Clinical monitoring of cellular metabolism during shock, based largely on traditional metabolic indicators, remains unsatisfactory. The purpose of this study was to compare venous oxygen tension and blood lactate gradients with blood gradients of purine nucleotide degradation products which are derived from tissue ATP catabolism during hypovolemic shock. Sixteen dogs were instrumented to sample arterial and venous blood. Measurements of arteriovenous lactate and PNPD gradients during spontaneous respiration were examined at four tissue sites: gut, kidney, hindlimb, and diaphragm. Hypovolemic shock (mean arterial blood pressure 35 to 40 mm Hg) was induced and maintained for one hour. The above parameters were remeasured at 30 and 60 minutes after induction of shock. Hypoxanthine gradients were greater than that of other PNPD, and so were used as the primary indicator of tissue ATP metabolism. In the hindlimb, the mean AV gradients for hypoxanthine (1 ± 1 μM) were not significantly greater than baseline, while the lactate gradient (700 ± 300 μM) rose markedly. In contrast, across the kidney there was a significantly greater AV hypoxanthine gradient (16 ± 3 μM, p<0.002) but no lactate gradient (~400 ± 200 μM). Both the hypoxanthine and lactate AV gradients were significantly elevated across the diaphragm and gut. Venous Po2 values less than 35 mm Hg predicted an increased hypoxanthine gradient across the kidney, but not across the hindlimb. We conclude that the metabolic response to hypovolemic shock as assessed by PNPD gradients, lactate gradients, and venous Po2 differs among tissues. Although resting muscle such as the hindlimb may be an important source of blood lactate, the viscera and working skeletal muscle (the diaphragm) are major contributors to circulating PNPD.

Metabolic function of organs and tissue beds reflects their structure and functional purpose. During times of stress, such as hypovolemic shock, the metabolic response of various tissue beds may therefore be expected to be nonhomogeneous. Arteriovenous gradients across tissue beds allow for comparison of different indices of metabolic activity. The pattern of metabolic response to hypovolemic shock, as characterized by AV metabolic gradients, has not been characterized simultaneously in multiple tissue beds.

Tissue metabolism during hypovolemic shock states

ATP = adenosine triphosphate; PNPD = purine nucleotide degradation products; TAN = total adenine nucleotides; HPLC = high pressure liquid chromatography; AV = arteriovenous

Compounds can diffuse into the extracellular space where they can be assayed.\textsuperscript{12} Release of these compounds reflects a fall in tissue ATP and in total adenine nucleotides intracellularly.\textsuperscript{13-15} Moreover, the release of PNPD during shock may also predict subsequent impairment of ATP resynthesis via the purine salvage pathway.\textsuperscript{16} This impairment can inhibit functional recovery following restoration of tissue oxygenation.

We compared PNPD gradients with lactate gradients and venous oxygen tensions during hypovolemic shock across tissue beds. Because these measurements are influenced by different metabolic events, we hypothesized the effect of hypovolemic shock on these three indices may be different in tissues with dissimilar metabolic characteristics. We report differences in these parameters in four tissue beds: gut, kidney, hindlimb, and diaphragm. We discovered dissimilar relationships between PNPD gradients and lactate gradients among these tissues, indicating a differential contribution by certain tissue to systemic levels of circulating purines and blood lactate. These differences could not be explained by differences in tissue oxygenation as measured by the venous Po\textsubscript{2}.

\textbf{METHODS}

\textbf{Surgical Preparation}

Sixteen mongrel dogs weighing between 12 and 24 kg were fasted overnight. This assured that the stomach would be decompressed to allow for optimal visceral and diaphragmatic catheter placement. They were anesthetized with 30 mg/kg of intravenous pentobarbital. Additional anesthesia was given to prevent shivering, yet allow spontaneous respiration. All dogs were tracheotomized and initially maintained on mechanical ventilation with a tidal volume of 25 ml/kg while further procedures were performed.

An arterial catheter was placed into the aorta via a right femoral cut down, and arterial pressures recorded on a strip chart. A right femoral venous catheter was placed for the administration of fluids and withdrawal of blood during hemorrhage. This catheter was attached to a peristaltic pump which emptied into a 1 L reservoir containing 4,000 units of sodium heparin. Subsequently, a laparatomy was performed using bilateral subcostal incisions. Venous catheters were placed in the left renal vein and left phrenic vein via the inferior vena cava. Catheters were also placed into the portal vein and the left femoral vein in a cephalad to caudal direction. All arterial samples were obtained from the single femoral arterial catheter. Dogs that had uncontrollable fibrillation or hemorrhage were killed (n = 2).

\textbf{Protocol}

All dogs were removed from mechanical ventilation to allow for a working diaphragm. Thereafter, arterial blood gas measurements were used to confirm adequate ventilation and oxygenation. (Due to persistent hypoventilation, two dogs were later placed on mechanical ventilation and therefore were excluded from phrenic vein sampling). After a 30 minute stabilization period during which time the dog demonstrated no sustained arrhythmias and stable hemodynamic parameters, baseline blood samples were obtained from all sites. Approximately 8 ml of blood was withdrawn from the arterial catheter, as well as from the left femoral, portal, phrenic, and renal veins. Blood gases were determined immediately on each sample, and the remainder stored on ice for assay of blood lactate and plasma PNPD. After baseline samples were drawn, animals were hemorraged over 10 to 15 minutes to reduce mean arterial blood pressure to 30 to 40 mm Hg. Additional blood was withdrawn or reinfused in order to maintain a stable pressure between 30 to 40 mm Hg. After 30 minutes of sustained hypotension, blood was sampled simultaneously from each site and blood gases, lactate, and PNPD were measured. Each aliquot was replaced with an equivalent amount of heparinized saline solution. Sampling was repeated one hour after induction of hypotension. Results obtained at the 30 minute and 60 minute time points were similar, and therefore, values are reported as pooled data.

\textbf{Biochemical Assays}

Blood for lactate measurement was precipitated using cold perchloric acid and assayed using a spectrophotometric kit. Blood for PNPD determination was preserved with 200 units of heparin, 2.5 nmol EHNA, 12.5 nmol of dipyridamole, and 100 nmol of allopurinol. The methods for determination of PNPD have been described elsewhere.\textsuperscript{17} Briefly, plasma was deproteinated by filtration. Samples were subsequently analyzed by high pressure liquid chromatography. Chromatography was performed using a c18 uBondapack column and an acetonitrile gradient. Peaks were identified by comparison of retention times to known standards, with enzymatic peak shift confirmation, for selected samples. Concentrations were calculated by comparison of peak areas to standards of known concentrations. This method has been shown to detect hypoxanthine, xanthine, inosine, and adenosine, with a 1 to 2 \textmu M lower limit of sensitivity. Values of PNPD and blood lactate are reported in micromoles/liter (\textmu M).

\textbf{Data Analysis}

Comparisons of PNPD, lactate and venous Po\textsubscript{2} between sites were initially done with a one way ANOVA. Subsequently statistical significance was determined using unpaired Student's t-tests with Bonferroni's correction for multiple comparisons. Unpaired Student's t-tests without a Bonferroni's correction factor were used for comparison between baseline values within each site. Linear regressions were performed on data relating hypoxanthine to lactate levels. Changes in blood flow before and after shock were compared with the Mann Whitney U test. All results are reported as mean ± standard error.\textsuperscript{18}

\textbf{RESULTS}

At baseline, mean AV gradients of all PNPD were 1 \mu M at each site. Lactate AV gradients did not exceed 200 \mu M across any tissue bed (renal = 200 ± 100 \mu M, diaphragm 200 ± 100 \mu M, femoral 100 ± 100 \mu M, portal 100 ± 100 \mu M), and did not differ significantly. Mean baseline values of venous Po\textsubscript{2} were all greater than 40 mm Hg, the renal vein being the highest (67 ± 5 mm Hg) and the femoral lowest (45 ± 5 mm Hg).

During hypotension, arterial levels of the measured PNPD rose only slightly (e.g., the mean arterial hypoxanthine level reached 2 ± 0.2 \mu M). There was, however, a rise in plasma PNPD at the venous sites (Table 1). Of the four PNPD measured, hypoxanthine and xanthine gradients were the highest. The mean xanthine gradient was less than the hypoxanthine gradient, e.g., the mean renal AV gradient for xanthine was approximately 40 percent less than the gradient for hypoxanthine. Mean AV gradients for inosine and adenosine were generally small. As the highest PNPD gradient was hypoxanthine, we chose it as the com-
bound for further comparisons.

Marked differences in the magnitude of the AV gradient for hypoxanthine existed at different sites. Resting muscle, as sampled at the femoral site, had a very low hypoxanthine gradient (Table 1). The AV gradients for hypoxanthine at the renal, phrenic, and portal sites were large and all significantly greater than from the femoral site (p < 0.002).

Lactate gradients also differed between sites, but in a different pattern. During hypotension positive AV gradients for lactate were detected across the femoral (700 ± 100 μM), portal (1,700 ± 300 μM), and phrenic (1,800 ± 400 μM) tissue beds. The AV gradient across the kidney (−400 ± 200 μM) was significantly different from the former three sites (p values < 0.002; Fig 1), but not different from baseline. Arterial blood lactate values during baseline and shock are shown in Table 2.

When lactate and PNDP gradients were compared, three patterns emerged. The kidney demonstrated no lactate gradient despite a marked renal PNDP gradient. In contrast, the femoral AV gradient for PNDP remained small yet was accompanied by a large lactate gradient. Portal and diaphragmatic tissue beds followed a third pattern, with tissue gradients for both lactate and PNDP rising significantly above baseline (p < 0.05). Thus, lactate gradients and PNDP gradients appeared most disparate in the kidney and hindlimb (Fig 2). When the hypoxanthine gradient is plotted against the lactate gradient, samples from the renal and femoral sites describe widely divergent lines which differ from each other significantly, (ie, the slope of the femoral curve is significantly different from zero, p < 0.05, while the slope of the renal curve is not).

As would be expected, the venous Po2 at all venous sites fell during shock (Fig 3). All mean values of venous Po2 were less than 35 mm Hg, and were also significantly less (p < 0.001) than baseline values. Systemic arterial values of blood gases during shock are presented in Table 2. When hypoxanthine gradients and venous Po2 values are compared, the renal and femoral sites were again the most disparate. At oxygen tensions below 35 mm Hg, there was a sharp increase in the renal hypoxanthine gradients. No such increase occurred at the femoral site, despite much lower values of venous Po2 (Fig 4).

We measured blood flow to the renal and femoral beds in the final eight dogs, because these tissue beds were most disparate in their metabolic response to shock. With hemorrhagic shock, Doppler probe measured blood flow in the renal and femoral arteries fell to 20 ± 7 percent and 17 ± 8 percent of baseline respectively. Although baseline renal blood flow per gram of tissue (140 ± 21 ml/100 g/min) was much greater than flow to resting muscle (6 ± 2 ml/100 g/min), the decrease with shock was comparable.

**DISCUSSION**

This study demonstrates important differences between tissues in their metabolic response to hypovo-
lemic shock, as assessed by PNDP gradients, lactate gradients, and venous oxygen tension. Each of these indices reflects a very different aspect of cellular metabolism. Lactate is dependent on the intracellular oxidation/reduction state, while venous oxygen tension in a specific organ effluent reflects mean tissue oxygen tensions.\(^5\)\(^6\) The PNDP gradients most likely reflect ATP degradation and may indicate ATP depletion.\(^1\)\(^3\) Therefore, the tissue metabolic response to shock is nonhomogeneous, and the changes seen in one tissue cannot be used to predict changes in another tissue.

During acute hemorrhagic shock, resting muscle had only a small PNDP gradient. This occurred despite venous Po\(_{2}\) low enough to stimulate glycolysis, as evidenced by the positive lactate gradient. The small PNDP gradient across the hindlimb in this setting most likely reflects relative preservation of tissue ATP, but we did not measure tissue concentrations. We believe this was due to low tissue energy demands and the ability of anaerobic glycolysis and creatine phosphate to support ATP levels. This is in keeping with work by Chaudry et al,\(^1\)\(^9\) which showed no significant fall in rat tissue ATP levels in resting muscle after one hour of hemorrhagic shock, despite significant decreases in hepatic and renal ATP levels. In contrast, working skeletal muscle appears to release large amounts of PNDP.\(^2\)\(^0\) An alternative explanation for the minimal release of PNDP is that extremely low skeletal muscle blood flow may have affected the washout of muscle metabolites. Changes in blood flow, however, should not cause a disparity between lactate release and PNDP release. Moreover, results of prior studies suggest that the preferential release of lactate is not due to diminished blood flow.\(^5\)\(^1\)\(^2\)\(^2\) During systemic hypoxia, a condition that does not reduce hindlimb flow,\(^8\) Thiringer et al\(^2\)\(^1\) noted that the hindlimb produced minimal gradients for hypoxanthine despite significant lactate efflux. It is, therefore, unlikely that this pattern of metabolite release is a result of very low blood flow alone.

In contrast to resting skeletal muscle, the kidney had a considerable PNDP gradient, yet had no lactate gradient. Although the kidney is an organ with high

![Figure 2. The A-V gradients for lactate and hypoxanthine during hemorrhagic shock. Values for the renal and femoral tissue bed are shown. R\(^2\)=0.4 for renal (0.015X−0.5= y) and R\(^2\)=0.4 for femoral (0.2X+0.5 = y).](image)

![Figure 3. Venous oxygen tensions in the effluent from tissue beds. Baseline and hemorrhagic shock values are presented. The bars represent the mean ± SE. All shock values are significantly less than baseline (p<0.001).](image)
baseline energy demands, there is evidence that it lacks a high degree of glycolytic activity.34 Daniel et al35 have shown that renal tissue lactate concentrations are less than those in arterial blood during shock, suggesting that the kidney can generate only a small amount of ATP by glycolysis during shock. Additionally, creatine phosphate levels are low in renal tissue, and creatine phosphate may not function as an emergency high energy phosphate source in the kidney.36,27 Therefore, the increased renal PNDP gradient may reflect the combination of greater energy demands and limited nonoxidative methods for maintaining renal ATP levels, thereby increasing the kidney’s susceptibility to ATP degradation during shock. This occurs despite renal venous Po2 being higher than the resting muscle venous Po2, an occurrence perhaps due in part to the countercurrent characteristics of renal blood flow.4

The portal and phrenic tissue beds had positive lactate and PNDP gradients concurrent with significant venous hypoxemia. These tissues have both high energy demands and high glycolytic activity during ischemia, at least when compared to the resting femoral tissue bed. Prior studies have demonstrated large increases in blood lactate with shock in spontaneously breathing dogs.28–30 This increase in lactate could be ameliorated by mechanically ventilating the dog. Both lactate and PNDP gradients are consistent with energy expenditures exceeding the ability of accelerated anaerobic metabolism to prevent net ATP degradation in these tissues. The present observations are consistent with Chaudry’s findings that diaphragmatic ATP levels fell in spontaneously breathing rats subjected to hemorrhagic shock for one hour.7 Of note, phrenic venous levels of inosine were appreciable during shock. A previous report suggested that ATP catabolism in working muscle may favor the formation of inosine monophosphate.31

Although prior investigations have shown that shock causes a redistribution of blood flow,35,36,38 it is unlikely that changes in blood flow alone caused the different pattern of metabolic gradients in our study. The percentage of decrease in measured blood flow with shock was comparable in the renal and femoral beds, the tissues most disparate in their metabolic response to shock. Our findings are consistent with the shock state in working diaphragm, where increases in blood lactate are not due to changes in blood flow but appear to be related to muscle work.32,38 During shock, it appears that the kidney contributes a larger share of circulating PNDP, while the hindlimb contributes to the circulating level of lactate. The latter concurs with prior work showing skeletal muscles to be a major contributor to the systemic lactate pool during shock.25 The portal and phrenic beds contribute both lactate and PNDP to the systemic circulation.

These findings may have considerable clinical correlation. Our data suggest that during acute hypotension, visceral organs (eg, gut and kidney), and not resting skeletal muscle, are a major source of PNDP found in blood. This is in contrast to blood lactate, which is conventionally believed to be produced largely by skeletal muscle. Thus, the detection of high levels of PNDP in acutely hypotensive patients performing minimal muscular work (eg, during mechanical ventilation) may indicate visceral ATP degradation.

Figure 4. The AV gradient for hypoxanthine and the effluent oxygen tension during hemorrhagic shock. The values for two tissue beds are presented. The lines were drawn by inspection.
This finding may be the basis for the clinical observations that elevated blood and urine PNDP levels predict a poor prognosis in the critically ill patient.\textsuperscript{17,33} Our data also suggest that in the spontaneously breathing patient, respiratory muscles may be an additional source of circulating PNDP. This could be explained by significant depletion of TAN in both the abdominal viscera and respiratory muscles.

The large PNDP gradients from some tissue beds during clinical shock suggest that therapy limiting TAN depletion may be advantageous. Preliminary work with infusions of substrates for nucleotide synthesis, \textit{e}g, inosine and phosphoribosylpyrophosphate, has had limited success.\textsuperscript{34} More recent interest has centered on xanthine oxidase. Xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine and uric acid. Following tissue hypoxia, the enzyme may concomitantly produce superoxide radicals as it degrades these PNDPs.\textsuperscript{35} Thus, the presence of large amounts of tissue or circulating PNDP may increase the risk of oxygen metabolite mediated tissue damage by providing increased substrate for xanthine oxidase. In these settings, inhibition of xanthine oxidase may then be useful in preventing oxygen radical-mediated postischemic damage.\textsuperscript{36-38} In addition, xanthine oxidase inhibition would alter the pattern of purine nucleotide degradation, preventing metabolism of xanthine and uric acid which are unable to be reutilized by purine biosynthetic pathways.

In summary, this study demonstrates that PNDP AV gradients, lactate AV gradients, and venous PO\textsubscript{2} may have dissimilar relationships in different tissues. The relevance of our study is that it alerts both the scientist and the clinician that one may not assume from the pattern of one metabolite that other metabolic markers are necessarily following the same pattern. It also points out that certain metabolic markers may be organ specific. Whether measurements of PNDP will ultimately aid in better directing therapy or determining prognosis in shock remains both intriguing and speculative.

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