Evaluation of the role of cellular hypoxia in sepsis by the hypoxic marker [^{18}F]fluoromisonidazole

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HOTCHKISS, RICHARD S., ROBERT S. RUST, CARMEN S. DENCE, TODD H. WASSERMAN, SHENG-KWEI SONG, DAH-REN HWANG, IRENE E. KARL, AND MICHAEL J. WELCH. *Evaluation of the role of cellular hypoxia in sepsis by the hypoxic marker [^{18}F]fluoromisonidazole.* Am. J. Physiol. 261 (Regulatory Integrative Comp. Physiol. 30): R965–R972, 1991.—Underlying cellular hypoxia, which may be difficult to detect, has been postulated to be a major cause of morbidity and mortality in sepsis. We employed the novel hypoxic marker [^{18}F]fluoromisonidazole to determine whether cellular hypoxia was present in a peritonitis model of sepsis in the rat. A second group of septic and control rats had organ blood flow measurements determined by the radiolabeled microsphere technique to relate possible ischemia to decreased organ perfusion. No evidence of cellular hypoxia was detected in skeletal muscle, brain, liver, heart, or diaphragm in the septic rats. Ligation of the femoral artery caused a greater reduction in flow (55% decrease vs. 20% decrease, $P < 0.05$) and an increased retention of [^{18}F]fluoromisonidazole in skeletal muscle of the septic rats. We conclude that sepsis does not invariably result in systemic, i.e., multiorgan, cellular hypoxia and that underlying cellular hypoxia is not the major pathophysiological abnormality in sepsis. The greater reduction in muscle blood flow and the increased retention of [^{18}F]fluoromisonidazole in the ischemic muscle of septic rats implies that they may be more vulnerable to hypoxia.

sepsis; fluoromisonidazole; hypoxia

ONE OF THE MOST fundamentally important and controversial issues concerning the pathophysiology of severe bloodstream-borne infection (i.e., sepsis) is the role of cellular hypoxia (1–3, 6, 23, 24). Many investigators believe that cellular hypoxia is a major underlying component of the sepsis syndrome and is responsible for the progression of sepsis and the resulting morbidity and mortality (2, 3, 6). Cellular hypoxia is presumed to result from defective microcirculatory blood flow due to either platelet and fibrin microthrombi obstructing selected capillary beds and/or defective autoregulation of blood flow. Some investigators propose that cellular hypoxia may occur despite elevated cardiac output and oxygen delivery because of maldistribution of flow (2, 23). The abnormal relationship of oxygen supply to oxygen utilization and the increase in plasma lactate, which are frequent findings in sepsis, are used to support the concept of underlying cellular hypoxia (2, 6, 23). A second competing theory on the pathophysiology of the sepsis syndrome is that sepsis induces a primary metabolic abnormality that occurs independently of cellular oxygen tension (22). Abnormalities in mitochondrial substrate metabolism and in specific enzymes such as pyruvate dehydrogenase have been implicated in the defective oxygen utilization and increase in lactate (22). Resolution of these two divergent theories is important not only in the daily management of this disorder but also in efforts to understand the cellular and subcellular effects of this often lethal disorder. A novel and exciting strategy for the detection of cellular hypoxia in vivo has evolved from studies of misonidazole, a 2-nitroimidazole, and its congeners (4, 5, 15, 18). These lipophilic, freely diffusible compounds are covalently bound in viable hypoxic cells in inverse proportion to cellular oxygen tension (4, 5, 26, 27). The use of these compounds has been extensively validated in various models including myocardial (18, 19, 29, 30), cerebral (9, 20), hepatic (21, 32), and tumor ischemia (4, 5). The purpose of this investigation was to determine the degree to which cellular hypoxia was present in an animal model of sepsis by using the positron-emitting hypoxic marker [^{18}F]fluoromisonidazole (Fig. 1).

MATERIALS AND METHODS

Animal Protocols

Septic model. The cecal ligation and perforation model (33) was used to induce peritonitis in thirteen 200- to 250-g female Sprague-Dawley rats. The cecum was punctured twice with a 19-gauge needle; further details including bacteriological results have been described previously (11). All surgery was performed using halothane anesthesia with air as the carrier gas. After surgery, the animals were housed in metabolic cages and kept fasted but allowed free access to water. (We kept both groups fasted because the septic rats eat little or no food.) Approximately 36–42 h after surgery, 11 surviving septic rats (2 septic rats died before this period) and the 7 sham-operated rats were again anesthetized, and the left femoral vein was surgically exposed. Each rat was injected via the femoral vein with [^{18}F]fluoromisonidazole (125 μCi radioactivity) and ^{125}I-labeled albumin (1 μCi radioactivity), which was used as a marker for blood volume. Three minutes later, both femoral vein and femoral artery were ligated, anesthesia was discontinued,

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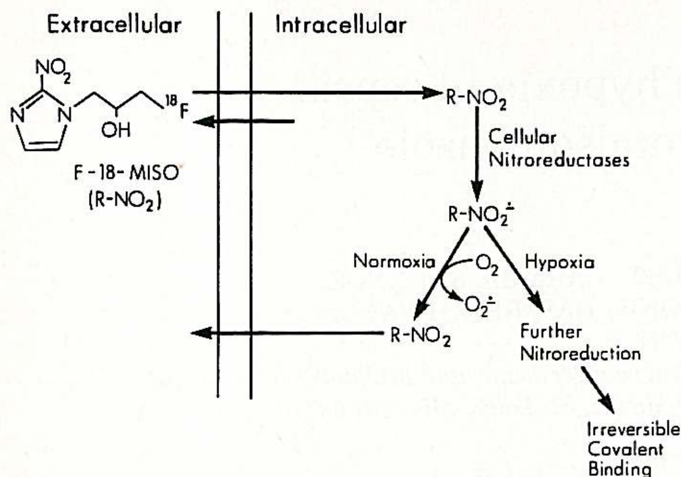

FIG. 1. Schematic diagram of the proposed metabolism of [^{18}F]-fluoromisonidazole (F-18-Miso) under normoxic and hypoxic conditions. R-$NO_2^{\cdot-}$, = reduced nitrate (modified from Ref. 29).

and the rats were returned to their cages where they promptly awoke. Ligation of the femoral artery was performed to determine the impact of decreased muscle blood flow and possible cellular hypoxia on retention of [^{18}F]fluoromisonidazole (see *Tissue Blood Flow* and DISCUSSION for the impact of femoral artery ligation on the hindlimb). Five hours after injection of [^{18}F]fluoromisonidazole, the nine remaining septic rats (2 septic rats died during this 5-h period) and seven control rats were again anesthetized, and the abdominal cavity was opened. All nine septic rats had evidence of gross peritonitis including foul-smelling purulent abdominal fluid, fibrinous exudates adhering to much of the large bowel, and a gray/black cecum. Abdominal examination of the control rats revealed normal healthy pink bowel with minimal peritoneal fluid.

After the blood specimens were obtained, the rats were killed and brain, heart, right and left lung, diaphragm, and right and left gastrocnemius muscles were obtained. All samples were weighed and counted for ^{18}F and ^{125}I (Table 3).

Reversible Ischemia

To determine the retention of [^{18}F]fluoromisonidazole in a reversible ischemic model, six control (i.e., nonoperated healthy) rats were examined. The rats were anesthetized, and [^{18}F]fluoromisonidazole and ^{125}I-albumin were injected via the femoral vein as previously described. Thirty minutes after injection and while the rats were still anesthetized with halothane and air, a modified rubber tourniquet was applied to the left upper leg, and ischemia was documented by appearance of cyanosis of the affected extremity. The tourniquet was left in place for 30 min and then removed. The rats were returned to their cages and promptly recovered from anesthesia. Four hours later, these rats were reanesthetized and blood, right and left gastrocnemius muscles, and heart were obtained, weighed, and counted for ^{18}F and ^{125}I.

Plasma Metabolite Concentrations

Arterial blood samples were obtained from the abdominal aorta and were used for arterial blood gas analysis and plasma metabolite analysis via fluorometric enzymatic technique (17) (Table 1). Analysis of selected plasma metabolites was performed to confirm the septic condition in the animals.

Ligation of the femoral artery in the hindlimb may induce cellular ischemia and elevated plasma lactate; see DISCUSSION. To determine plasma lactate values from sham-operated and septic rats that did not have ligation of the femoral artery, a separate group of 11 sham and 11 septic rats that were prepared in an identical fashion (except that they did not have ligation of the femoral artery) also had blood samples obtained and plasma lactate values measured.

[^{18}F]fluoromisonidazole Determinations

Synthesis, stability, purity and specific activity of no-carrier-added [^{18}F]fluoromisonidazole were as previously described (13). The percent injected dose per gram of tissue (%ID/g) of all samples was determined. To correct for the amount of the [^{18}F]fluoromisonidazole contained in the blood that was present in the various tissue samples, the following formula was used to obtain "corrected" %ID/g ^{18}F-labeled tissue:

$$\text{corrected } \%ID/g\ ^{18}F\text{-tissue} = \%ID/g\ ^{18}F\text{-tissue} - [(\%ID/g\ ^{18}F\text{-blood} \times \%ID/g\ ^{125}I\text{-tissue}) \div \%ID/g\ ^{125}I\text{-blood}]$$

Effect of sampling time on [^{18}F]fluoromisonidazole retention. The biological half-time of the bound [^{18}F]-fluoromisonidazole, present in the tissues after the initial washout, is ~40 h and is consistent with irreversible covalent binding. Therefore, longer time intervals between administration of tracer and sampling of tissues will improve the discriminating ability of the [^{18}F]fluoromisonidazole to identify hypoxic tissue because of continued elimination of the unbound [^{18}F]fluoromisonidazole from normoxic tissues. The 110-min physical half-time of the ^{18}F will limit the degree to which tissue sampling can be delayed. To determine the effects on tissue-to-blood ratio of sampling at different postinjection time points, in addition to the 5-h group, we also sampled at 2 h (7 septic rats and 7 sham rats) and 8 h (8 septic rats and 8 sham rats). The tissues that were sampled included heart, right and left gastrocnemius muscle, liver, and right kidney (Table 3).

Tissue Blood Flow Measurement

To relate changes in organ blood flow during sepsis to possible cellular ischemia, a third group of 10 septic and 7 control rats had determination of tissue blood flow using radiolabeled microspheres using the technique of Heyman et al. (8) and as previously described (12). Each rat had two determinations made using alternately ^{46}Sc and ^{103}Ru (Table 2).

Statistical analysis. The significance of difference test between two samples of data was carried out by using RS/1 system from BBN Software. The paired *t* test was performed on the data measured from the same animal, i.e., fluoromisonidazole retention in normal vs. ischemic

TABLE 1. *Arterial plasma metabolite concentrations and arterial blood gases*

	Glucose	Aceto-acetate	β-Hydroxy-butyrate	Lactate	Glutamate	Glutamine	Alanine	Arterial P_{O_2}, mmHg	Arterial P_{CO_2}, mmHg	Arterial pH	Base Excess/Deficit, meq/l
Sham	7.8±0.4	50.2±5.6	1754.9±364.7	2818.4±241.8	132.7±35.7	1042.2±156.4	318.2±60.2	109.3±16.3	31.6±2.2	7.45±0.02	−0.6±1.9
Septic	10.8±1.0	30.1±9.3	648.8±66.6	4117.8±772.2	83.8±15.3	817.4±146.5	302.3±72.4	94.7±16.3	34.0±4.6	7.46±0.04	1.6±2.0
P	0.0001	0.0002	0.0001	0.0015	0.0089	0.0123	ND	ND	ND	ND	ND

Values are means ± SD; for metabolic analysis, $n = 7$ for sham and $n = 8$ for septic. Glucose values are in mM; all other metabolite values are μM. For blood gas analysis, $n = 7$ sham and $n = 6$ septic. ND, not statistically different.

TABLE 2. *Tissue blood flow*

	n	Gastrocnemius		Diaphragm	Brain Stem	RH	LH	Right Heart	Liver	Kidney
		Normal	Ischemic							
Sham	14	0.081±0.031	0.065±0.052‡	0.479±0.099	1.547±0.0478	1.296±0.0404	1.694±0.0462	3.40 ±1.74	0.532±0.201	5.039±1.061
Septic	20	0.073±0.031	0.033±0.019*‡	0.614±0.0367	1.226±0.0579	1.139±0.0429	1.274±0.0375	3.230±1.389	0.542±0.365	3.759±1.305†

Values are means ± SD in $ml \cdot min^{-1} \cdot g\ tissue^{-1}$; *n*, number of measurements (each rat had 2 measurements). RH, right cerebral hemisphere; LH, left cerebral hemisphere. * $P < 0.05$ for ischemic septic muscle vs. ischemic control muscle. † $P < 0.01$ for septic kidney vs. control kidney. ‡ $P < 0.01$ for normal vs. ischemic gastrocnemius muscle.

gastrocnemius. For unpaired data, an *F* test was first employed to test for the equality of variance. The pooled variance *t* test was employed for data with equal variances. The unpooled variance *t* test was employed for data with unequal variances. Statistical significance was accepted at the 95% confidence limit. The data are presented as means ± SD.

RESULTS

Metabolic and Arterial Blood Gas Analysis

Examination of the plasma metabolites revealed the characteristic findings of the sepsis syndrome including increased glucose (22), increased lactate (24), decreased ketone bodies (28), and decreased glutamine (7) (Table 1). Interestingly, despite the increased lactate in the septic rats, arterial blood pH and calculated base deficit were normal and not statistically different from the control rats (see DISCUSSION).

The plasma lactates in the 11 sham-operated and 11 septic rats that did not have ligation of the femoral artery were 1,548.8 ± 117.5 and 3,639.1 ± 695.4 μmol/l, which were statistically different ($P < 0.001$) and comparable to the plasma lactates in the sham and septic rats that had ligation of the femoral artery.

Tissue Blood Flow

There were no statistical differences in septic vs. control rats in blood flow to brain, heart, diaphragm, or the gastrocnemius muscle in the leg that did not have ligation of the femoral artery (Table 2). Blood flow to the kidney was decreased by 25% in the septic rat compared with control ($P < 0.01$). There was a statistically significant decrease in blood flow between the gastrocnemius in the normal leg vs. the gastrocnemius muscle in the leg that had the femoral artery ligated for both control ($P < 0.01$) and septic ($P < 0.0001$) rats. However, the decrease in flow was more profound in the septic rat (55%) than in the control rat (20%) ($P < 0.05$). As previously reported (12), there were no differences in hematocrits (~39%) or mean arterial pressure (~80 mmHg) between the septic and control rats. The cardiac index and stroke volume index were decreased by ~20% ($P < 0.03$) in the septic rats compared with controls (12).

[^{18}F]fluoromisonidazole

Five-hour experiment. There were no significant differences in retention of [^{18}F]fluoromisonidazole in lung, heart, diaphragm, or normal skeletal muscle in septic and control rats (Fig. 2). Ligation of the femoral artery caused an increase in [^{18}F]fluoromisonidazole retention in the ischemic gastrocnemius muscle compared with the normal gastrocnemius muscle (i.e., paired muscles from the same animal, in both septic and control) (Fig. 3).

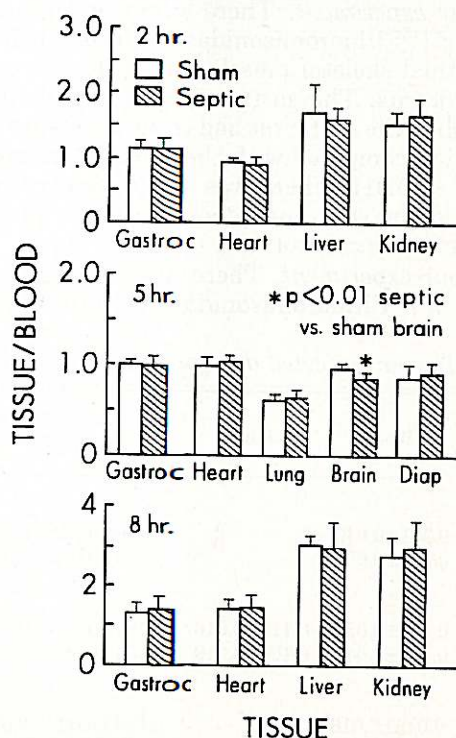

FIG. 2. Tissue-to-blood ratios for [^{18}F]fluoromisonidazole for septic and sham-operated rats. Gastroc, gastrocnemius muscle; $n = 7$ sham and 7 septic rats, 2 h; $n = 7$ sham and 9 septic rats, 5 h; $n = 8$ sham and 8 septic rats, 8 h.

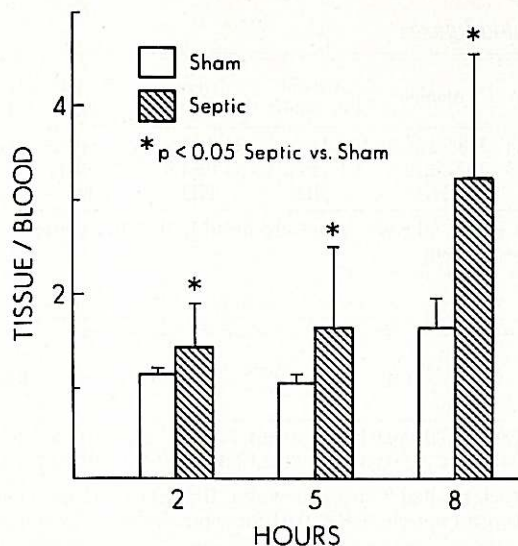

FIG. 3. Tissue-to-blood ratios for [^{18}F]fluoromisonidazole of the ischemic gastrocnemius muscle for septic and sham-operated rats. No. of rats at each time point as in Fig. 2.

Furthermore, the septic animals had a greater increase in [^{18}F]fluoromisonidazole retention in the ischemic gastrocnemius muscle than did the control rats (58% and 6%, respectively). Comparison of the retention of [^{18}F]-fluoromisonidazole in the brains of the septic vs. control rats revealed that the septic brains actually had a lower retention of the hypoxic marker. Although there was no difference in the septic and control brains when expressed as the %ID/g (Table 3), the septic brains did have a lower retention of the hypoxic marker when expressed as the brain-to-blood ratio ($P < 0.01$).

Two-hour experiment. There were no differences in retention of [^{18}F]fluoromisonidazole in heart, liver, kidney, or normal skeletal muscle in septic rats compared with control rats. The gastrocnemius muscle in the ischemic limb of the septic rat had a 20% increase in tracer concentration compared with the normal gastrocnemius muscle ($P < 0.01$). There was no difference in tracer concentration between the ischemic muscle and the normal muscle in the control rats.

Eight-hour experiment. There was an increased concentration of [^{18}F]fluoromisonidazole in the blood of the septic rats compared with control rats ($P < 0.04$) (Table 3). The increased concentration of [^{18}F]fluoromisonidazole in the blood of the septic rats is most likely due to delayed renal excretion in the septic rats compared with the control rats. Renal blood flow is decreased in the septic rats (see Table 2). The concentration of [^{18}F]-fluoromisonidazole in the tissues reflects to some extent the circulating concentration in the blood, and consequently the septic rats would tend to have higher values for the %ID/g compared with control rats for most of the organs (Table 3). Nevertheless, the difference between septic and control tissues was only statistically significant for the kidney ($P < 0.03$). To avoid the confounding effect of the elevated blood levels of [^{18}F]-fluoromisonidazole in the septic rats, the tissue-to-blood ratios can be used to compare the septic and control rats for differences in retention of [^{18}F]fluoromisonidazole and thus the presence of cellular hypoxia (Fig. 2). Examining the tissue-to-blood ratios, we found that there were no differences in liver, kidney, normal gastrocnemius muscle, or heart between the septic and control rats at the 8-h time period. The ischemic muscle in the septic rats had a greater retention of [^{18}F]fluoromisonidazole (146%) than did the ischemic muscle in the control rats (52%).

Reversible ischemia. [^{18}F]fluoromisonidazole retention was significantly greater in the reversible ischemic hindlimb. The [^{18}F]fluoromisonidazole tissue-to-blood ratios were 0.995 ± 0.099 and 1.196 ± 0.139 for the normal gastrocnemius muscle and the reversibly ischemic gastrocnemius muscle, respectively, and were highly significantly different ($P < 0.003$).

DISCUSSION

Sepsis and the resulting multiorgan failure that it induces are now the most common cause of death in the surgical intensive care unit. One of the most important issues is whether underlying cellular hypoxia, which may be difficult to detect, is an important abnormality in sepsis (1–3, 6, 24). Many investigators believe that sepsis causes defective microcirculatory blood flow due to either abnormal autoregulation of flow or capillary endothelial obstruction (2, 3, 6, 23). These investigators propose that

TABLE 3. *Percent injected dose per gram of tissue of [^{18}F]fluoromisonidazole*

	n	Blood	Lung	Gastrocnemius Normal	Gastrocnemius Ischemic	Heart	Liver	Brain	Kidney	Diaphragm
2-h Experiment										
Sham	7	0.301±0.036		0.346±0.061	0.348±0.047	0.266±0.035	0.501±0.086		0.458±0.036	
Septic	7	0.288±0.080		0.326±0.072	0.392±0.072^{ab}	0.258±0.070	0.465±0.140		0.477±0.163	
5-h Experiment										
Sham	7	0.167±0.023	0.110±0.016	0.167±0.025	0.177±0.021^{b}	0.164±0.025		0.161±0.023		0.148±0.012
Septic	9	0.157±0.041	0.104±0.019	0.154±0.033	0.244±0.082^{ab}	0.165±0.049		0.135±0.033		0.140±0.026
8-h Experiment										
Sham	8	0.048±0.016		0.064±0.027	0.097±0.003^{ab}	0.070±0.031	0.144±0.044		0.129±0.034	
Septic	8	0.070±0.029^{c}		0.095±0.039	0.234±0.061^{ab}	0.099±0.043	0.194±0.071		0.197±0.073^{d}	

Values are means ± SD in percent injected dose per gram of tissue. [a] Control ischemic muscle less than septic ischemic muscle, $P < 0.05$. [b] Normal gastrocnemius muscle less than ischemic gastrocnemius muscle (paired muscles from same rat), $P < 0.05$. n = number of rats. [c] Control blood less than septic blood, $P < 0.04$. [d] Control kidney less than septic kidney, $P < 0.03$.

a primary abnormality in sepsis is the cellular hypoxia that results from the abnormal blood flow distribution. The abnormal supply dependence of oxygen uptake, which has been demonstrated in animal models of endotoxic (6) and septic shock (1, 23) and in patients with septic shock (16), is felt by some investigators to be a reflection of inadequate cellular oxygen delivery. The cellular oxygen deficiency is presumed to be a major driving force responsible for progression of the sepsis syndrome and the resulting morbidity and mortality (2, 3). The major finding of the present study is that sepsis does not result a priori in systemic, i.e., multiorgan, cellular hypoxia and makes it unlikely that unrecognized cellular hypoxia is responsible for the progression of the sepsis syndrome. This study does not exclude the possibility of cellular hypoxia occurring in the kidney (because of the presence of large amounts of the excreted drug present in the urine) or in other organs, such as the gastrointestinal tract, that were not examined.

The use of the nitroimidazole compounds as hypoxic markers has been well validated in numerous laboratories (4, 9, 15, 20, 26, 30). It has been shown that fluoromisonidazole identifies hypoxic cells in tumors (5, 27), spheroids (26), monolayered cells (4), brain (9, 20), and myocardium (18, 19, 29, 30). Because these nitroimidazole compounds are lipophilic and freely diffusible, they can readily traverse cell membranes and identify hypoxic cells even if flow is markedly reduced. Retention of the compounds is inversely related to cellular oxygen tension. The presumed mechanism of accumulation and retention of fluoromisonidazole in hypoxic tissues involves diffusion across the plasma membrane where the nitro group undergoes reduction by ubiquitous cellular nitroreductase enzymes to form the reduced nitrate radical anion (4, 5, 29) (Fig. 1). In the presence of oxygen, the favored subsequent reaction is donation of the electron to oxygen to form the superoxide anion with regeneration of the lipophilic parent compound, which is free to diffuse back across the cell membrane. With hypoxia, however, the reactive nitro anion is further reduced and eventually binds covalently to intracellular macromolecules, thereby becoming irreversibly trapped within the cell.

The nitroimidazoles are ideally suited for detecting intracellular hypoxia because their binding is quite sensitive over the range of oxygen levels that exist within the cell. Although somewhat dependent on the concentration of the drug and the particular type of cells studied, drug binding, in general, progressively increases as oxygen concentration is lowered below ~3–10%, with maximum binding occurring under anoxic conditions. For both misonidazole and fluoromisonidazole, the oxygen concentration required for 50% inhibition of binding (relative to anoxia) for most cells is usually between 1,000 and 10,000 ppm (~0.8–7.6 mmHg) (26, 27).

The ability of the nitroimidazole to identify regional variations in cellular oxygenation has been confirmed in many autoradiographic studies. In liver, oxygenated blood enters the sinusoidal vessels from the portal tracts and becomes deoxygenated as it flows toward hepatic veins. Cells surrounding hepatic veins are therefore likely to be the most hypoxic hepatocytes in vivo (21). Autoradiographs of livers from mice injected in vivo with labeled nitroimidazoles demonstrate characteristic regional differences in binding of the tracer (21, 32). Binding of the nitroimidazoles is most intense in regions near hepatic veins but sparse in areas surrounding portal tracts. This pattern of binding has been striking and consistent and is thought to reflect the regional oxygen tension variation that exists in vivo in healthy organs.

A potential mechanism whereby [^{18}F]fluoromisonidazole would fail to identify hypoxic tissues in septic animals would exist if sepsis caused inactivation of the intracellular enzymes responsible for nitroreduction of the fluoromisonidazole. There is no evidence that such enzyme inactivation has occurred in any of the studies in which nitroimidazoles have been employed. Furthermore, as other investigators have reported, nitroaromatic compounds can interact with electron transfer processes in cells by means of a variety of enzymes whose normal function is subverted to that of a nitroreductase in the presence of nitroaromatic compounds (25). These enzymes include xanthine oxidase, aldehyde oxidase, NADPH cytochrome P_{450} reductase, NADPH cytochrome *c* reductase, and lipolyl dehydrogenase (25). Thus many enzymes would have to be affected by sepsis for fluoromisonidazole not to undergo nitroreduction and therefore not identify hypoxic tissues. This would seem unlikely. Second, the purpose of ligation of the femoral artery and femoral vein in the septic and control rats was to validate the ability of the fluoromisonidazole to detect hypoxia in the experimental rats. The rat hindlimb has good collateral blood supply, and the microsphere flow results indicated that a significant amount of blood flow remained in the affected hindlimb despite femoral artery ligation. Nevertheless, fluoromisonidazole did detect reduced oxygen tension in the affected hindlimb and, in fact, identified the septic hindlimb to be more susceptible to hypoxia than the control hindlimb (Fig. 3). Thus there is no evidence that sepsis disturbed the enzymes responsible for nitroreduction of the fluoromisonidazole.

The higher concentration of nitroimidazoles in the kidney relative to other organs is presumably due to inclusion of variable amounts of urine (18). The kidney is the primary pathway for the drug's excretion, and urine concentrations are 50–80 times plasma concentration. In mice injected intraperitoneally with fluoromisonidazole, the drug is rapidly cleared from the plasma by urinary excretion with a fast component (half-time = 1 h) (Ref. 26). The higher concentration of nitroimidazoles retained in the liver has been investigated in a number of studies and is believed primarily to reflect the unusual circulatory supply to the liver and the resulting lower oxygen tension gradients in hepatocytes surrounding hepatic veins (21, 32).

Although there were no differences in the retention of the fluoromisonidazole in septic and control organs, the findings in the ischemic hindlimb suggest that the septic tissues may be more susceptible to cellular hypoxia (Fig. 3). Although the rat hindlimb has good collateral blood flow, and previous experience with ligation of the femoral artery (performed for chronic placement of aortic cannulas) at the iliac fossa in many control rats has usually

resulted in no physical evidence of ischemia or compromised function (i.e., the extremity is not cyanotic and the rats ambulate normally on the affected hindlimb), there is a reduction in flow to the affected limb (see microsphere flow results, Table 2) that is usually not great enough to cause profound ischemia or cell death. This difference between septic and control retention of fluoromisonidazole in the ischemic leg may represent a decreased ability of the septic tissue to regulate or recruit collateral blood flow. The microsphere blood flow data confirmed the greater decrease in blood flow to the ischemic leg in the septic rats and would support this conclusion. This decreased ability to recruit blood flow is consistent with the abnormality in microcirculatory blood flow regulation that other investigators have reported (2, 23). It also implies that the septic animals may be more vulnerable to events that decrease blood flow.

An alternative explanation for the increased fluoromisonidazole in the septic rat muscle is that it is due to delayed washout of the tracer. This issue also has been addressed by Hoffman et al. (9) and Shelton et al. (30). It seems unlikely that washout of this very diffusible drug would be substantially inhibited by the amount of flow reduction in the septic and control muscles at the 5-h and 8-h postinjection time periods. Results from the experiment on reversible ischemia employing the rubber tourniquet are consistent with this conclusion. After removing the tourniquet and reestablishing blood flow, the fluoromisonidazole retention was still greater ($P <$ 0.003) in the ischemic muscle, thus implying cellular binding of the fluoromisonidazole and not delayed washout.

Results from the timed experiments indicate that 2 h after [^{18}F]fluoromisonidazole administration may not be a sufficient time interval to detect cellular hypoxia in tissues such as muscle that have less blood flow than heart or brain. Fluoromisonidazole did not detect significant cellular hypoxia in the control ischemic hindlimb (the hindlimb in which the femoral artery and vein were ligated) at 2 h, but it did detect hypoxia at 5 h and 8 h. Shelton et al. (30), using positron emission tomography in an ischemic dog model, reported increased accumulation of [^{18}F]fluoromisonidazole in ischemic myocardium compared with normal myocardium at 30 min. In vivo studies in a gerbil stroke model found distinct differences in fluoromisonidazole concentrations in normal vs. ischemic brain at 2 h (20). Rasey et al. (27) did not find much improvement in the ratio between hypoxic tumor cells and normal tissue in tumor-bearing mice when the sampling time was extended beyond 4 h to 18 h. Presumably, the lesser amount of blood flow to muscle relative to heart and brain will result in slower washin and washout of unbound tracer. Extending the time interval to 8 h resulted in continued improvement in the hypoxic tissue (ischemic gastrocnemius)-to-blood ratios (Fig. 3). The important point is that for tissues with reduced baseline blood flow (such as resting skeletal muscle) and mild cellular hypoxia (such as existed in the ischemic hindlimb in the present study), 2 h may not be a sufficient time period to allow definitive detection of cellular hypoxia by fluoromisonidazole.

One surprising finding was the decreased retention of the fluoromisonidazole in the brains of the septic rats. In a previous study, normal rats over a wide range of time periods had a brain-to-blood ratio of ~1 (15). The sham-operated rats in the present study had a brain-to-blood ratio of 0.967, which is essentially identical to the previous study. Therefore, the real question is why the septic rats had a lower brain-to-blood ratio (0.861) than the sham-operated rats. There is no readily apparent explanation for this difference, and whether such a minor change is physiologically significant is questionable. However, binding of fluoromisonidazole is exquisitely sensitive at the low levels of oxygen that occur within the cell, and it is possible that if minor differences in intracellular oxygen tension exist in septic and control brains they would be detected. One conceivable mechanism for an increase in intracellular oxygen tension in septic brains would be that neuronal cellular oxygen consumption is decreased because of metabolic downregulation. The resulting decrease in cellular oxygen consumption would result in increased intracellular oxygen tension and therefore less retention of fluoromisonidazole during sepsis. Neurological symptoms including somnolence, stupor, and obtundation are hallmarks of sepsis (31) and are consistent with depressed metabolic function. In support of this concept of metabolic suppression, Jepson et al. (14) documented decreased aerobic metabolism in skeletal muscle in endotoxic rats that was not due to inadequate oxygen delivery.

Additional recent work in the rat supports the hypothesis that cellular hypoxia is not present in brain (10), heart (12), or skeletal muscle (30a). Cellular hypoxia causes increased anaerobic metabolism, intracellular acidosis, and a fall in tissue high-energy phosphates. In vivo ^{31}P-nuclear magnetic resonance spectroscopy performed on brain and skeletal muscle in septic rats demonstrated that intracellular pH was normal, i.e., ~7.1, and not significantly different between sham-operated rats (10). The high-energy phosphates PCr and ATP were well maintained in both brain and skeletal muscle during sepsis. Analysis of cardiac metabolites obtained from septic rats demonstrated no decrease in high-energy phosphates, normal lactate-to-pyruvate ratio, and none of the characteristic changes in the tricarboxylic acid cycle intermediates or amino acids that occur during cardiac ischemia (12).

Whether or not the findings from this animal study can be applied to the clinical arena involving critically ill patients must be determined by future investigations. Even before attempting to generalize from this study, a few points must be strongly emphasized. Cellular hypoxia can and will result if intravascular volume losses, which are likely to occur during sepsis, are not adequately replaced. In fact, there is some evidence from this study that muscle in the septic rats may be more susceptible to decreases in flow. Second, it should be reiterated that this study does not exclude significant cellular hypoxia occurring in kidney (because of the high concentration of excreted drug present) or in organs such as skin or gastrointestinal tract that were not included.

A second intriguing question that arises is the origin of the increase in plasma lactate that is a frequent finding in sepsis. There is no evidence of cellular hypoxia in the

present study, but because not all organs were sampled, it is possible that the increased plasma lactate in the septic rats was due to cellular hypoxia that existed in some of the organs that were not examined. It does not appear that ligation of the femoral artery in the rats had a significant effect to increase plasma lactate because the lactate values determined in the sham-operated and septic rats that did not have ligation of the artery were reasonably comparable to the values for the sham-operated and septic rats that did undergo femoral artery ligation. Furthermore, the plasma lactates for the sham-operated and septic rats that did have ligation of the femoral artery were virtually identical to the values obtained by Hasselgren et al. (7), i.e., 2.6 ± 0.2 and 4.1 ± 0.3 mmol/l, respectively, using a similar cecal ligation and perforation model of sepsis that did not have ligation of the femoral artery. The degree of reduction in flow to the hindlimb that had the femoral artery ligated (~20% decrease in sham-operated and ~50% decrease in septic) was probably not sufficient to cause anaerobic metabolism even though the [^{18}F]fluoromisonidazole retention was greater in that limb. In agreement with this statement is the work of Jepson et al. (14), who found that despite a 65% reduction in skeletal muscle blood flow during endotoxemia, intracellular pH was normal and therefore anaerobic metabolism was not occurring.

Although another possible explanation of the increased lactate could involve decreased uptake of lactate by the gluconeogenic organs, i.e., liver and kidney, Wolfe et al. (35) found an increase in the rate of lactate uptake by the liver in an endotoxin dog model. In addition, we have found a consistently greater increase in release of lactate (30–50%) from isolated incubated epitrochlearis muscles from septic rats compared with control rats (unpublished observations). These incubated epitrochlearis muscles are not hypoxic (maintain normal levels of ATP and PCr for over 8 h), and therefore a major cause of the increased lactate in sepsis must be increased production not due to anaerobic metabolism.

Recently, a possible explanation for the increased lactate production in the absence of cellular hypoxia has been provided. Cells that have become virally infected respond by translocation of glucose transporters from the perinuclear region to the plasma membrane (34). Thus an abnormality involving increased glucose uptake could be the driving force behind the increased lactate production. If the increase in lactate that occurs in sepsis is due to a primary disorder in glucose uptake and accelerated glycolysis, no change in intracellular pH or base deficit would occur (36). Once again, it is vitally important that cellular hypoxia due to inadequate oxygen delivery be ruled out as a cause of the increased lactate. The presence of a metabolic acidosis (which was not present in the septic rats in this study; see arterial blood gas results) would be a helpful finding in suggesting that the increased lactate is due to anaerobic metabolism and cellular hypoxia. The response of the plasma lactate to measures that increase oxygen delivery would also be a means of distinguishing the etiology of the increased lactate.

In summary, systemic multiorgan cellular hypoxia is not an invariable component of sepsis and is unlikely to be responsible for the progression of the sepsis syndrome. These findings indicate that the major pathophysiological abnormality in sepsis is not cellular hypoxia but rather a primary metabolic disorder.

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