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SMALL VOLUME RESUSCITATION OF HYPOVOLEMIC SHOCK

ANNUAL AND FINAL REPORT

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Studies on unanesthetized sheep were performed to evaluate the effectiveness of hypertonic saline dextran formulations and studies on anesthetized rat measured changes in intracellar metabolism of skeletal muscle, kidney and brain during shock and resuscitation. Based on presented data we conclude: 1) Hypertonic 7.5% saline 6% dextran (HSD) can safely and effectively be delivered via peripheral vessels. 2) Hypertonic saline hetastarch works almost as well as hypertonic saline dextran. 3) Higher concentrations of dextran added to hypertonic saline increase the volume expansion and cardiovascular improvement. 4) NMR shows that falls in ATP occur very late in kidney, brain and skeletal muscle during hemorrhagic shock. The P-Cr/Pi ratio decreases in shock and was decreased more in animals that were in irreversible shock. 5) Multiple small bolus infusions (1 ml/kg) of HSD can be used for resuscitation. 6) A 29% NaCl/24% dextran solution given in a volume of 40ml fully resuscitated a 1.6 liter hemorrhage. 7) Large volume isotonic resuscitation and small volume hypertonic resuscitation resulted in similar distributions of cardiac output.

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8) HSD resuscitation was found safe and effective in dehydrated animals, although dehydration causes animals to tolerate less blood loss.

Foreword

In conducting research using animals, 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) 86-23, Revised 1985).

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Summary

Studies on unanesthetized sheep and anesthetized rats were performed to evaluate the effectiveness of hypertonic saline dextran formulations. Based on presented data we conclude:

1. Hypertonic 7.5% saline 6% dextran (HSD) can safely and effectively be delivered via peripheral vessels.
2. Hypertonic saline hetastarch works almost as well as hypertonic saline dextran.
3. Higher concentrations of dextran added to hypertonic saline increase the volume expansion and cardiovascular improvement.
4. NMR shows that falls in ATP occur very late in kidney, brain and skeletal muscle during hemorrhagic shock. The P-Cr/Pi ratio decreases in shock and was decreased more in animals that were in irreversible shock.
5. Multiple small bolus infusions (1 ml/kg) of HSD can be used for resuscitation.
6. A 29% NaCl/24% dextran solution given in a volume of 40 ml fully resuscitated a 1.6 liter hemorrhage.
7. Large volume isotonic resuscitation and small volume hyeprtonic resuscitation resulted in similar distributions of cardiac output.
8. HSD resuscitation was found safe and effective in dehydrated animals, although dehydration causes animals to tolerate less blood loss.

1. Statement of Problem

Animal investigations in our lab (1,2,3,4) and those of others (5,6,7,8) have established that small volume infusions of hypertonic saline can effectively restore cardiovascular function after hypovolemic shock. It was our overall goal to extend our experience and knowledge of small volume resuscitation. The specific aims of this project for year 01 through 03 were:

1) Evaluate the importance of the amount and type of colloid in hypertonic resuscitation.

2) Evaluate the possibility of using peripheral vein access for hypertonic resuscitation.

3) Determine the effectiveness and safety of multiple bolus injections of hypertonic saline.

4) Measure the distribition of cardiac output during hypertonic resuscitation.

5) Evaluate the efficacy of hypertonic resuscitation in dehydrated animals.

6) Determine the physiological mechanisms of hypertonic resuscitation.

7) Use NMR to measure intracellular energy stores during hemorrhage and hypertonic saline resuscitation.

2. Background

Basic and clinical research stimulated by World Wars I and II established the basis for current treatment of hypovolemic shock as 1) control of hemorrhage and 2) restoration of vascular volume (9). A hemorrhaged soldier arriving at a field hospital in Vietnam was given definitive care - prompt surgical control of bleeding and intravenous infusions of physiological salt solution and/or blood as needed (10). Despite the availability of effective resuscitation therapy in hospitals exsanguination remained the main cause of mortality, being responsible for 50% of all deaths even with a highly efficient system of rapid helicopter evacuation (11,12). A recent model analysis based on Vietnam casualty statistics concluded "for there to be significant improvement in combat casualty care there must be a renewed emphasis on field medical care, with special attention to management of hemorrhage" (12). Successful field resuscitation has been limited by the large volumes required of solutions of crystalloid (2-4x shed blood volume) and colloid (1-1½x shed blood). Logistically feasible field therapies are needed which will reestablish near normal cardiovascular function, and protect against the deleterious metabolic alterations of tissue ischemia.

A novel approach to resuscitation is suggested by the studies of Rocha e Silva and his colleagues (5,6) in which hemorrhaged dogs were successfully resuscitated with a small bolus infusion of hypertonic saline. A 2400 milli-osmolar solution of sodium chloride equal in volume to only 10% of shed blood rapidly returned cardiac output and blood pressure to normal (5). These significant and rapidly beneficial effects of hypertonic infusions have been generally verified in our studies of hemorrhage and resuscitation of the unanesthetized sheep (1,2). Improved survival has been demonstrated in an anesthetized dog by Rocha e Silva and more recently in conscious swine by Traverso et al. (5,7). Resuscitation with equal volumes of 1200 mosm and 4800

mosm sodium chloride were not as effective as with 2400 mosm. The exact mechanisms of hypertonic resuscitation remain undefined but are at least partly due to plasma volume expansion subsequent to a cellular/extracellular fluid shift and a significant reduction in peripheral resistance. In addition, stimulation of a pulmonary osmoreceptor may initiate a reflex decrease in venular capacitance, thus effectively increasing cardiac output to normal levels despite blood volumes less than normal (6).

We have shown that the initial rapid improvement in cardiovascular function is a function of the increased osmolality per se and does not require either sodium or chloride (4). Figure 1 shows a study in which we performed a screening evaluation of the effectiveness of five different hypertonic solutions.

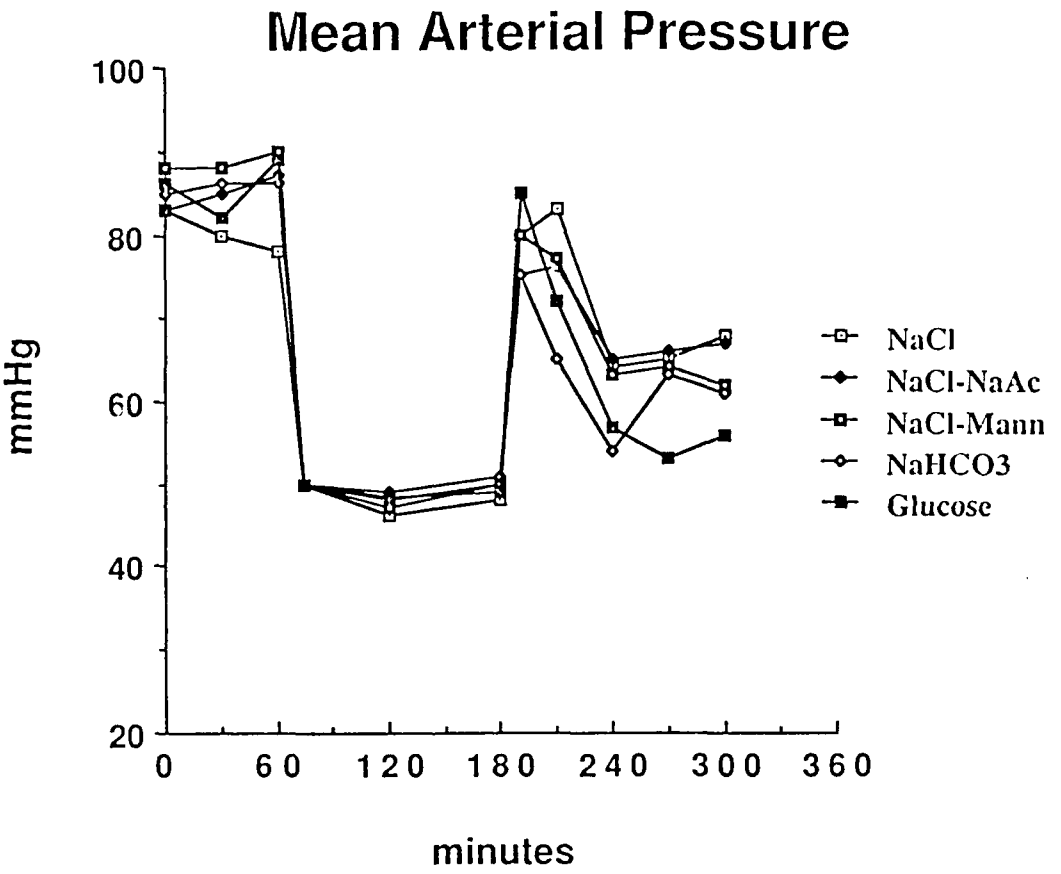


Figure 1.

Mean arterial pressure is plotted for baseline conditions, during 2 hours of hemorrhage (blood loss = 40-45 ml/kg) and for 3 hours after a 4 ml/kg bolus of 2400 mosm solutions of sodium chloride (NaCl); sodium acetate-sodium chloride mix 50:50 (Acetate); Glucose; Mannitol-sodium chloride mix 60:40; and sodium-bicarbonate (Bicarb). Clearly all solutions rapidly returned cardiac output to normal levels within minutes of bolus injection. Thereafter, blood pressure slowly declined with all solutions. The most effective solution was 2400 mosm saline which caused rapid and full restoration of cardiovascular function, but the improvement was only transitory.

At this time we began to consider the possibility of adding a hyperoncotic colloid to the formulation. We reasoned that the hypertonic sodium chloride would pull water out of the cell while a hyperoncotic colloid would selectively partition this water in the

vascular space. This idea was suggested by our earlier work on dextran 70 in burn resuscitation in which we found dextran to be a highly efficient plasma volume expander associated with a good cardiovascular response (13). In a detailed study (Figure 2) we compared a mixture of hypertonic sodium chloride mixed with 6% dextran 70, (HS-Dex) against hypertonic saline alone (HS) and 6% dextran 70 in normal saline (Dex) and no resuscitation. Hypertonic saline-dextran (NaCl-Dex) resulted in significantly higher values of sustained cardiac output, mean arterial pressure, and measured plasma volume while the total peripheral resistance was lower when compared to hypertonic saline alone or dextran alone. In short, while the hyperosmotic solutions caused a large and immediate improvement in cardiovascular function the addition of the dextran was required to sustain the effectiveness.

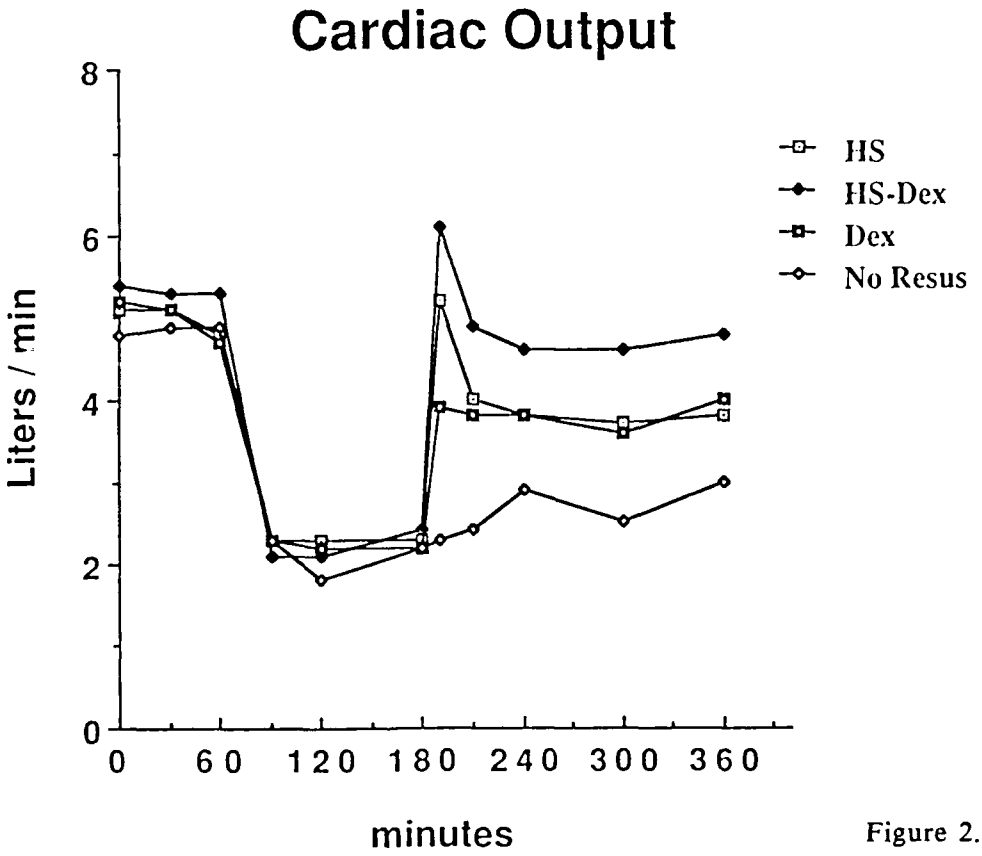


Figure 2.

In the past three years, we have completed further analysis of small volume hyperosmotic/hyperoncotic resuscitation of dextran. Summaries of these studies are presented in this final report.

3. Rationale

The overall rationale of our study is to evaluate the efficacy and safety of small volume hypertonic resuscitation in experimental animals. Specifically, we will quantitate the cardiovascular response and metabolic response of vital organs during shock and after therapy with different hypertonic formulations and resuscitation regimens. We believe that these experiments will establish potential clinical therapeutic regimens. It is our hope that these regimens will provide field corpsmen with a logistically feasible and effective means to stabilize cardiovascular function in wounded soldiers until definitive care at a field hospital can be provided.

4. Experimental Methods

All experimental procedures outlined below have been previously used by the investigators (1,2,4,13,14). Our laboratory is equipped for aseptic survival surgery and cardiovascular monitoring of unanesthetized and anesthetized animals.

Sheep were used to study the cardiovascular responses to different resuscitation regimens: a) effects of multiple boli infusion of hypertonic saline dextran, and b) comparisons of hypertonic saline versus hypertonic saline dextran. Sheep were anesthetized with halothane/ nitrous oxide for placement of silastic catheters in the thoracic aorta and vena cava and a Swan Ganz thermodilution catheter in the pulmonary artery. Experiments on awake sheep were performed 4-7 days after surgery. Sheep offer several advantages. They are a relatively inexpensive large animal and are easy to study in the unanesthetized state. The awake sheep's cardiovascular response to hemorrhage and resuscitation is similar to man (9), and its response is more applicable than those measured in anesthetized animal preparations. At a blood pressure of 60 mm Hg and lower sheep lie down in their cages. They experience no apparent pain during hypotension, are generally lethargic but conscious for the entire experimental protocol. All sheep experiments consisted of measurements made during a 2 hr baseline period; 2 hr of hemorrhagic hypotension (50 mm Hg) maintained by bleeding without reinfusion, and 2-4 hrs of post-resuscitation monitoring.

Rats were used for in vivo monitoring of intracellular high energy phosphates of skeletal muscle. The low cost of rats allowed the many experiments required to establish statistical significance in survival studies, while their small size allowed them to fit into the topical NMR for non-invasive monitoring. Rat experiments typically consisted of a baseline period, 60-90 minutes of hemorrhage (40-50 mm Hg) and a post resuscitation period.

Measured Variables:

Sheep: Pressures of the aorta, pulmonary artery, pulmonary wedge and right atrium were measured with P23 Gould transducers. Pressures, and heart rate were monitored on a multi-channel strip chart recorder. Cardiac output was determined by thermal dilution using an Edwards Cardiac Output Computer. Arterial blood gases and pH were measured with an Instrumentation Laboratories Blood Gas Analyzer. Arterial and mixed venous oxygen content were determined on an Instrumentation Laboratories Co-oximeter. Oxygen consumption was calculated as the average cardiac output times the difference in A-V oxygen content. Urine output was determined by continuous collection from a Foley retention catheter. Plasma and urine concentrations of Na^+, K^+ and Cl^- as well as total osmolality were measured by flame photometry, acid titration and freezing point depression respectively. Lactate was measured on protein precipitated blood samples with enzyme assay. Creatinine clearance was determined by enzymatic assays of blood and urine. Plasma volume was measured as the distribution volume of Evans Blue dye.

Rat: Arterial and central venous pressure and heart rate were determined by placement of PE 50 catheters and pressure monitoring. Intracellular metabolic status was evaluated by using topical NMR to monitor relative levels of phosphocreatine, ATP, inorganic phosphate and pH in skeletal muscle. HPLC and enzymatic assays on excised tissue samples were performed to quantitate high energy phosphates, lactate and ammonia and other metabolites.

Results:

Our research on hypertonic saline dextran (HSD) resuscitation over the last 3 years has resulted in 7 published manuscripts and 9 published abstracts. The following is a summary of our work which is organized to relate directly to the numbered specific aims listed in the Statement of Problem section.

1) The importance of the amount and type of colloid (published manuscript M4, abstract A1)

Infusion of hypertonic 7.5% NaCl (HS) in small volumes, 4-6 ml/kg, rapidly improves arterial pressure and cardiac output in hemorrhaged animals (1). The addition of 6% dextran 70 (Dex) sustains the improved cardiovascular function and significantly improves survival (4,8). To further evaluate and confirm the importance of the added dextran to the hypertonic saline, we compared resuscitation with 3 solutions - 2400 mosm hypertonic saline alone (HS-0% dex), hypertonic saline with 6% dextran 70 (HS-6% Dex) and hypertonic saline with 24% dextran 70 (HS-24% Dex). After a 2 hour period of hemorrhagic hypotension in conscious sheep we resuscitated with 100 ml (~2 ml/kg) of test solution. We used these smaller volumes because our previous studies had established that 200 ml of hypertonic saline/6% dextran resulted in near normal restoration of blood pressure and cardiac output. We reasoned that with the smaller volumes, we were more likely to establish differential responses. Results of 6 experiments each are shown for arterial pressure, cardiac output and blood volume expansion slotted against dextran dose, figures 3. Results clearly show a dose response effect due to the added dextran. The blood volume expansion and the cardiovascular response are both proportional to the amount of dextran given.

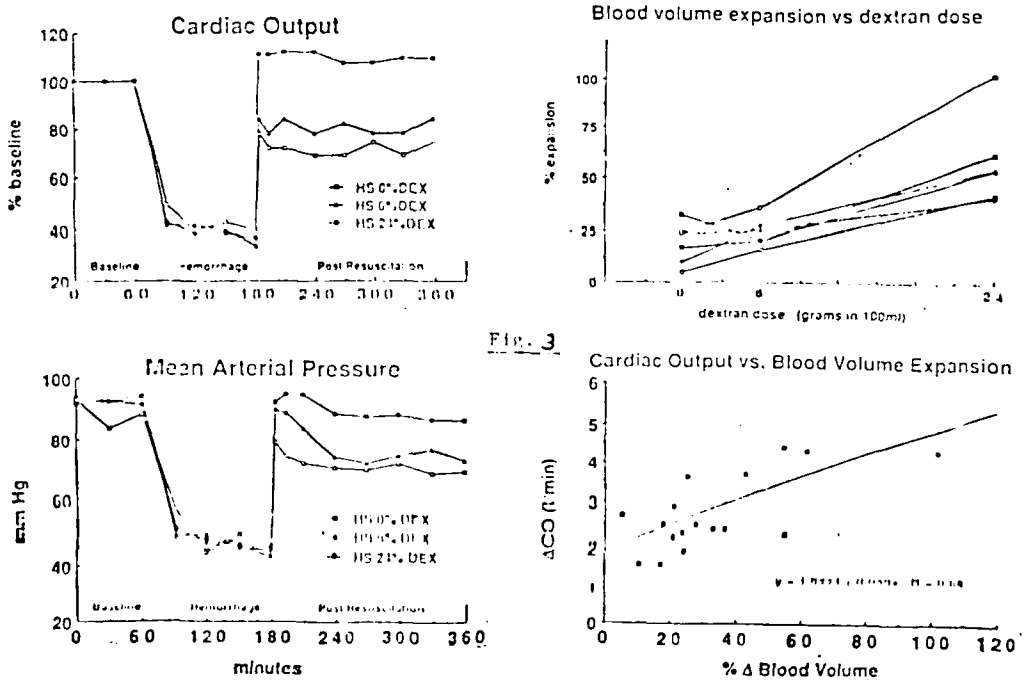


Fig. 3

Another study was performed to compare the effectiveness of HS-Dex with HS-6% hetastarch. Unanesthetized sheep were bled for 2 hrs to maintain arterial pressure (AP) at 50 mm Hg and then resuscitated with a 2 min infusion of 200 ml of either solution. Mean AP, and cardiac output (CO) are shown for baseline, end of hemorrhage, and at specified minutes post infusion, figure 4. Both colloids sustained cardiovascular function longer than in previous studies of hypertonic saline alone. The somewhat better response with HS Dex may be attributed to a higher oncotic pressure and a slower vascular clearance of dextran compared to hetastarch.

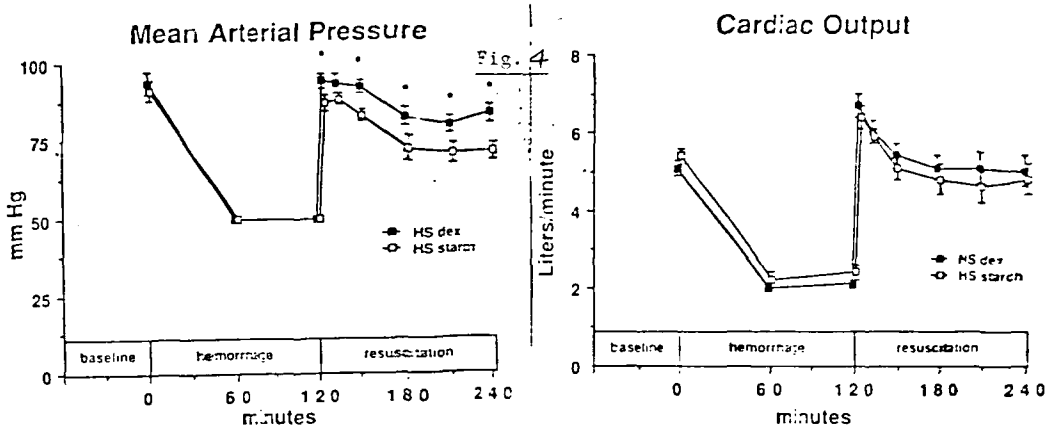
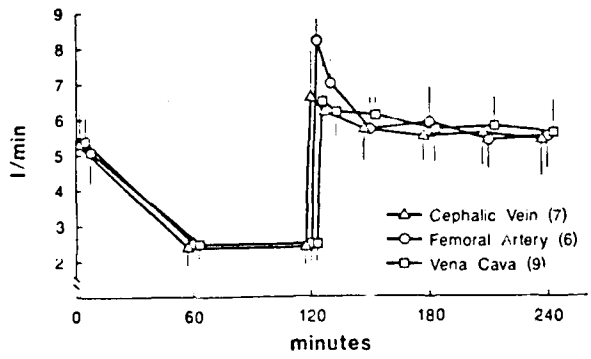


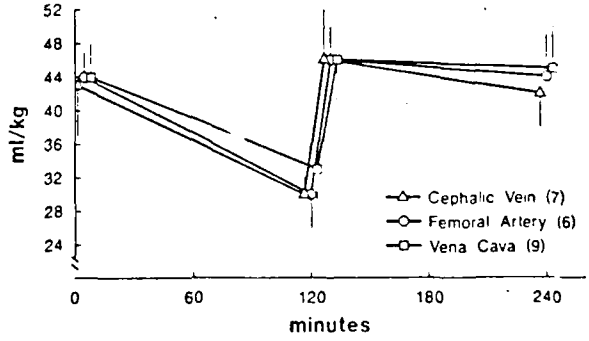
Fig. 4

2. The efficacy and safety of peripheral infusions of HSD (published manuscript M2)

These studies were designed to determine if 2400 mosm NaCl could be safely and effectively infused via a peripheral vessel. After a 2 hour baseline period conscious sheep were hemorrhaged to maintain mean arterial pressure at 50 mm Hg for 2 hours (blood loss equal to 38-46 ml/kg). Thereafter each animal was treated with 200 ml of 7.5% hypertonic saline - 6% dextran 70 given by a 2 minute infusion into either the right atrium (RA), cephalic vein (CV) or femoral artery (FA). Six experiments were performed for each route of infusion. Figure 5 shows cardiac output and plasma volume for all 3 groups. Histological examination of peripheral vessels sampled immediately or several days after infusions showed no significant pathology. We conclude that small volume resuscitation with hypertonic saline-dextran is equally effective and safe if given by either central or peripheral vascular catheters.



Cardiac outputs. (See legend for Fig. 1.)

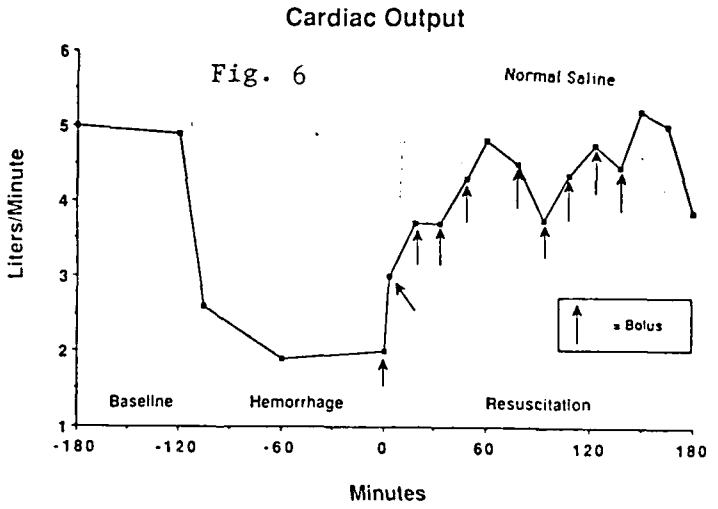


Plasma volumes. Measurements made at baseline, at 120 minutes after hemorrhage, and at 10 and 120 minutes after initiation of resuscitation.

Fig. 5

3. Effectiveness and safety of multiple bolus injection of HSD (published abstract A2)

The dose for HSD (7.5% NaCl/6% dextran 70) of 4 ml/kg or 250 ml per patient has been found to be effective for treatment of moderate hemorrhage. However, a single fixed volume dose of HSD does not provide for any titration of efficacy. Clearly, some wounded soldiers will require less volume expansions - others will require more. In this study we examined the effectiveness of infusing small single syringe 40 ml boli of HSD given at 15 minute intervals as needed to maintain mean arterial pressure above 70 mm Hg. Results were compared with a control group given 40 ml boli of normal saline and another group given 40 ml boli of a near saturated salt solution for titration to a suitable arterial pressure if hemorrhage volume is outside the range effectively treated by the 250 ml bolus a 4 ml/kg done (28% NaCl/24% dextran 70). Resuscitation of conscious sheep was begun after 2 hrs of hemorrhage, bleed volume 30-47 ml/kg. Arterial pressure could not be maintained above 70 mm Hg with 40 ml boli of isotonic saline given at 15 minute intervals as shown in figure 6. However, arterial pressure was well maintained with two to three boli of 7.5% NaCl/6% dextran 70 or a single injection of 28% NaCl/24% dextran 70. Figure 7 shows a representative experiments from an animal resuscitated with 3 infusions of HSD. Figure 8 shows averaged results from 6 sheep each. These data show that small multiple boli (~1 ml/kg) of 7.5% NaCl/6% dextran 70 can be safely and effectively used to maintain arterial pressure and cardiac output. An important finding is that a 1.5 ℓ blood loss was effectively replaced with only 40 ml of 28% NaCl/24% dextran for three hours. The efficacy of smaller volumes of more concentrated solutions is clearly suggested and merits further investigation. This concentrated NaCl/dextran solution could greatly decrease the weight and size of backpacks carried by medical corpsmen.



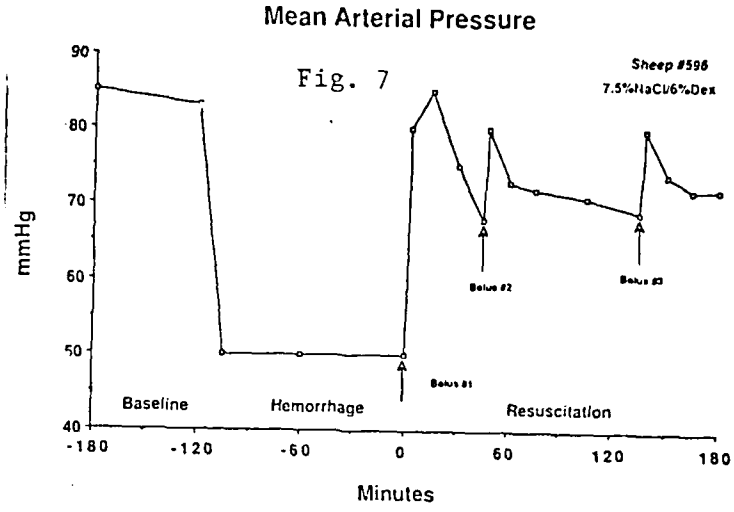

Mean Arterial Pressure
Sheep #596
7.5%NaCl/6%Dex
mmHg
90
80
70
60
50
40
Bolus #2
Bolus #3
Bolus #1
Baseline
Hemorrhage
Resuscitation
-180
-120
-60
0
60
120
180
Minutes

Fig. 7

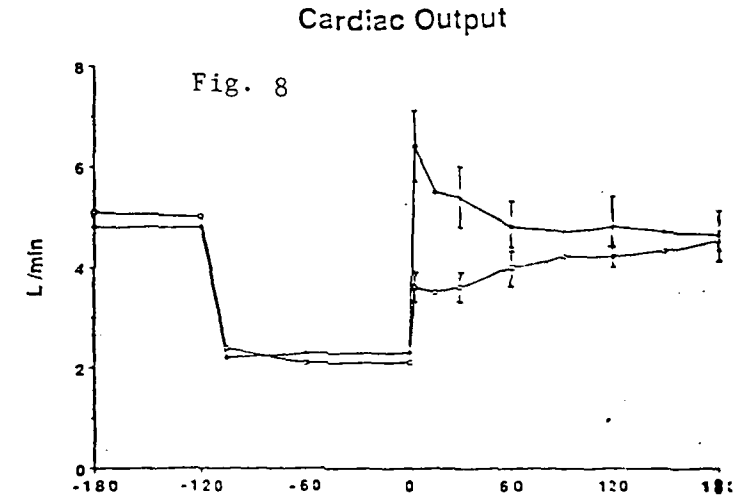
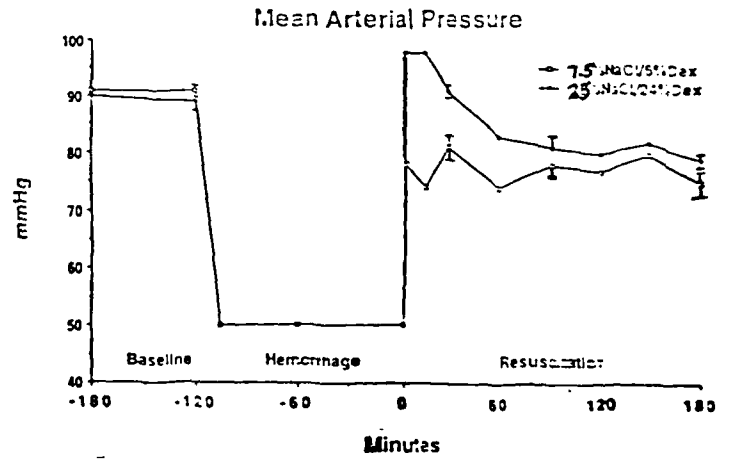

Cardiac Output
L/min
8
6
4
2
0
-180
-120
-60
0
60
120

Fig. 8


Mean Arterial Pressure
mmHg
100
90
80
70
60
50
40
Baseline
Hemorrhage
-180
-120
-60
0
60
120
180
Minutes

4. The distribution of cardiac output after hypertonic resuscitation

We measured distribution of cardiac output using radioactive microspheres in 6 hemorrhaged awake sheep resuscitated with 200 ml of HSD and 6 hemorrhaged sheep resuscitated with an equal solute load, 1600 ml, of normal saline. In both groups arterial pressure (97 ± 15 versus 93 ± 6 mm Hg), cardiac output (6.9 ± 2.5 vs. 7.1 ± 1.2 liters/min), and urine output (120 ± 110 vs. 90 ± 78 ml/30 min) returned to baseline levels 5 mins after resuscitation and remained near baseline for 2 hrs. Regional blood flow ± SD (ml/min per 100g) is shown in Table 1 for baseline (BL), late hemorrhage (Hem), and 5 (R5) and 120 (R120) min after resuscitation:

NS (n=3)	Kidney	Heart	Jejunum	Pancreas	Brain
BL	817±243	156 ± 20	76 ± 39	264 ± 126	103 ± 20
Hem	147±127	196 ± 95	31 ± 4	23 ± 17	72 ± 17
R5	447 ± 271	997 ± 476	207 ± 49	233 ± 142	188 ± 63
R120	560 ± 193	411 ± 131	60 ± 18	145 ± 93	128 ± 50
HSD (n=3)					
BL	749 ± 230	186 ± 17	68 ± 8	206 ± 187	91 ± 16
Hem	264 ± 125	131 ± 29	37 ± 16	31 ± 32	67 ± 33
R5	450 ± 71	902 ± 501	158 ± 90	140 ± 117	164 ± 30
R120	725 ± 293	846 ± 456	97 ± 30	189 ± 162	167 ± 34

There are no significant differences ($p \geq .05$) for flows at baseline, hemorrhage, or at early resuscitation with either HSD or NS. However, flows at 2 hrs post resuscitation tended to be better maintained with HSD, but the differences are not statistically significant. We conclude that: (1) An equivalent renal and hemodynamic response can be achieved with 200 ml of HSD or eight times the volume of NS; (2) Redistribution of cardiac output to visceral organs was maintained for 2 hrs post-resuscitation with both regimens; and (3) The effectiveness of hypertonic resuscitation does not depend on a unique redistribution of cardiac output.

5. The efficacy of HSD resuscitation in dehydrated animals (published abstract A5)

HSD (7.5% NaCl/6% dextran 70) resuscitates hypovolemia by rapid repartitioning cellular water into the vascular space. However, it is unclear if HSD resuscitation is effective in dehydration. Adult sheep were studied in paired experiments with normal (312±2 mosm) and elevated (330±3 mosm) plasma osmolalities (Posm) due to water deprivation for 3-4 days. Sheep were bled to a mean aortic pressure (AO) of 50 mmHg for 2 hr and resuscitated with 200 ml of HSD. With dehydration the bled volume was less (32±2.5 ml/kg) compared to controls (41±2.7 ml/kg), Table 2.

Mean results ± SEM are shown.

				Min Post Resuscitation		
		Baseline	Hemorrhage	30	60	120
AO	Control	91±4	50±0	95±4	83±4	81±3
mmHg	Dehydrated	92±2	50±0	100±4	88±3	88±4
CO	Control	4.9±3	2.1±2	5.1±3	5.1±3	4.9±3
L/min	Dehydrated	3.9±2	1.6±1	4.6±3	4.2±2	4.0±2
UO	Control	15±1	5.0±2	88±8	61±16	48±7
ml/30'	Dehydrated	13±3	3.0±1	33±19*	22±4*	14±2*

Posm was elevated by 30-35 mosm after HSD infusion in both groups. Measured plasma volume expansion after HSD (18-22 ml/kg) was similar in both groups; AO and cardiac output (CO) were equally improved in both groups. Dehydration blunted the diuresis of HSD. HSD restored cardiovascular function in dehydrated sheep following hemorrhage.

6. <u>The physiological mechanisms of hypertonic resuscitation</u> (published manuscripts M5, M6 and M7, abstracts A4, A6, A8 and A9)

We have performed studies directed towards evaluating various physiological mechanisms important for hypertonic resuscitation. Specifically, we evaluated the importance of plasma volume expansion; hypertonic effects on cardiac function; the nature of the natriuresis/diuresis; and the extent of resuscitation induced hypokalemia.

Plasma volume expansion was measured during and after a ten minute infusion of 1 ml/kg 25% NaCl/24% dextran 70 in 6 euvolemia sheep using Evans blue dye dilution and changes in protein concentration. Plasma volume expansion began immediately upon infusion and was 90% complete by the end of the infusion, figure 9. Each ml of HSD expanded PV 6.4±2.2 ml while blood pressure rose 8-15 mm Hg, cardiac output increased 0.8-1.5 l/min and right atrial pressure increased 3-6 mm Hg. Pre-femoral lymph flow increased 2-3x. These data suggest that the predominant mechanism of action of HSD is rapid plasma volume expansion caused by a sudden shift of intracellular fluid into the interstitial and intravascular spaces in response to a large increase in plasma osmotic pressure.

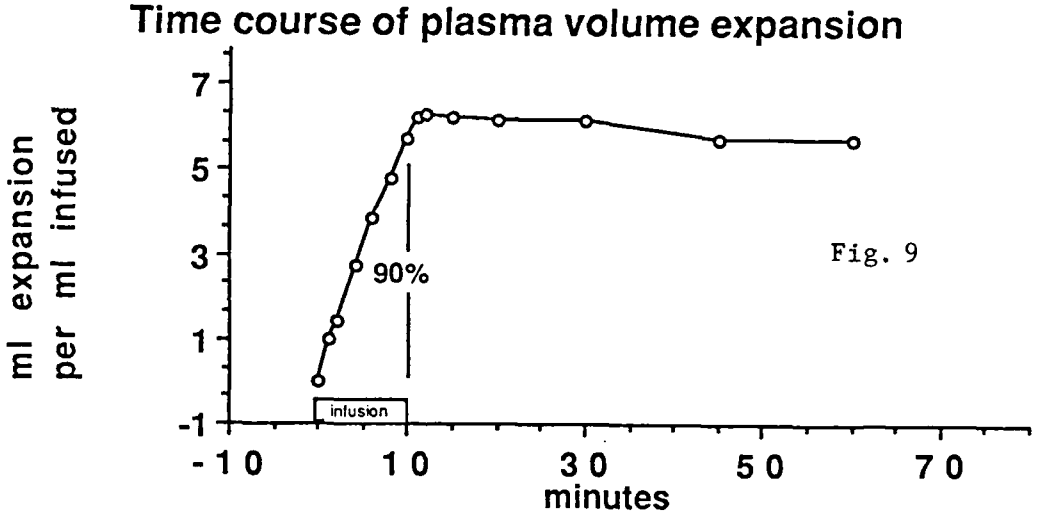


Fig. 9

The rapid effectiveness of hypertonic resuscitation has been partially attributed to direct or reflex induced enhacement of cardiac performance. In this study, we measured cardiovascular function before and after either intravenous or intra-arterial infusion of hypertonic saline (7.5% NaCl) in 7 halothane anesthetized dogs. A high fidelity micromonometer and ultrasonic dimension transducers were implanted to measure pressure and wall motion of the left ventricle (LV). Cardiac output (CO) was measured using electromagnitic flowmeter and thermodilution. The slope (Ees) of the linear regression of the LV pressure-diameter relationship was used as an index of cardiac contractility. Regional myocardial function was examined using velocity of shortening (VS) and percent of systolic shortening (%SS). Hypertonic saline (3 ml/kg) produced increases in mean arterial pressure (MAP, 102 ± 6 to 111 ± 9 mm Hg), heart rate (HR, 121 ± 23 to 135 ± 17), CO (3.1 ± 0.8 to 4.1 ± 1.0 l/min), and Ees (11.6 ± 2.0 to 15.1 ± 1.8 mm Hg/mm). Systemic vascular resistance fell by 18%, while both VS and %SS increased slightly, but not significantly. The above responses were similar whether infusion was intravenous or intra-arterial into innervated or denervated hindlimbs. While nerve blockade at T-4 (xylocaine) attenuated the increases in MAP and CO and completely prevented the tachycardia; the ionotropic and vasodilatory responses remained intact. These studies suggest that the cardiac effects of hypertonic saline infusion are not mediated by stimulation of a pulmonary or peripheral osmoreceptor and the

increased contractility results from circulating catecholamines or a direct effect of increased osmolality.

Resuscitation of hemorrhagic hypovolemia with 7.5% NaCl/6% dextran 70 (HSD) is associated with a robust diuresis. We hypothesized that the diuresis was due to release of atrial natriuretic factor (ANF). In the study we measured ANF by RIA in 5 euvolemic and 6 hemorrhaged sheep before and after infusion of 200 ml HSD. Mean results (±SEM) for arterial pressure (AP), mm Hg; left atrial pressure (LAP), mm Hg; urine output (UO), ml/30min; and ANF, pg/ml, are shown before and at 15 and 30 minutes post infusion, Table 3.

Euvolemia	AP	LAP	UO	ANF	Hem.	AP	LAP	UO	ANF
baseline	99±5	1±3	33±10	46±13		101±6	2±3	46±8	39±17
hemor.	- - -	-	- - -	- - -		51±2	-2±3	10±2	45±12
15 min	108±6	5±2	146±45	194±87		101±2	1±3	68±17	45±11
30 min	104±5	4±2	140±44	121±38		99±2	1±3	85±34	43±11

A diuresis occurred after HSD infusion in both euvolemic and hemorrhaged sheep. Plasma ANF was increased only in the euvolemic sheep. We conclude that the diuresis following HSD resuscitation is not primarily modulated by ANF. Increased ANF release may require atrial stretch and pressures above baseline levels.

A marked hypokalemia develops after HSD resuscitation and the mechanism responsible remains unclear. In this study we measured potassium excretion (UVk^+) and plasma potassium (Pk^+) following resuscitation from hemorrhagic hypotension. After 2 hrs of hypotension, aortic pressure (AO) = 50 mmHg; we infused over 10 min either HSD, 200 mls, or normal saline (NS), 1600 mls. Mean results ± SEM are shown in Table 4.

		Baseline	Hemorrhage	Min Post Resuscitation 15	30	60
UVk^+	NS	.18±.04	05±.02	.29±.07	.23±.08	.17±.05
μeq/min	HSD	.18±.04	06±.02	.34±.11	.29±.08	.21±.06
AO	NS	100±6	48±2	102±6	92±6	87±6
mmHg	HSD	96±6	48±2	87±6	87±6	84±7
Pk^+	NS	4.0±.2	4.0±.4	2.8±.3	2.9±.4	3.2±.3
mEq/L	HSD	4.2±2	4.5±.3	3.0±3	3.2±.2	3.4±.1

HSD or NS produced the same increase in AO and cardiac output. Both HSD and NS resuscitation were associated with a significant decrease in Pk^+. About half of the decrease in Pk^+ could be attributed to extracellular volume expansion. Potassium excretion was not different between groups and cannot account for the hypokalemia. We conclude that expansion of plasma volume after hemorrhage with either NS or HSD resuscitation produces marked hypokalemia.

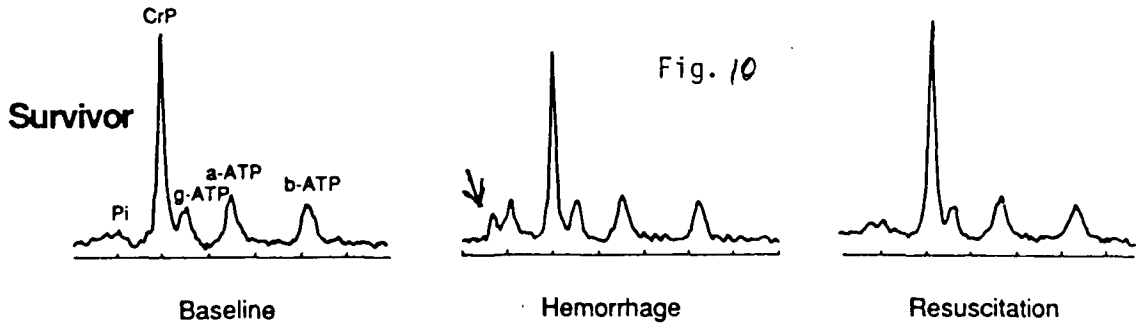


Fig. 10

Table 5

Results of MR and tissue analysis

Period	Parameter evaluated									
	PCr	ATP	ADP	AMP	Pi	G6P	Ammonia	Lactate	IMP	H^+
					Magnetic Resonance Spectroscopy					
Baseline	100%	100%	. . .	. . .	100%	. . .	. . .	. . .	. . .	83 ± 8
Shock	67 ± 10%	89% ± 10%	. . .	. . .	274% ± 26%	. . .	. . .	. . .	. . .	120 ± 30
	$P < 0.01$				$P < 0.01$					$P < 0.01$
					Tissue Analysis					
Baseline	54 ± 9[2]	17 ± 2[1]	3.2 ± 0.3[1]	1.1 ± 0.1[1]	. . .	0.8 ± 0.4[2]	1.4 ± 0.5[2]	5 ± 1[2]	0.2 ± 0.03[1]	. . .
Shock	26 ± 5	15 ± 3	4.2 ± 0.5	0.9 ± 0.3	. . .	10.9 ± 2.6	4.2 ± 1.2	36 ± 11	1.4 ± 0.8	. . .
	$P < 0.01$					$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	

PCr = intracellular skeletal muscle phosphocreatine, ATP = adenosine triphosphate, ADP = adenosine diphosphate, AMP = adenosine monophosphate, Pi = norganic phosphate, G6P = glucose-6-phosphate, IMP = inosine monophosphate, H^+ = hydrogen ion. Concentrations were measured at baseline and after 60 inutes of shock. Values are in means ± 1 SD. Significance determined by two-tailed Mann-Whitney test.

MR spectroscopy values are expressed as percentage of baseline values. Tissue analyses were performed with high-performance liquid chromatography[1] or standard nzyme assay[2] and are expressed in μmoles/g dry weight. Hydrogen ion concentration is expressed in nmoles/L.

7. NMR measurements of intracellular metabolic function (published manuscripts M1 and M3)

a) Skeletal muscle

We used tissue biopsies and topical NMR to monitor intracellular metabolism of skeletal muscle during shock and resuscitation. Table 5 shows NMR measured changes in phosphocreatine (PCr), inorganic phosphorous (Pi), ATP and intracellular pH of biceps femoris muscle after 60 minutes of severe hemorrhage (MAP = 40-50 mm Hg) in anesthetized rat. Figure 10 shows typical phosphorous spectra during baseline and hemorrhage. The appearance of an additional sugar monophosphate peak (arrow) occurred in late hemorrhage and its occurrence seemed to coincide with the fall in intracellular pH. To substantiate these results and to identify the compound responsible for the new peak we measured phosphorous and other metabolites in freeze clamped samples of skeletal muscle from 6 control rats and the 6 shock rats used for NMR measurements, Table 5. The difference in PCr and ATP confirm our NMR measurements. Also, the data suggests that glucose 6-phosphate is the sugar monophosphate peak which appears in late shock.

Phosphorus nuclear magnitic resonance (NMR) spectroscopy allows noninvasive monitoring of intracellular high-energy metabolites. In this study we used topical NMR to monitor intracellular levels of ATP, creatine phosphate (CrP), inorganic phosphate (Pi), and pH in the biceps femoris muscle of rats during hemorrhagic shock and resuscitation. Twelve rats weighing 300-500 mm Hg for 90 minutes. Then they were resuscitated with lactated Ringers' until MAP returned to normal or resuscitation fluid equaled four times the shed blood volume. During resuscitation, the rats fell into one of two groups: survivor group (n=5) which could be successfully resuscitated for 60 minutes or longer; or nonsurvivor group (n=7) which died during resuscitation. In both groups, ATP levels were maintained during hemorrhage and resuscitation. Intramuscular pH dropped about 0.2 pH units in both groups at the end of hemorrhage; however, pH was restored back toward baseline in the survivor group. CrP levels were lower in the nonsurvivor group at the end of hemorrhage. After resuscitation, CrP returned to nearly baseline levels in the survivor group; in the nonsurvivor group, CrP was further depleted after resuscitation. Pi levels were increased in both groups at the end of hemorrhage, but in the survivor group Pi decreased during the first 15 min of resuscitation; in the nonsurvivor group Pi increased further to four times baseline levels. This study demonstrated that topical NMR can quantitate a metabolic deficit in skeletal muscle during hemorrhage and resuscitation. The results in figure 11 show that improvement of intracellular Pi and CrP levels correlated with survival.

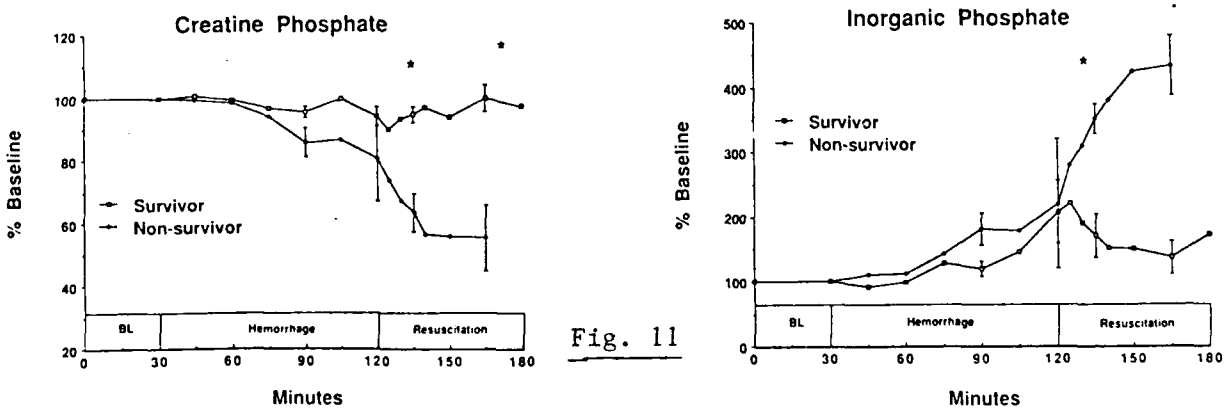


Fig. 11

b) Brain - NMR and Brain Metabolism

We combined a rat shock model and nuclear magnetic resonance to study cerebral high energy phosphate cerebral metabolism during shock and resuscitation. Intracellular pH was determined from phosphate spectra using the shift between phosphocreatine and inorganic phosphate. Results from a series of experiments in rats without brain injury subjected to hemorrhagic shock and subsequent resuscitation with either lactated Ringer's or 7.5% hypertonic saline are seen in Table 6.

Table 6. Phosphorus Spectra Shock/Resuscitation Experiments

Parameter	Baseline	Shock	Resuscitation	
pH	7.12 ± 0.03	7.06 ± 0.04	LR	7.21 ± 0.04
			HS	7.08 ± 0.05*
Pi/PCr	0.34 ± 0.02	0.39 ± 0.04	LR	0.29 ± 0.04
			HS	0.38 ± 0.05

Table 6: Values for intracellular pH (pH) and the ratio of inorganic phosphate to phosphocreatine (PCr/Pi) in rats shocked to a mean arterial pressure of 40 mm Hg for one hour followed by one hour of resuscitation with either lactated Ringer's (LR, n=8) or 7.5% hypertonic saline (HS, n=11).

Perhaps because our model allows for spontaneous ventilation and respiratory compensation of intracellular acidosis, we did not find that pH and Pi/PCr decrease significantly with shock. We continued our study into the resuscitation phase and discovered differences in the relative abilities of LR and HS to restore intracellular pH. LR also returned Pi/PCr towards baseline levels; HS resuscitation did not.

We believe that an animal model utilizing spontaneous ventilation more accurately mimics the clinical situation during shock and early field resuscitation of trauma and we will continue using this model. Previous work with hemorrhagic shock and head injury using nuclear magnetic resonance high energy phosphate spectroscopy has been done in paralyzed and ventilated animals and has not focused on different forms of resuscitation.

We have also done a smaller number of experiments in animals with head injury added to hemorrhagic shock. Although these experiments are very few in number, they suggest greater deterioration in cerebral metabolism with shock when a brain injury is present as compared to animals without brain injury. They also have suggested that, as with non-brain injured animals, LR more completely resuscitates cerebral high energy phosphate metabolism than does HS.

c) Kidney

We subjected 16 rats to 60 minutes of hemorrhagic shock while monitoring intracellular phosphate levels of kidney. Nine rats were subjected to an initial resuscitative infusion of 6 ml/kg of HS. Control rats were given normal saline. Additional fluid, lactated Ringers, was given to all rats as needed to keep arterial pressure at 80 mm Hg or above. While the HS group did display better blood pressure and less additional volume requirements, there was no significant difference between NMR spectra. ATP levels generally were unchanged; Pi increased during hemorrhage, but were not corrected during resuscitation.

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List of Publications

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Manuscripts:

M1. Moore, J.S., R.T. Bogusky, G.C. Kramer and J.W. Holcroft. Nuclear magnetic resonance spectroscopy and tissue analysis of skeletal muscle during hemorrhagic shock. Surgical Forum 36:59-61, 1987.

M2. Hands, R., J.W. Holcroft, P.R. Perron and G.C. Kramer. Comparison of peripheral and central infusions of 7.5% NaCl/6% dextran 70. Surgery 103:684-689, 1988.

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M7. Kramer, G.C. and J.C. Walsh. Emergency fluid resuscitation. The Role of Hemodilution in Optimal Patient Care. (In press), 1989.

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A1. Walsh, J.C., P.R. Perron, D.C. Lindsey, J.W. Holcroft and G.C. Kramer. Improved resuscitation of hemorrhagic shock after adding high concentrations of dextran 70 to hypertonic saline. Circulatory Shock 21:338-339, 1987.

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