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For the wounded soldier in combat, the time between his injury and treatment can be quite long and variable depending on the combat circumstance. The duration and severity of this hypotensive period are critical factors in determining his responsiveness to resuscitative efforts when aid does arrive. Due to the venous nature of the bulk of its blood flow, the liver is particularly susceptible to decreases in its blood flow and has been found to suffer high energy phosphate depletion relatively early in the course of hemorrhagic shock, and thus metabolic anomalies in this organ are especially relevant to the pathogenesis of the shock state. We have explored the effects of graded hypotension and isobaric hemorrhagic hypotension on hepatic oxygen delivery, defined by portal flow, hepatic tissue PO_2 and arterial B-hydroxybutyrate/acetoacetate ratio measurements and function as assessed specifically by ethanol clearance. The results show that the B-hydroxybutyrate/acetoacetate ratio and the lactate/pyruvate ratios are equivalent in their indication of the hepatic aerobic to anaerobic transition within the fed and fasted groups but the absolute values of the ratio attained in this transition were significantly different although hepatic high energy phosphate status was equivalent. Due to these nutritional effects, single point determinations will have little value in evaluating hepatic oxygenation state during hypotension but may be valuable if

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followed serially. Hepatic clearance of ethanol was decreased proportionately with decreases in blood pressure down to 60 mm Hg and hepatic PO_2 values < 2 mm Hg where clearance ceased. Ethanol clearance may be an excellent non-invasive means of assessing hepatic metabolic integrity. At a blood pressure of 40 mm Hg, there is no significant gluconeogenisis or ethanol clearance. Resuscitation with whole blood caused only partial return of hepatic PO_2 after hypotensive periods of duration longer than 45 min although portal venous flow returned to normal levels indicating a persistent defect in oxygen delivery after resuscitation to control blood pressures. These studies form the basis of developing an approach to assessing the degree of hepatic dysfunction in the acute and post-resuscitative phases of injury in the wounded soldier and will serve as a basis for comparative evaluation of future resuscitative fluids and therapies designed to accelerate the rate of recovery of hepatic function and an expedient return of the soldier to duty.

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 (NIH Publication No. 86-23, Revised 1985).

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INTRODUCTION:

For the wounded soldier in combat, the time between his injury and treatment can be quite long and variable depending on the combat circumstance. The duration and severity of this hypotensive period are critical factors in determining his responsiveness to resuscitative efforts when aid does arrive. Due to the venous nature of the bulk of its blood flow, the liver is particularly susceptible to decreases in its blood flow and has been found to suffer high energy phosphate depletion relatively early in the course of hemorrhagic shock (2,3,10,11), and thus metabolic anomalies in this organ may be especially relevant to the pathogenesis of the shock state. Of particular interest in regard to the current studies is the effect of this compromised hepatic function on ketone body metabolism. Engel, who was the first to describe changes in total ketone body levels during hypovolemia, reported an "early and sustained decrease" in blood ketone levels during "fatal hemorrhage" and speculated that this was due both to decreased production by the liver, and changes in utilization by various tissues (4). Since ketone bodies are important "glucose-sparing" energy substrates (especially for heart, brain and muscle), insight into their role during hemorrhage could prove useful in trying to piece together the cellular and metabolic correlates of the homeostatic response to severe blood loss. The intra-mitochondrial location and high concentration of B-hydroxybutyrate dehydrogenase makes the B-hydroxybutyrate/acetoacetate ratio a reliable indicator of the mitochondrial redox state of the liver cell (15) which in turn depends on oxygen availability. Recently, the arterial B-hydroxybutyrate/acetoacetate ratio has been proposed to be a useful indicator of hepatic high energy phosphate status in vivo (12) as this ratio correlated with the hepatic ATP level during hypovolemia while the L/P ratio did not. These results suggested the possibility that the arterial B-hydroxy-butyrate/acetoacetate ratio might be a useful dynamic monitor of the hepatic oxygenation state during the progression of shock, although these authors did not study this. It was a goal of the present studies, therefore, to examine the correlation of the B-hydroxybutyrate/acetoacetate ratio with hepatic function (i.e. ethanol clearance), hepatic tissue PO2, hepatic blood flow and hepatic ATP levels in a well-defined, phased model of hemorrhagic shock to evaluate its potential usefulness in serving as a indicator of hepatic oxygenation state.

BODY:

This past year we have explored the effects of graded hypotension and isobaric hemorrhagic hypotension on hepatic oxygen delivery, defined by portal flow, hepatic tissue PO2 and arterial B-hydroxy-butyrate/acetoacetate ratio measurements and function as assessed specifically by ethanol clearance.

PHASE-DEPENDENT CHANGES IN HEPATIC BLOOD FLOW AND ARTERIAL KETONES DURING ISOBARIC HEMMORHAGE

Sprague-Dawley rats weighing between 290 and 360 grams were anesthetized using sodium pentobarbitol (50 mg/kg), then a cannula (PE-50 tubing) was placed in the femoral artery for removal of blood and monitoring of mean arterial blood pressure. Animals were allowed time to stabilize, then bleeding was induced over a period of ten minutes until a MABP of 40 mm Hg was achieved. Blood was subsequently either removed or reinfused to maintain the MABP at 40 mm Hg. Rectal temperatures were maintained at 37 degrees Celsius throughout the protocol. Blood samples (0.3 ml) for determinations of hematocrit and plasma glucose, lactate, acetoacetate and ß-hydroxybutyrate concentrations were drawn at prespecified intervals based upon the hemodynamic status of the animal rather than at discrete time intervals. Four phases of shock were defined experimentally as follows: Phase I (early compensatory phase), which ends when approximately 50% of the projected maximal blood removal has been achieved and during which homeostatic mechanisms are being rapidly recruited; Phase II (maximal compensatory phase), corresponding to the point at which 100% of the projected maximal blood loss has been achieved and during which compensatory adjustments for blood loss are maximal; Phase III (early decompensatory

phase), defined as the point at which 20% of the shed blood has been reinfused, and beyond which the shock is irreversible; and Phase IV (late decompensatory phase), defined as the point at which 75% of maximal shed blood volume has been reinfused, and which is well into the irreversible stage of shock (9,10). Blood removed for sampling was replaced by blood taken from a donor animal (if necessary).Fed animals were allowed free access to food and water prior to experimentation. Fasted animals were denied food for 24 hours before the protocol, but since fasting causes dehydration, the animals were induced to drink normal amounts of water through inclusion of 0.1N NaCl in their drinking water. Previous work has shown that survival times for fed and fasted hydrated animals are comparable (1). Plasma acetoacetate and ß-hydroxybutyrate were assayed fluorometrically using a slight variation of the method described by Young and Renold (16).

Tables 1 and 2 below show the mean acetoacetate and ß-hydroxybutyrate concentrations for fed and fasted animals in relation to shed blood volume. The maximal decrease in the concentration of plasma acetoacetate occurred during phase I (early compensatory) for both fed and fasted animals. In fasted animals, there was also a significant decrease during phase II. Fed animals reached a final value equivalent to 46% of the control value while fasted animals decreased to a final value of 29% of control. Changes in ß-hydroxybutyrate concentrations were discrepant for fed and fasted animals. Fed animals showed an overall increase in plasma ß-hydroxybutyrate (to 268% of control), with the maximal increase occurring during phase II. In fasted animals, plasma ß-hydroxybutyrate values decreased through the compensatory phases (to 58% of control), but increased (to a final value at 74% of control) through decompensation. Changes in total ketones paralleled changes in ß-hydroxybutyrate for both groups. The mean values of ß-hydroxybutyrate/acetoacetate ratios for fed and fasted animals show that both fed and fasted rats exhibited overall increases (to 687% and 304% of control values, respectively) with the most significant increases occurring during late decompensation. Both groups showed a secondarily large increase during phase II.

KETONE RESULTS

FED ANIMALS (Table 1)

% Maximum Shed Volume	[ß-OHB]	[AcAc]	Total Ketones	B/A ratio
0	0.25	0.14	0.39	1.98
41	0.36	0.11	0.47	3.54
57	0.41	0.09	0.50	4.74
79	0.61	0.09	0.70	7.97
100	0.64	0.08	0.72	8.00
84	0.54	0.07	0.61	10.14
55	0.62	0.06	0.68	14.73
30	0.67	0.06	0.73	13.61

FASTED ANIMALS (Table 2)

0	1.37	0.89	2.28	1.60
52	1.18	0.60	1.78	2.16
72	0.87	0.41	1.28	2.29
87	0.70	0.38	1.08	2.05
99	0.80	0.34	1.14	2.60
80	0.93	0.31	1.24	3.46
52	1.01	0.26	1.27	4.86

Tables 3 and 4 below, describe the changes in blood flow to various tissues for fed and fasted animals. Blood flow was determined by injection of differentially labelled microspheres into the left ventricle before bleeding and during each of the four phases of shock. Total hepatic flow was similar in both fed and fasted groups falling to levels which were about 30% of control values. Portal venous flow was identical in the fed and fasted groups during the compensatory phase, but rose in the fasted group during decompensation. Hepatic arterial flow was two-fold greater in fasted animals and was well maintained until the peak shed blood volume was reached. Adipose tissue flow diminished to undetectable levels with low vascular conductance maintained throughout the shock period.

BLOOD FLOW DATA
(ml/100 g tissue)

FED ANIMALS

%Maximum Shed Volume	Hepatic Artery	Vein	Portal	Adipose Tissue
0	26.7		198	0.23
50	30.7		74	0.05
100	18.0		50	0.02
80	17.0		59.3	0.04
20	12.5		51.3	0.08

FASTED ANIMALS

0	58.0		200	0.84
50	57.7		74.9	0.42
100	27.5		35.8	0.03
80	35.4		60.4	0.05
20	18.5		109.9	0.11

Significance of changes in ß-hydroxybutyrate and acetoacetate

Originally, ketone bodies were considered waste products indicative of an abnormal metabolism, however, it is now clear that they serve as important alternative fuels for various tissues when carbohydrate is in short supply or cannot be utilized efficiently. Ketones are an especially important alternative substrate for the brain, which does not have the capacity to utilize fatty acids for energy. In fact, during prolonged starvation, ketone bodies may actually replace glucose as the major energy substrate in the brain. In addition, they can serve to minimize the breakdown of muscle protein for purposes of gluconeogenesis during periods of hypoglycemia (7). The rate-limiting enzyme for ketone production (hydroxymethylglutaryl-CoA synthase) is present in large quantities only in the liver, and hence this is the primary site of ketogenesis. During hemorrhage, blood flow to the liver is decreased to about one-third of control values, for both fed and fasted animals, by the maximal compensatory stage. Other investigators have reported similar flow decreases to the liver during early phases of shock (6,8). Concomitant with this decreased blood flow is compromised hepatic integrity, as evidenced by a depletion of hepatic ATP levels occurring between the early and maximal compensatory phases (10). Such depleted ATP levels certainly result in compromised gluconeogenic capacity, but ketone body production must also be compromised since ATP is required in the early steps of fatty acid catabolism.

Flow to adipose tissue is even more greatly compromised during shock, even at the earliest time point examined. Farnebo, et. al., reported similar results using a comparable model of shock. In fasted

rats bled to a MABP of 35 mm Hg and maintained for a period of four hours, plasma free fatty acid levels fell significantly to less than 50% of control within 2 hours. It was speculated that this effect was a consequence of decreased blood flow to adipose tissue which is known to inhibit lipolysis and free fatty acid outflow. Also, adipose tissue demonstrated compromised metabolic integrity as evidenced by large decreases in levels of high energy phosphates (5). Since the rate of ketogenesis is determined primarily by the rate of delivery of free fatty acid precursors to the liver (7), such circumstances must also have profound effects on ketone body production.The compensatory phase in this model of shock is also characterized by dramatic increases in plasma glucose and lactate concentrations, especially during phase II (9,10), and in fasted animals, there is a concomitant decrease in plasma total ketone body levels. Plasma substrate levels reflect a balance between production and utilization. Thus, while glucose and lactate production exceeds their utilization during phases I and II, ketone bodies show the opposite trend. In fasted animals, then, ketone bodies are probably more than just a minor energy substrate during the initial stages of shock. Blood flow to brain, heart, and muscle, the major consumers of ketone bodies, is not severely compromised as is that to adipose tissue, and delivery of ketone bodies for use as energy substrates to these tissues is quite significant. Thus, a combination of increased utilization and decreased production probably accounts for the initial decrease in total ketones.

In contrast to fasted animals, total ketone bodies in fed animals increase in parallel to plasma glucose and lactate during the compensatory phase. Since production by the liver is decreasing during this period, the plasma concentration must rise due to a relatively greater decrease in ketone utilization. The decompensatory phase is characterized by a dramatic decrease in plasma glucose to hypoglycemic levels and but no further increase in plasma lactate fron the maximum level attained during the peak of compensation (9,10). Throughout decompensation, hepatic ATP concentrations are stable at 30% of normal and blood flow to liver and adipose tissue is sustained at the low values reached during maximal compensation. It is probably reasonable to assume, then, that ketone production by the liver also plateaus, but at some low value. Thus, changes in plasma total ketone levels during decompensation probably reflect changes in utilization, only.

ß-hydroxybutyrate to Acetoacetate Ratios

The ß-hydroxybutyrate dehydrogenase system may be useful for assessing the oxidoreduction state of liver. During hemorrhage, the hepatic energy charge (which is indicative of energy status) changes concomitantly with NAD/NADH ratios. Using this principle, other investigators have correlated ratios of ß-hydroxybutyrate/acetoacetate in the liver with hepatic energy status. Even further, they have shown a precise correlation between plasma ß/A ratios ahd hepatic ß/A ratios (15).The results of the present study do not comfirm the efficacy of plasma ß/A ratios as accurate indicators of hepatic energy status (as determined by hepatic levels of high-energy phosphates), at least not during all stages of shock. During compensation, arterial ß/A ratios seem to correlate with hepatic ATP levels for fed animals. That is, hepatic ATP shows a slight decline during phase I, and a more significant decline to 30% of control by phase II (10), which would correspond to a slight increase in ß/A during phase I and a more significant increase during phase II to approximately 3 times the control ratio. During decompensation, however, there seems to be little correlation. Hepatic ATP levels do not drop any further through decompensation, while the ß/A ratio increases significantly.

For fasted animals, the correlation is not as clear since the ß/A ratio does not rise much at all during the compensatory period compared to the change in fed animals, although the ATP contents in fasted animals are depleted to the same extent as in fed animals and the total hepatic flow is decreased to equivalent levels as well . Thus, in spite of previous reports, it would seem erroneous to conclude that arterial levels of ß-hydroxybutyrate and acetoacetate always correlate with hepatic levels.

Effect of graded hypotension on hepatic PO_2 and ethanol clearance

Since the majority of the alcohol dehydrogenase in the body resides in the liver, we proposed that whole body alcohol clearance measurements might be used to assess this metabolic hepatic function. Previous results have shown that the vasoconstriction induced in the splanchnic bed by severe blood loss which lowers the blood pressure to 40 mm Hg, diminishes hepatic O_2 delivery and ATP levels to levels which reached 30% of normal [1] before the onset of decompensation. However, the functional impact of this high energy phosphate depletion, either during the hypotensive period or after resuscitation following varying periods of hypotension, has not been determined. Determination of the functional impact of various degrees of hypotension on hepatic metabolism was a major goal of the present studies. These studies used ethanol clearance (Cl_{EtOH}) as a functional marker of hepatic metabolism to be related to hepatic PO2 (HPO2) and mean arterial blood pressure (MABP). Pentobarbital anesthetized, Sprague-Dawley rats were bled in steps of MAP equaling 100, 80, 70, 60, 50, and 40 mm Hg each of 15 min. duration. HPO2, was measured every 10 sec. with an 8-channel MDO oxygen electrode placed on the liver surface. A second experimental series was conducted to measure Cl_{EtOH} using the same graded hemorrhage protocol but in steps lasting 30 min.. A plasma [EtOH] of 22 mM was maintained using a primed constant rate infusion protocol and the ClETOH at each pressure calculated from the difference between the infusion rate and EtOH accumulation rate assuming a volume of ETOH distribution equal to the total body water (600 ml/kg BW). ClEtOH declined at MAPs below 100 mmHg and ceased at HPO2s < 2 torr and at MAPs < 60 mm Hg. The data indicate that blood pressure reductions which do not significantly affect hepatic high energy phosphate stores cause significant reductions the clearance of ethanol. These studies together with our previous results show that hepatic function as assessed by gluconeogenesis and alcohol clearance effectively cease at a blood pressure of 40 mm Hg.

CONCLUSIONS:

The results of our studies described here show:

1.) The ß-hydroxybutyrate/acetoacetate ratio and the lactate/pyruvate ratios are equivalent in their indication of the hepatic aerobic to anaerobic transition within the fed and fasted groups but the absolute values of the ratio attained in this transition were significantly different although hepatic high energy phosphate status was equivalent. Due to these nutritional effects, single point determinations will have little value in evaluating hepatic oxygenation state during hypotension but may be valuable if followed serially.

2.) Hepatic clearance of ethanol was decreased proportionately with decreases in blood pressure down to 60 mm Hg and hepatic PO2 values < 2 mm Hg where clearance ceased. Ethanol clearance may be an excellent non-invasive means of assessing hepatic metabolic integrity. At a blood pressure of 40 mm Hg, there is no significant gluconeogenisis or ethanol clearance.

3.) Resuscitation with whole blood caused only partial return of hepatic PO2 after hypotensive periods of duration longer than 45 min although portal venous flow returned to normal levels indicating a persistent defect in oxygen delivery after resuscitation to control blood pressures.

These studies form the basis of developing an approach to assessing the degree of hepatic dysfunction in the acute and post-resuscitative phases of injury in the wounded soldier and will serve as a basis for comparative evaluation of future resuscitative fluids and therapies designed to accelerate the rate of recovery of hepatic function and expedient return of the soldier to duty.

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APPENDIX:

Abstracts:

Iannoli, P., N. Lund, and F.J. Pearce. Relationship of mean arterial pressure (MAP) to hepatic tissue PO2 (HPO2) and ethanol clearance during graded hemorrhage. FASEB Journal 5: A1502, 1991. (presented at the Federation of American Societies for Experimental Biology in Atlanta, Georgia in April, 1991).

Lund, N., P. Iannoli, P. Papadakos and F.J. Pearce. The effects of graded hemorrhage on liver oxygenation and ethanol clearance. (presented at the meeting of the American Society of Anesthesiologists in San Francisco, California on October 29, 1991.