Sepsis-induced depression of rat glucose-6-phosphatase gene expression and activity

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Deutschman, Clifford S., Kenneth M. Andrejko, Barbara A. Haber, Lisa Bellin, Eric Elenko, Rachel Harrison, and Rebecca Taub. Sepsis-induced depression of rat glucose-6-phosphatase gene expression and activity. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1709–R1718, 1997.—Sepsis in rats decreases the hepatic expression of the gluconeogenic enzyme glucose-6-phosphatase (G6Pase). The aim of this study was to investigate the relationship among G6Pase transcription, mRNA, enzymatic activity, and serum glucose levels at different intervals during mild or fulminant sepsis. Both fulminant and mild sepsis immediately decreased hepatic G6Pase mRNA levels. In mild sepsis, levels began to recover late in the time course. Serum glucose levels were maintained in mild sepsis but decreased markedly in fulminant sepsis. G6Pase transcription after fulminant sepsis decreased and never recovered. A similar transcriptional decrease was noted in mild sepsis, but some recovery occurred in this state. Histochemistry after mild sepsis revealed a decrease in G6Pase protein and enzymatic activity that paralleled transcription. These studies suggest that changes in G6Pase transcription and activity are early markers for sepsis-induced alterations in hepatic function. Mechanisms other than gene expression and enzymatic activity serve to maintain glucose levels in mild sepsis, but in the fulminant disorder, compensatory mechanisms fail and hypoglycemia develops.

Materials and Methods

Induction of SIRS-sepsis by cecal ligation and puncture. Studies were performed in male, 200- to 300-g Sprague-Dawley rats (CD strain, Charles River, Boston MA). All protocols were approved by the University Laboratory Re-
source Center of the University of Pennsylvania and con-
formed to National Institutes of Health standards. As previ-
ously described, an inflammatory state secondary to fecal
peritonitis was induced using cecal ligation and puncture
(CL P) under methoxyflurane anesthesia (8, 11, 13, 36). Two
septic models were used. In the first, fulminant sepsis was
induced using cecal ligation and double puncture with an
18-gauge needle on the antimesenteric border. In the second,
a single 18-gauge puncture was used to induce a hyperdy-
namic septic state. In sham-operated controls, the cecum
was manipulated but not ligated or perforated. In each case,
the incision was closed, and the animals were resuscitated
with 40 ml/kg saline subcutaneously. Animals were returned
to their cages, allowed to awaken, and given free access to water
but not food. After recovery for varying periods of time,
animals were reanesthetized with pentobarbital sodium (50
mg/kg ip). Blood was obtained from the inferior vena cava and
hepatic tissue isolated as detailed below. Only Northern blot
analysis and serum glucose assays were performed on double-
puncture CLP animals. Single-puncture CLP and sham-
operated animals were subjected to all procedures detailed
below.

Northern blot hybridization analysis. Tissue for Northern
blot analysis was obtained from three animals at each time
point as previous described (11, 13). In animals subjected
to double-puncture CLP, tissue was harvested 6, 16, and 24 h
after operation. In animals treated with single-puncture CLP
or sham operation, tissue was obtained at 0 (unoperated), 3,
6, 16, 24, 48, and 72 h after the initial procedure. Total hepatic
RNA was isolated using the sodium acetate-acid-phenol-
chloroform method of Chomczynski and Sacchi (7). Total RNA
(5 µg) from each animal was subjected to formaldehyde gel
electrophoresis and transferred to nitrocellulose (Micron Sepa-
rations, Westborough, MA). A G6Pase cDNA was radiolabeled
with [32P]dCTP using the random primer method of Feinberg
and Vogelstein (14). Blots were incubated with the this probe
overnight, then washed, and autoradiographed. Signal intensi-
ity was quantified using laser densitometry (Molecular
Dynamics, Sunnyvale, CA). Blots were stripped and rehybrid-
ized with a CDNA complementary to ATP synthase, a constitu-
tively expressed gene. G6Pase signal density was normalized
to ATP synthase density at the same time point. Means ± SD
were determined.

Determination of glucose in blood. Vena caval blood was
centrifuged promptly after venipuncture, and plasma was
separated and placed on ice. Glucose was determined using a
hexokinase-based kit (Sigma Diagnostics, St. Louis, MO).
Insulin and glucagon were determined by a core laboratory
(Diabetes Center, University of Pennsylvania) by radioimmu-
noassay (Linco Research, St. Louis, MO).

In situ hybridization. Tissue from three single-puncture
CLP animals was obtained at each time point and prepared
for in situ hybridization as previously described (13). Briefly,
at 0, 3, and 24 h after single-puncture CLP, animals were
reanesthetized, and the liver was perfusion fixed with Histo-
choice (Amresco, Solon, OH). The perfused organ was re-
moved, cut into slices, and fixed for 1 h at 4°C. Slices were
embedded in paraffin, and 7-µm sections were cut, adhered to
poly-L-lysine-coated slides, and dried. Just before use, sec-
tions were dewaxed, rehydrated, digested with 0.2 N HCl,
rinsed in buffered saline-0.3% Triton X-100 followed by
glycine and distilled water, acetylated with 0.25% acetic
anhydride-0.1 M triethanolamine, and prehybridized with a
50% formamide-based buffer solution. Linearized G6Pase
cDNA was labeled with digoxigenin-11-dUTP. Sections were
subjected to hybridization with this probe for 3 h at 42°C. The
sections were then washed and colorimetric detection was
carried out with anti-digoxigenin-alkaline phosphatase solu-
tion containing antibody, X phosphate, and nitro blue tetra-
zolium (Genius DNA Labeling and Detection Kit, Boehringer
Mannheim, Indianapolis, IN).

Transcript elongation analysis (nuclear run-on). In single-
puncture CLP and sham-operated animals, nuclei from three
animals at each time point were isolated, and nuclear run-on
was performed using a modification of a previously reported
method (1, 34). All procedures were performed at 4°C. Briefly,
to isolate nuclei, liver tissue was harvested, minced, homog-
ernized, filtered, layered onto 50 mM tris(hydroxymethyl)ami-
nomethane (Tris)-HCl (pH 8), 2 M sucrose, 5 mM magnesium
acetate, 0.1 mM EDTA, and 0.1 mM phenylmethylsulfonyl
fluoride (PMSF), and ultracentrifuged at 100,000 g for 30
min. The pellet was resuspended in 25% glycerol-5 mM
magnesium acetate-0.1 mM EDTA-5 mM dithiothreitol (DTT)
and stored at −80°C. Nitrocellulose-immobilized targets
were prepared using 10 µg each of plasmids containing
G6Pase and ATP synthase cDNAs suspended in 50 µl deoxiri-
bonucleic (DNA)-free water. Plasmid without insert served
as a negative control and Eco R1-digested genomic DNA as a
positive control. Reaction mix (4×, 250 µl; 100 mM N-2-
hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES, pH
7.4), 10 mM MgCl2, 10 mM DTT, 300 mM KCl, and 20% glycerol)
combined was added to 1 ml [32P]UTP and 125 µl of 8×
triphosphate mixture (2.8 µM each ATP, GTP, and CTP,
Boehringer Mannheim) and diluted to a volume of 500 µl to
yield the 2× reaction cocktail. For each reaction, 100 µl of
thawed nuclei were added to an equal volume of 2× reaction
cocktail and incubated at room temperature for 30 min. The
reaction was stopped by adding 2 µl DNase 1 (10,00 U/ml in
50% glycerol, Boehringer Mannheim) and incubating at 37°C
for 30 min. Stop buffer (600 µl; 2% sodium dodecyl sulfate
(SDS), 7 M urea, 0.35 M LiCl, 1 mM EDTA, and 10 mM Tris
(pH 8.0)), proteinase K, and 100 µg RNA were added, and the
mixture was incubated at 40°C for 1 h. The mixture was
phenol-chloroform extracted, trichloroacetic acid precipitated,
etanol washed. The pel was resuspended in 100 µl of
10 mM Tris-1 mM EDTA-0.5% SDS, added to 5 ml of
formamide hybridization solution, and exposed to the targets
at 42°C for 40-48 h. Targets were washed, incubated with 10
µl proteinase K, extracted with phenol-chloroform-isoamyl
alcohol, ethanol precipitated, and resuspended in 10 µl of
stop buffer (600 µl; 2% SDS) at 37°C, and washed again for 1 h. Targets were autoradio-
graphed for 5 days at −80°C and quantitative laser densitom-
etry (Molecular Dynamics) was performed. G6Pase density
was normalized to ATP synthase density, and means ± SD
were determined. Normalized density at zero time (T0) was
artificially set at unity.

Immunohistochemistry. In animals subjected to single-
puncture CLP or sham operation, tissue from three animals
at each time point was harvested, and immunohistochemistry
was performed as previously described (13, 17). At 0, 3, 6, 16,
24, 48, and 72 h after the initial procedure, animals were
reanesthetized and livers were perfusion fixed with 2% para-
formaldehyde. At the conclusion of the perfusion, the
liver was excised, sliced, and fixed in parafomaldehyde for 1 h
at 4°C. Slices were subjected to fixation, sectioning, mount-
ing, dewaxing, and rehydration as above. Endogenous peroxi-
dase activity was quenched with methanol-hydrogen perox-
ide. Slices were rinsed and then treated overnight with
3% goat serum to block nonspecific binding, and incubated for 2 h
with an affinity-purified polyclonal rabbit antibody to rat G6Pase.
After a wash in 2× PBS, sections were stained secondarily
with a the Vectastain Elite ABC kit (Vector Laboratories),
rinsed in 2× PBS, and metal-enhanced immune-pure 3,3-

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diaminobenzidine (Sigma) was added for 5 min. Sections were again immediately washed, treated briefly with ethanol and xylene, dried, and mounted.

Cytochemical detection of G6Pase activity. Enzymatic activity was determined as previously described (17). In brief, 10-μm frozen sections slides were washed with sucrose, exposed to G6Pase substrate mixture [40 mM Tris maleate (pH 6.5), 0.27 M sucrose, 10 mM glucose 6-phosphate, and 3.6 mM lead nitrate] for 30 min at 37°C, washed briefly in 0.22% ammonium sulfide, and fixed with 1:1 acetone-methanol.

Statistical methods. Means ± SD for normalized densities (Northern analysis and nuclear run-on), glucose, insulin, glucagon, and the insulin-to-glucagon (I/G) ratio were calculated and significance tested using analysis of variance with the Bonferroni correction at P < 0.05. Power analysis was used to determine the ability to detect a difference should one be present.

RESULTS

Clinical responses to cecal ligation and double puncture differ from those to cecal ligation and single puncture. As previously reported, 6 h after CLP single-puncture, animals differed little from double-puncture or sham-operated animals (36). At later times, behavior diverged. Sham-operated animals recovered uneventfully. Single-puncture CLP resulted in a mild decrease in spontaneous movement and less fastidious grooming relative to sham-operated controls, findings that persisted up to 72 h. Twelve animals in each of these two groups were allowed to survive beyond 48 h. One sham-operated rat and one single-puncture CLP rat died between 48 and 72 h. Thus mortality in these groups was equal at 8.3%. In marked contrast, ~8–12 h after double-puncture CLP, animals became severely ill (36). Water intake decreased, spontaneous movement was lost, eyes became encrusted, and animals did not groom themselves. At 16 h, double-puncture CLP animals were in a state previously described as characteristic of late sepsis (8, 9, 36). They became severely lethargic, stopped drinking, and had diarrhea, piloerection, and tachypnea. Six double-puncture CLP animals were observed up to 24 h and three died.

Steady-state levels of G6Pase mRNA decrease in mild and fulminant sepsis. Previous work demonstrated that single-puncture CLP is associated with a decrease in G6Pase mRNA (1). We postulated that G6Pase expression would also be reduced in fulminant sepsis. Six, sixteen, and twenty-four hours after double-puncture CLP, steady-state levels of G6Pase mRNA were almost undetectable (representative autoradiogram of representative Northern blots. Liver tissue harvested from overnight-fasted unoperated (T0), septic (single- or double-puncture CLP), or sham-operated male Sprague-Dawley rats at various time points after initial operation. Total RNA isolated as per text; 10 μg total RNA/lane. Blots were first hybridized against a [32P]dCTP-labeled cDNA complementary to G6Pase mRNA. Autoradiography and quantitative laser densitometry were performed, and blots were stripped and then probed with a random labeled probe for ATP synthase. Left: double-puncture CLP; middle: single-puncture CLP; right: sham operation. Numbers above each panel indicate time after CLP or sham operation. Three independent densitometric measurements of G6Pase were determined at each time point with same results. B: graphic representation of serum glucose levels as determined by hexokinase assay. Data represent means ± SD. *P < 0.05 relative to T0; †P < 0.05 relative to sham operation at same time point; #P < 0.05 relative to single-puncture CLP at same time point.
gram, Fig. 1A, left). At the same time, expression of ATP synthase, a constitutive mRNA, was maintained (Fig. 1A), whereas that of α2-acid glycoprotein, an acute-phase reactant, was elevated (data not shown). A representative Northern blot demonstrating previously reported results (1) after single-puncture CLP is shown for comparison (Fig. 1A, middle). Relative to both T0 (starved) and sham-operated controls, a decrease in steady-state G6Pase mRNA levels was observed at 3 through 72 h. These data demonstrate that the nadir in steady-state levels of G6Pase mRNA occurred 6 h after single-puncture CLP, representing a 50-fold decrease relative to normal liver (T0) (1). Recovery after single-puncture CLP was present but incomplete at 72 h, when levels were still decreased fourfold relative to T0.

As previously reported, sham operation was associated with a small but significant time-dependent increase in G6Pase mRNA levels, similar to that observed after fasting (2). These data document that decreases in G6Pase mRNA occurred early in either mild (single puncture) or fulminant (double puncture) sepsis. Some recovery occurred in mild sepsis but none in fulminant sepsis.

The potential functional importance of this decrease in steady-state levels of G6Pase mRNA is illustrated by determination of serum glucose levels (Fig. 1B). After sham operation, glucose was unchanged. Single-puncture CLP, as previously reported, also caused a mild hyperglycemia that did not differ from that observed at T0 (11, 12). Glucose levels were initially normal in double-puncture CLP. However, by 16 h after double-puncture CLP, hypoglycemia developed and by 24 h levels had fallen precipitously. The decrease in glucose level paralleled the clinical development of late sepsis described above. Thus mild sepsis decreased steady-state G6Pase mRNA levels, but there was some recovery by 72 h after the initial insult, and serum glucose levels were maintained. In contrast, fulminant sepsis resulted in a persistent decrease in G6Pase transcription, hypoglycemia, and ultimately death.

Sepsis-induced decrease in G6Pase mRNA reflects a decrease in transcription rate. We have previously shown that the mechanism underlying the drop in steady-state levels of G6Pase mRNA in mild sepsis involved decreased gene transcription (1). In this study, we performed transcript elongation analysis (nuclear run-on) 0, 4, and 24 h after double-puncture CLP and 0, 4, 24, 48, and 72 h after single-puncture CLP and sham operation. Figure 2A is a composite of a representative run-on experiment, and Fig. 2B is a graphic representation of normalized elongation analysis data from septic and sham-operated animals obtained over time. Both

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**Fig. 2. Transcription elongation analysis of G6Pase in liver nuclei.**

A: representative transcription elongation analysis blot. Subscript after T indicates time after single- or double-puncture CLP or sham operation. B: graphic representation of data from transcription elongation analysis in septic animals. Analysis performed on nuclei from 3 septic (single- or double-puncture CLP) and 3 sham-operated animals at each time point. Autoradiograms subjected to quantitative densitometry and G6Pase density normalized to ATP synthase density. Normalized value at T0 for both septic and sham-operated animals was arbitrarily set at unity. Data represent means ± SD. *P < 0.05 relative to T0; †P < 0.05 relative to sham operation at same time point; #P < 0.05 relative to single-puncture CLP at same time point.
mild and fulminant sepsis resulted in a rapid decrease in G6Pase transcription to undetectable levels by 4 h. This decrease persisted through 24 h (when half the animals had died) in double-puncture CLP but began to resolve by 24 h after single-puncture CLP. The rate of transcription paralleled steady-state mRNA levels (Fig. 1). Transcription increased by 1.3-fold at 16, 24, 48, and 72 h after sham operation. The transcription of ATP synthase remained constant in both single- and double-puncture CLP. These studies indicate that initial changes in G6Pase gene transcription are similar in single- and double-puncture CLP. Because of the high mortality in double-puncture CLP and because the decrease in transcription is similar in single or double-puncture CLP, further studies were conducted after single-puncture CLP only.

Changes in transcription of G6Pase are inappropriate for hormonal milieu. G6Pase expression is decreased by insulin and increased by glucagon (2, 29). Therefore circulating levels of glucagon and insulin were determined and the I/G ratio was calculated 0, 3, 6, 16, 24, 48, and 72 h after single-puncture CLP. As in previous studies (11), glucagon levels after mild sepsis were markedly and persistently elevated (Fig. 3A), insulin levels more modestly elevated (Fig. 3B) and the I/G ratio was decreased (Fig. 3C). Studies by others in double-puncture CLP have revealed a similar hormonal milieu (20). Sham-operated animals had an early (3 h) elevation of both glucagon (Fig. 3A) and insulin (Fig. 3B). These increases were followed by a modest increase in glucagon and a decrease in insulin, findings consistent with starvation. Because G6Pase expression is increased by glucagon and decreased by insulin (2, 29), the decrease in steady-state levels of G6Pase is inappropriate and indicates that hormonal modulation of G6Pase is altered in sepsis.

Mild sepsis alters zonal distribution of G6Pase mRNA in liver. We have previously reported that, in normal and regenerating liver, G6Pase is predominantly localized to the periportal region (17). Sepsis-induced decreases could alter the steady-state distribution of G6Pase mRNA, which was determined at 0 (unoperated), 3 (modest reduction in G6Pase), and 24 h (major reduction in G6Pase) after CLP. In Fig. 4, periportal (left) and pericentral (right) photomicrographs of liver sections revealed the predominant localization of G6Pase mRNA to the periportal region. Some decrease in staining corresponding to G6Pase in the periportal region was apparent 3 h post-CLP. By 24 h, hybridization in both regions was undetectable. In situ hybridization on sections from sham-operated animals were unchanged over time (data not shown).

Sepsis-induced decreases in G6Pase protein levels paralleled steady-state mRNA levels and transcription rate. Although steady-state levels of G6Pase mRNA demonstrably decrease in sepsis, these data must correlate with protein levels to be functionally meaningful. We therefore examined changes in G6Pase protein abundance in septic liver (Fig. 5). Staining on T0 sections revealed the periportal distribution of G6Pase under normal conditions. Levels of the G6Pase cata-

lytic subunit decreased after CLP. This change was most pronounced 16 h after single-puncture CLP and did not resolve fully in the time period studied. Protein levels increased over time after sham operation. The time course and distribution of the decrease in protein after single-puncture CLP (Fig. 5A) paralleled the loss of mRNA (Fig. 4). Close examination of high power magnifications (Fig. 5B) revealed that the protein is localized to the perinuclear region (black arrows), a finding consistent with previous results (17).
Total G6Pase activity in perinuclear region parallels changes in mRNA and protein. Because both G6Pase mRNA and protein are decreased in sepsis, enzymatic activity should also decrease. We therefore investigated G6Pase activity after single-puncture CLP. By 6 h after single-puncture CLP, a loss of G6Pase activity in the perinuclear region was observed (Fig. 6). Both the decrease in activity and distribution of this loss were consistent with the distribution of the protein (Fig. 5; Refs. 6, 17) and mRNA (Fig. 4). No change was observed in sham-operated animals (data not shown).

**DISCUSSION**

The studies detailed here demonstrate that both mild and fulminant sepsis result in a rapid decrease in hepatic G6Pase transcription. In mild (single-puncture CLP) sepsis, G6Pase transcription, along with activity, begins to resolve by 72 h. Blood glucose levels remain steady throughout the time course studied after single-puncture CLP. In double-puncture CLP, there is no recovery of transcription. Glucose levels are maintained initially, but hypoglycemia develops soon after the insult. In double-puncture CLP, the persistent decrease in G6Pase transcription is clearly inappropriate, since hypoglycemia should stimulate hepatic glucose production and therefore G6Pase transcription and activity. We have hypothesized that an inappropriate decrease in transcription is one important aspect of hepatic pathophysiology in sepsis (1).

Discussion of the relationship between G6Pase expression and serum glucose levels in sepsis is essential. Disordered metabolism is a major component of septic pathophysiology and perhaps a cause of death (3, 10). The hypermetabolic septic state is characterized by increases in peripheral glucose utilization and breakdown of nitrogen stores (3, 19, 20, 25, 26, 32, 33). This
enhances delivery of gluconeogenic substrate to the liver. In addition, early, hypermetabolic sepsis results in hyperglucagonemia, high levels of circulating catecholamines, and increases in the activity of the sympathetic nervous system (8, 9, 18, 38). These extrahepatic factors drive hepatic gluconeogenesis. Using an isolated perfused liver preparation, Clemens et al. (9) have shown that, for any given level of substrate delivery, the glucose produced by a septic liver is less than that produced by a control liver. Other investigators (8, 9, 18, 23, 38) have demonstrated that when hormonal influences are removed or blocked, hepatic gluconeogenesis is less in septic animals than in control animals. This implies that, even in early, hypermetabolic sepsis, there is an hepatocellular defect in glucose production (16) which is masked by high levels of substrate delivery and sympathohormonal tone. In late sepsis or septic shock, serum glucose levels fall as glucose production is exceeded by peripheral consumption (3, 15, 30, 33). Hepatic glucose production is ultimately determined by the activity of G6Pase, which catalyzes the release of glucose from the liver (2, 22, 27). Decreased G6Pase transcription and activity, coupled with loss of compensatory mechanisms, could explain decreased gluconeogenesis in late sepsis and the intrinsic defect in early sepsis. However, the de-
creased expression of G6Pase may not be a critical determinant of hepatic glucose production in early sepsis, when extrahepatic factors predominate.

G6Pase transcription in the liver decreases before the development of hypoglycemia, and the fall in glucose in fulminant sepsis does not stimulate an increase in hepatic G6Pase transcription. Coupled with our previous demonstration of decreased transcription of a number of hepatic metabolic genes, this suggests that the decrease in G6Pase transcription is pathophysiological (1). G6Pase expression is increased by fasting (2), hemorrhagic shock (21), and after partial hepatectomy (17), other states that can potentially cause hypoglycemia. Our findings therefore suggest that decreased expression of G6Pase is an important early marker of altered liver function in sepsis.

Sepsis-induced decreases in G6Pase transcription do not reflect a global defect in hepatic gene expression. Constitutive ATP synthase transcription is maintained and acute-phase gene expression is increased after both single- and double-puncture CLP. Metabolic and hormonal regulation of G6Pase in sepsis also is inappropriate. Massillon et al. (22) contend that G6Pase expression is influenced by blood glucose levels; yet our data reveal no correlation between blood glucose levels and G6Pase expression. Similarly, increases in glucagon and a decreased I/G ratio, as demonstrated here for single-puncture CLP and by others in double-puncture CLP (20), should increase G6Pase transcription. We have demonstrated that sepsis renders transcription unresponsive to glucagon or 8-bromoadenosine 3',5'-cyclic monophosphate (12). The same may be true of G6Pase. The decrease in both PEPCK and G6Pase expression might indicate that downregulation by insulin is maintained in sepsis while upregulation by glucagon is depressed. Alternatively, a change in the response of both the PEPCK and G6Pase genes to common transcription factors modulating adenosine 3',5'-cyclic monophosphate-insulin responses could be involved. The G6Pase promoter contains putative binding sites for transcription factors that regulate PEPCK expression (29). Links such as these have been shown to be important in other metabolic diseases such as diabetes (6).

The sepsis-induced alterations in G6Pase transcription are more pronounced than those of PEPCK (1). An explanation lies in the significant regulation of PEPCK expression by posttranscriptional modification of the message and by a specific RNA-binding protein, which increases posttranscriptional mRNA stability (24). In addition, G6Pase is subject to regulation by mechanisms other than control of mRNA levels (27). However, the correlation between G6Pase transcription and enzymatic activity tightly parallels the behavior of PEPCK in sepsis.

These studies also provide insight into the correlation of G6Pase gene expression with enzymatic activity, which has been the subject of controversy (2, 5, 31). As demonstrated recently, most of the G6Pase activity is found in the nuclear membrane fraction (17, 39), a finding not surprising, since these structures are in continuity with the endoplasmic reticulum. Data in this report confirm this by demonstrating that both G6Pase protein abundance and enzymatic activity reside in the perinuclear region. Therefore correlation of...
activity with transcription must specifically focus on this region.

Finally, these data may be important in defining the precise pathological effect of sepsis on the liver. Most assays for hepatic damage, such as measurement of serum transaminases, are insensitive. Decreased indocyanine green clearance, a better index of hepatocyte dysfunction, can be found early in sepsis. This alteration indicates that significant hepatocellular dysfunction precedes necrosis (35). However, clearance of an exogenously administered toxin may simply reflect saturation or a reprioritization of hepatocyte function in the face of systemic inflammation. In contrast, gluconeogenesis is an intrinsic, essential hepatic function. We believe that decreased G6Pase expression, as well as decreased expression of other genes, is an early marker of hepatic dysfunction in sepsis.

The results detailed here and the similarities in sepsis-induced changes in G6Pase and PEPCK support the hypothesis that sepsis alters hepatic regulation of metabolism via decreased transcription of a number of genes involved in essential metabolic pathways (1). Our data add credence to the proposal espoused by Pilikis and Granner (27) that modulation of the expression of multiple genes involved in a single metabolic pathway is coordinate and that pathway and organ dysfunction, as occurs in disease states such as sepsis, may also be coordinate. Exploration of the mechanisms mediating decreased G6Pase transcription may explain abnormalities in other hepatic pathways as well as altered function in other organ systems.

C. S. Deutschman gratefully acknowledges the continued support and collaboration of Mark G. Clemens, PhD, University of North Carolina-Charlotte and Drs. David E. Longnecker and Bryan E. Marshall, Dept. of Anesthesia, University of Pennsylvania, for creating an environment of academic opportunity.

This study was supported in part by National Institutes of Health Grants K08–02179 (C. S. Deutschman) and DK-49210 (R. Taub).

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Received 29 April 1997; accepted in final form 28 July 1997.

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