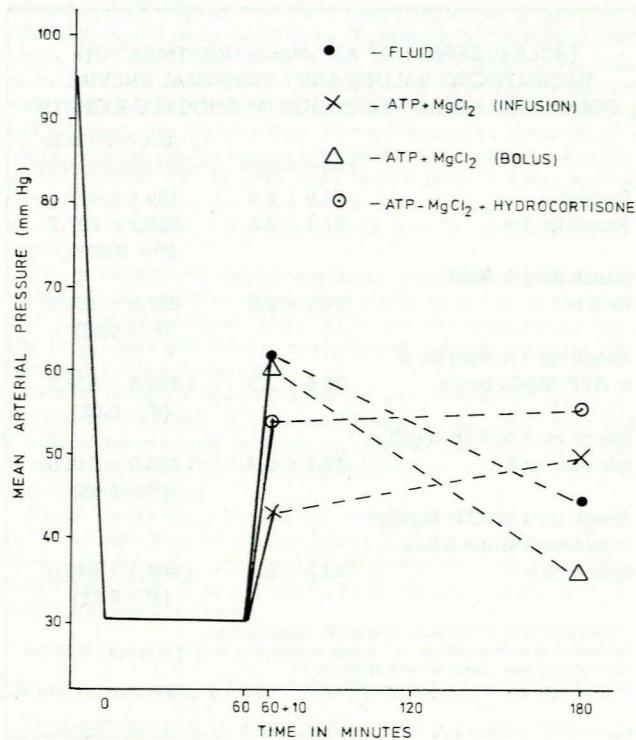
When serum  $\beta$ -glucuronidase activity was compared, a significant increase was found after 1 hour of severe hypovolaemia. Fluid therapy at this stage (volume of fluid equal to the volume of blood lost from the circulation) led to a further but insignificant increase 2 hours after therapy started. Following the administration of therapeutic agents either as a bolus or in the infuse, an insignificant further increase was found in lysosomal enzyme activity after 2 hours.

In Fig. 2 the average mean arterial pressures of the rabbits in each group are set out (no SD values shown). The control mean arterial pressure for all groups was  $95,9 \pm 7,4 \text{ mmHg}$ . In all groups the pressure dropped sharply to  $30 \text{ mmHg}$  within 10 minutes after controlled haemorrhage. Thereafter the pressure was kept at  $30 \pm 5 \text{ mmHg}$  for 1 hour to induce shock. At this stage fluid or fluid plus several forms of therapy were administered. Fluid alone produced an immediate increase in arterial pressure to  $58,1 \pm 15,8 \text{ mmHg}$  followed by a gradual decline over 2 hours to  $43,9 \pm 10,9 \text{ mmHg}$ . When ATP-MgCl<sub>2</sub> was infused, the arterial pressure immediately increased to  $44,4 \pm 12,8 \text{ mmHg}$  and stabilized gradually at a value of  $49,0 \pm 5,8 \text{ mmHg}$  2 hours later. With an infusion of ATP-MgCl<sub>2</sub> plus hydrocortisone a very similar trend was observed and the mean arterial pressure in the terminal stage was raised to  $53,4 \pm 14,9 \text{ mmHg}$ . ATP-MgCl<sub>2</sub> administered as a bolus increased the mean arterial pressure to  $58,6 \pm 11,6 \text{ mmHg}$ . However, it gradually declined to  $36,8 \pm 6,6 \text{ mmHg}$  in the terminal stage, which was lower than the value for fluid therapy alone.

## Discussion

Recently it was shown<sup>12</sup> that the capacities of tissue from animals in haemorrhagic shock to metabolize glucose and to utilize oxygen *in vitro* are sensitive parameters in assessing the degree of shock. This principle was applied in the present evaluation of the beneficial effects, if any, of ATP-MgCl<sub>2</sub> therapy using a well-standardized shock model. The metabolic responses of lung, liver and kidney tissue from the same animal were separately evaluated after adjunct treatment.

From previous experience<sup>12</sup> the degree and duration of the haemorrhage induced was such that all the rabbits were still in



**Fig. 2.** Mean arterial pressure (mmHg) of control and experimental groups ( $N > 6$ ) to demonstrate the long-term effects of adjunct therapy (for details and SD values see text).

a reversible phase of shock before adjunct therapy was started. Fluid therapy therefore led to a remarkable recovery of lung, liver and kidney tissue as judged by their capacities to oxidize glucose *in vitro*. It would appear that the plasma-saline infusion (v/v) we used made a special contribution to improving the perfusion of the microcirculation as well as to the partial hydration of the dehydrated tissues. This improvement is reflected in increased metabolic rates of all three tissues.

The beneficial effect of ATP-MgCl<sub>2</sub> administered either as a bolus or in combination with fluid was highly significant and the metabolic rates of lung and liver tissue with glucose as substrate were increased to levels well above normal control values. An infusion of a cocktail of ATP-MgCl<sub>2</sub>-hydrocortisone had an even more marked effect in lung tissue than ATP-MgCl<sub>2</sub> alone. These findings substantiate the results of Chaudry<sup>5</sup> and Ohkawa *et al.*<sup>7</sup> but are in direct contrast to the findings of Schloerb *et al.*<sup>9</sup> in a rat shock model. ATP-MgCl<sub>2</sub> treatment had a minor effect on the recovery of kidney tissue as judged by our criteria. However, the results showed the same tendency in kidney tissue as was observed for lung and liver, but owing to large intervariation between animals no significant improvement in the metabolic capacity of kidney tissue could be established after treatment. Our results are therefore in contrast with claims<sup>7</sup> that ATP therapy had a beneficial effect in acute renal failure.

ATP therapy had no significant effect on haematocrit values. However, when a cocktail of ATP and hydrocortisone was administered a marked increase was constantly observed. This increase could probably be due to the effect of the hydro-

cortisone on the microcirculation, and implies that either more red cells are mobilized in the circulation or fluid has left the circulation.

Concerning the effect of ATP-treatment on lysosomal enzyme release as represented by  $\beta$ -glucuronidase activity in the blood, no significant beneficial effect was found. In fact, the general trend was an increase in lysosomal enzyme activity. A similar observation<sup>1</sup> was made with hydrocortisone therapy. It is suggested that the increase in lysosomal enzyme activity could be accounted for by a wash-out effect due to improved microcirculation or an extremely long half-life of this enzyme.

The short-term effect of ATP-MgCl<sub>2</sub> on the mean arterial pressure is one of vasodilatation. When given as a bolus an overdose could be fatal due to severe hypotension, bradycardia and respiratory failure. The long-term effect of ATP-MgCl<sub>2</sub> on the arterial pressure is thus dependent on the dose and the way it is administered. When it was given in a single dose following an infusion of fluid, the arterial pressure gradually fell to levels which seemed to be incompatible with survival. When it was given as a gradual infusion, the immediate rise in pressure due to volume expansion was lower than that obtained with the same volume of fluid alone, but the arterial pressure rose gradually over a period of 2 hours to levels very favourable for survival. This finding could therefore explain the controversy<sup>4,9</sup> regarding the beneficial effects of ATP therapy when judged by survival as one of the important criteria.

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