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Metabolic control of hepatic gluconeogenesis in response to sepsis

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The regulation of hepatic gluconeogenesis was studied in rats made septic by cecal-ligation and puncture technique. Blood glucose was not significantly different in septic rats, but lactate, pyruvate, and alanine were markedly increased. Conversely, blood ketone body concentrations were markedly decreased in septic rats. Both plasma insulin and glucagon were markedly elevated in septic rats. The maximal activities of glucose 6-phosphatase, fructose 1,6-biphosphatase, pyruvate carboxylase, and phosphenolpyruvate carboxykinase were decreased in livers obtained from septic rats suggesting a diminished hepatic gluconeogenesis. Hepatic concentrations of lactate, pyruvate, and other gluconeogenic intermediates were markedly increased in septic rats, whereas those of fructose 2,6-bisphosphate and acetyl-CoA were decreased. The rate of gluconeogenesis from added lactate, pyruvate, alanine, and glutamine was decreased in isolated incubated hepatocytes from septic rats. It is concluded that the diminished capacity of hepatic gluconeogenesis of septic rats could be the result of changes in the maximal activities or regulation of key nonequilibrium gluconeogenic enzymes or both but do not exclude other factors (e.g., toxins). (J Lab Clin Med 1989;114:579-586)

Abbreviations: ADP = adenosine 5'-diphosphate; AMP = adenosine 5'-monophosphate; ATP = adenosine 5'-triphosphate; CoA = coenzyme A

Clinical sepsis develops after major trauma such as burns or abdominal surgery, and the septic episode is not merely limited to the bacterial insult but has been described as an acquired disease of intermediary metabolism.[1,2] In humans and experimental animals sepsis causes several changes in carbohydrate, lipid, and protein metabolism.[1,3,4]

Hepatic gluconeogenesis has been reported to either increase or decrease[7-11] after the induction of sepsis. Such differing findings are related to several factors: source and type of sepsis, nutritional states of animals, and the stage or phase of infection or both.[12]

A considerable amount of work has been performed on the control of hepatic gluconeogenesis in different physiologic conditions (e.g., starvation[13] and exercise[14]). However, limited information is available on the regulation of hepatic gluconeogenesis in response to sepsis.[4,15]

The present work was designed to obtain more information about the regulation of hepatic gluconeogenesis in septic rats with a cecal-ligation and puncture technique.[16] This has been done by measurements of key nonequilibrium enzymes and concentration of key metabolites in the pathways of glycolysis and gluconeogenesis together with the extent of hepatic gluconeogenesis in vitro in septic and corresponding sham-operated rats. The relevance of these changes to the overall regulation of hepatic gluconeogenesis in sepsis is discussed.

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Table I. Body weight, liver weight, rectal temperature, hemodynamic parameters, nitrogen-balance, and blood concentrations of glucose, pyruvate, lactate, alanine, ketone-bodies, plasma nonesterified fatty acids and plasma insulin and glucagon for 48-hour septic and corresponding control rats

	Animals	
	Sham-operated	Septic
Initial body weight (gm)	187.2 + 5.33 (8)	192.1 ± 4.60 (10)
Final body weight (gm)	173.8 ± 1.98 (8)	177.4 ± 3.49 (10)
Liver weight (gm)	6.67 ± 0.77 (8)	5.33 ± 0.47 (10)
Rectal temperature (° C)	35.50 ± 0.55 (8)	37.48 ± 0.43 (8)
Heart rate (beats/min)	380 ± 24 (8)	475 ± 50* (8)
Mean arterial pressure (mm Hg)	110 ± 7 (8)	125 ± 11 (8)
Cardiac out-put (ml/Kg/min)	310 ± 25 (8)	395 ± 30* (8)
Nitrogen-balance (mg of N/day/100 gm body weight)	−31.56 ± 6.62 (8)	−51.70 ± 7.22 (8)**
Blood glucose (mmol/L)	4.46 ± 0.37 (8)	4.11 ± 0.28 (8)
Blood lactate (mmol/L)	1.13 ± 0.10 (8)	2.57 ± 0.36 (8)**
Blood pyruvate (mmol/L)	0.07 ± 0.01 (8)	0.15 ± 0.01 (8)**
Blood alanine (mmol/L)	0.13 ± 0.03 (8)	0.36 ± 0.08 (8)**
Blood ketone-bodies (mmol/L)	1.23 ± 0.21 (8)	0.58 ± 0.18 (8)*
Plasma nonesterified fatty acids (mmol/L)	0.87 ± 0.16 (8)	0.78 ± 0.18 (8)*
Plasma insulin (u-units/ml)	12.91 ± 2.75 (8)	31.55 ± 4.60 (8)**
Plasma glucagon (pg/ml)	572 ± 186 (8)	1805 ± 642 (8)**

Values are presented as mean ± SD with the number of animals used given in parenthesis. Rats were starved for 48 hours after shamoperation or cecal-ligation as described in the experimental section. Statistical significance: *$p < 0.01$; **$p < 0.001$.

METHODS

Animals. This study was conducted in accordance with the National Institutes of Health guidelines for the use of experimental animals. Male Wistar albino rats (170 to 195 gm) were supplied by King Fahd Medical Research Center, College of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were maintained on a standard diet [commercial rat cubes containing (w/w) approximately 18% protein, 3% fat, 77% carbohydrate, and 2% of an inorganic-salt mixture with a vitamin supplement] (Grain Silos and Flour Mills Organization, Jeddah, Saudi Arabia) and water ad libitum. Animals were kept in a controlled environment (constant temperature 24° C, and a light cycle of 12 hours on/12 hours off). Animals were starved for 48 hours after cecal-ligation to induce sepsis but allowed water and housed individually. Septic and corresponding sham-operated control rats were killed by cervical dislocation at 48 hours after cecal-ligation.

In the present work, sepsis was induced by means of cecal-ligation and puncture technique.[16] All operations were carried out with the rats under pentobarbital (40 mg/kg body weight) anesthesia. A midline laparotomy was performed. In the sham-operated animals the cecum was mobilized by incising the mesocecum, and it was returned to the abdominal cavity. In the septic groups after mobilization of the cecum, the feces were milked into the cecum, which was then ligated with a single 3.0-silk ligature in such a manner that the bowel continuity was maintained. The antimesenteric surface of the cecum was punctured once with a 21-gauge needle, and the bowel was returned to the abdominal cavity. The abdominal wall was closed in two layers and all rats received 0.9% (w/v) NaCl (2.5 ml/100 gm body weight) subcutaneously. With this procedure an experimental peritonitis was created in which rats experienced the hypermetabolic hyperdynamic septic state as described by Wichterman et al.,[16] and the peritonitis developed at a slower rate allowing animals to starve and survive for 48 hours. Water was offered ad libitum. Rectal temperature of rats was measured with a temperature monitor (Ellab, type te-3-5, Ellab, Copenhagen) with thermoelectrodes. Three different temperature readings were made over 30 minutes for each rat, and the mean value was taken.

For the measurements of hemodynamic parameters, rats were anesthetized with ether, and a 22-gauge polytetrafluoroethylene catheter was inserted into the left common carotid artery. Blood pressure and heart rate were recorded with a Gould P23ID transducer (Gould Inc., Recording Systems Division, Cleveland, Ohio). The mean arterial pressure was derived electronically through an integrator circuit. Cardiac output was measured by the thermodilution technique as described previously.[17]

Chemicals and enzymes. All chemicals and enzymes were obtained from the sources described previously.[18-19]

Preparation of homogenates and assay of enzyme activities. Animals were killed by cervical dislocation, and the livers were rapidly removed, weighed, cut into several pieces, and homogenized in 5 to 10 vol of appropriate extraction medium in a Polytron homogenizer (Kinematica GmbH, Kriens-Lucerne, Switzerland) (PCU-2, at position 4) for 0 to 20 seconds at 0 to 4° C. Homogenates for all enzymes, except phosphoenolpyruvate carboxykinase and pyruvate carboxylase, were centrifuged at 13800 *g* for 3 minutes (Beckman, Beckman Instruments, Fullerton, Calif.) and the su-

Table II. Maximal activities of key glycolytic and gluconeogenic enzymes in livers of sham-operated and septic rats

Enzyme	Activity (μmol/min per gm fresh weight)	
	Sham-operated	Septic
Glucokinase	2.54 ± 0.24 (6)	2.39 ± 0.32 (6)
Glucose 6-phosphatase	10.89 ± 1.22 (6)	7.40 ± 0.71 (6)*
6-Phosphofructokinase	1.17 ± 0.18 (12)	1.29 ± 0.14 (12)
Fructose 1,6-bisphosphatase	6.80 ± 0.57 (7)	4.80 ± 0.44 (7)**
Pyruvate kinase	39.02 ± 7.47 (6)	39.28 ± 4.90 (8)
Pyruvate carboxylase	5.12 ± 0.54 (8)	4.11 ± 0.97 (9)*
Phosphoenolpyruvate carboxykinase	1.39 ± 0.21 (6)	0.87 ± 0.24 (6)**

Activities are presented as mean + SD with the number of separate animals used given in parenthesis. For extraction and assay procedures see the experimental section in text. Rats were starved for 48 hours after sham-operation or cecal-ligation as described in the experimental section. Statistical significance: *$p < 0.05$; **$p < 0.01$.

pernatants were used for enzyme assays. For the assay of phosphoenolpyruvate carboxykinase, the extraction medium contained 30 mmol/L mercaptoethanol. For the assay of pyruvate carboxylase, the extraction medium contained 50 mmol/L triethanolamine, 0.3 mmol/L sucrose, and 1 mmol/L ethylenediaminetetraacetic acid, pH 7.2. Homogenates were centrifuged at 13800 *g* for 10 minutes and pellets were resuspended in an equal volume of homogenization buffer plus 0.05% (v/v) Triton-X-100. Resuspended pellets were used for assay of the enzyme. Extraction media for all enzymes studied were as described previously.[20]

Enzymes were assayed either spectrophotometrically at 25° C with a recording spectrophotometer, model 260 (Gilford, Oberlin, Ohio) or radiochemically at 30° C with a Beckman scintillation counter, model LS 7500. Enzyme activities are assayed as described previously.[21] The final volume of assay mixtures in all cases was 1.0 ml. For all enzymes studied, preliminary experiments established that extraction and assay procedures were such as to provide maximum activities.[20]

Extraction of blood for metabolite determinations. Rats were anesthetized with ether, and blood was withdrawn into heparinized syringes by cardiac puncture. Samples (4.0 ml) were quickly added to ice-cold $HClO_4$ (10% v/v) and used for metabolite determinations after deproteinization and neutralization.[22]

Extraction of liver for assay of metabolites. Animals were killed by cervical dislocation. The liver was quickly removed and freeze-clamped between aluminum tongs precooled in liquid nitrogen. The frozen liver samples were stored in liquid-nitrogen until required for extraction. Frozen samples were weighed and ground into a powder in liquid-nitrogen with a mortar and pestle. Samples were then homogenized with 0.7 mmol/L $HClO_4$ in a Polytron homogenizer (PCU-2, at position 4) [in a ratio of tissue to $HClO_4$ as 1:2 (w/v)] for 10s at 0 to 4° C. The precipitate was removed by centrifugation at 13800 *g* for 5 minutes and the supernatant was neutralized as described previously.[22] The $KClO_4$-free supernatant was used for metabolite determinations, which were completed on the same day as preparation of the extract.

Preparation and incubation of hepatocytes. Hepatocytes were prepared essentially as described by Berry and Friend[23] with the modifications that were described by Krebs et al.[24] Hepatocyte viability was tested by the maintenance of constant cellular ATP/AMP ratios throughout the incubation periods and by using the Nigrosine-exclusion-test (exclusion >92%). Cells were suspended in incubation medium.

Incubations were performed at 37° C in 20 ml Erlenmeyer flasks that had been treated with silicone. Freshly prepared hepatocytes were incubated in a total volume (4.0 ml) containing 70 to 100 mg wet weight of cells in an incubation medium, which consisted of Krebs-Henseleit saline solution[25] containing essentially fatty acid-free albumin [final concentration 2.5% (w/v)] and added substrates. The gas phase was O_2/CO_2 (19:1), and flasks were shaken continuously (90 to 110 oscillations/min). Incubations were initiated by the addition of cell suspension and terminated by the addition of 0.4 ml of $HClO_4$ (20%, w/v), the mixture was centrifuged to remove protein and the supernatant was neutralized with KOH. The precipitate of $KClO_4$ was removed by centrifugation at 13800 *g* for 5 minutes. Neutralized extracts were used for assay of metabolites.

Determination of concentrations of metabolites, plasma insulin and glucagon. Concentrations of metabolites in neutralized extracts of livers, hepatocytes plus medium, and plasma were determined spectrophotometrically (with a Beckman DU-6 recording spectrophotometer) by standard enzymic methods as described previously.[21] Nonesterified fatty acids were determined according to the method of Shimizu et al.[25] Plasma insulin and glucagon were measured with radioimmunoassay technique and RIA kits were obtained from Diagnostic Products Corp., Los Angeles, Calif. (for insulin) and from ICN Biomedicals, Inc., Calif. (for glucagon), respectively. Fructose 2,6-bisphosphate was measured in liver extracts as described by Van Schaftingen et al.[27] with stimulation of pyrophosphate-dependent phosphofructokinase (EC 2.7.1.90) purified from potato tubers.

Nitrogen-balance measurements. For the determination of the nitrogen-balance of sham-operated and septic rats, animals were placed in metabolic cages that allowed the sep-

Table III. Concentrations of metabolites in livers of sham-operated and septic rats

Metabolite	Metabolite concentration (μmol/gm fresh weight of liver)		
	Sham-operated	Septic	(% change)
Glucose	5.79 ± 0.63 (12)	6.22 ± 0.70 (12)	+7
Glucose 6-phosphate	0.078 ± 0.016 (10)	0.133 ± 0.031 (10)**	+70
Fructose 6-phosphate	0.050 ± 0.014 (10)	0.103 ± 0.014 (10)**	+106
Fructose 1,6-bisphosphate	0.008 ± 0.002 (10)	0.015 ± 0.002 (10)**	+87
Dihydroxyacetone phosphate	0.011 ± 0.003 (10)	0.014 ± 0.003 (10)*	+27
D-3 phosphoglycerate	0.457 ± 0.089 (10)	0.716 ± 0.155 (10)**	+57
D-2 phosphoglycerate	0.041 ± 0.010 (10)	0.063 ± 0.008 (10)**	+54
Phosphoenolpyruvate	0.148 ± 0.027 (10)	0.281 ± 0.058 (10)**	+90
Pyruvate	0.031 ± 0.006 (10)	0.075 ± 0.013 (10)**	+142
Lactate	0.350 ± 0.059 (10)	1.246 ± 0.132 (10)**	+256
Acetyl-CoA	0.107 ± 0.025 (10)	0.047 ± 0.015 (10)*	−56
ATP	2.174 ± 0.171 (10)	1.908 ± 0.129 (10)	−12
ADP	0.409 ± 0.058 (10)	0.356 ± 0.015 (10)	−13
AMP	0.716 ± 0.251 (8)	0.863 ± 0.212 (8)*	+20
Fructose 2,6-bisphosphate[a]	10.120 ± 1.41 (5)	7.282 ± 2.590 (5)*	−28

Concentrations are presented as mean ± SD with the number of animals used given in parenthesis. Rats were starved for 48 hours after sham-operation or cecal ligation as described in the experimental section in text. Statistical significance: $^*p < 0.05$; $^{**}p < 0.001$.
[a]Expressed as nmol/gm fresh weight of liver.

arate collection of urine and feces. Urine was collected during a 48-hour period (from 8 A.M. to 8 A.M.) in a vessel containing 0.5 ml of concentrated H_2SO_4. The daily H_2SO_4-urine volume was measured and a sample was frozen at −100° C. Feces were collected at 24-hour intervals and weighed. The nitrogen content of urine and feces was determined by the micro-Kjeldahl method.[28] Nitrogen-balance was determined over the 48 hours after cecal-ligation or laparotomy.

RESULTS

The decrease in body weight of septic (7.7%) rats was similar to that of sham-operated controls (7.2%) (Table I). Sepsis resulted in an increase in rectal temperature of rats at 48 hours after cecal-ligation. Sepsis resulted in increases in heart rate (25%, $p < 0.01$) and cardiac output (27.4%, $p < 0.01$) but with no marked changes in mean arterial pressure (Table I). Blood lactate (127%), pyruvate (114%), and alanine (177%) concentrations in septic rats were significantly higher than that found in corresponding controls, respectively (Table I). Blood ketone-bodies concentrations (3-hydroxybutyrate and acetoacetate) in septic rats were markedly lower (53%) than those of sham-operated control rats (Table I). No significant changes in the concentrations of blood glucose and plasma nonesterified fatty acids were observed in septic or sham-operated rats (Table I). Similar findings have been reported by others with this model of sepsis, for example, increased blood alanine[29] and increased plasma lactate but no change in plasma glucose.[11,30]

Maximal enzyme activities. The maximal activities of key enzymes of the pathways of glycolysis and gluconeogenesis in livers of sham-operated and septic rats are presented in Table II. The activities of glucose 6-phosphatase (32%, $p < 0.05$); fructose 1,6-bisphosphatase (29%, $p < 0.01$); pyruvate carboxylase (17.5%, $p < 0.05$) and phosphoenolpyruvate carboxykinase (37%, $p < 0.01$), were decreased in livers obtained from septic rats, respectively (Table II). These findings suggest a diminished hepatic gluconeogenic capacity. Moreover, there was no marked change in the maximal activities of glucokinase, 6-phosphofructokinase and pyruvate kinase in livers of septic rats when compared with those of sham-operated animals (Table II).

Concentrations of hepatic gluconeogenesis intermediates in vivo. Livers obtained from septic rats showed marked increases in most of the intermediates involved in the pathway of gluconeogenesis (Table III). The largest increase was in the concentration of lactate (256%) and pyruvate (142%), respectively. However, there was no significant change in hepatic glucose concentration. This pattern of change in the concentrations of intermediates in sepsis is consistent with the suggestion of diminished rates of glucose synthesis (via hepatic gluconeogenesis), with a new steady state being achieved at higher concentrations of precursors, particularly lactate. These findings are consistent with those obtained for maximal enzyme activities (see Table II).

Moreover, in septic rats, hepatic total adenine nucleotide content was maintained almost unchanged compared to that of sham-operated rats (Table III). However, the calculated hepatic energy charge (i.e., [ATP + 0.5 ADP]/[ATP + ADP + AMP]) fell from 0.85 (the value of normal control rats) to 0.72 and 0.67 for sham-operated and septic rats, respectively. The

Table IV. Effects of sham-operation or sepsis (48 hours) on the rates of glucose synthesis from various precursors in incubated hepatocytes of the rat

Added substrate	Rates of glucose synthesis in: (μmol/min per gm fresh weight of hepatocytes)	
	Sham-operated	Septic
None	0.109 ± 0.029 (10)	0.114 ± 0.031 (10)
Lactate (5 mmol/L)	0.665 ± 0.116 (10)	0.341 ± 0.074 (10)*
Pyruvate (5 mmol/L)	0.442 ± 0.095 (10)	0.321 ± 0.061 (10)*
Alanine (5 mmol/L)	0.352 ± 0.041 (10)	0.208 ± 0.047 (10)*
Glutamine (5 mmol/L)	0.464 ± 0.084 (10)	0.235 ± 0.045 (10)*

Hepatocytes were isolated from sham-operated and septic rats after 48 hours of starvation as described in the experimental section. Hepatocytes were incubated for 60 minutes at 37° C in incubation medium containing added substrates as indicated. Rates of glucose synthesis (μmol/min per gm wet weight of hepatocytes) are presented as mean ± SD with the number of experiments with separate cell preparations shown in parenthesis. Statistical significance compared with sham-operated values: *$p < 0.001$.

changes in hepatic energy charge values calculated for rats of the present work are similar to that previously reported for septic rats.[31,32]

Rates of glucose synthesis by hepatocytes. The rates of glucose synthesis in hepatocytes isolated from sham-operated and septic rats in the presence of various gluconeogenic precursors are presented in Table IV. The rates of glucose synthesis by hepatocytes of septic rats were markedly decreased when compared to corresponding sham-operated rats in the presence of various substrates. Hepatocytes isolated from septic rats showed a decrease in the rates of glucose synthesis in the presence of lactate (48.7%, $p < 0.001$); alanine (41%, $p < 0.001$) and glutamine (49%, $p < 0.001$), respectively (Table IV). However, if hepatocytes were incubated with lactate at concentrations found in blood in vivo (septic: 2.49 mmol/L; sham-operated: 1.16 mmol/L), the rate of glucose production was about the same (i.e., 0.42 μmol/min/gm of wet weight of cells). This suggests that gluconeogenesis in vivo may be maintained in sepsis by increasing the availability of gluconeogenic precursors (e.g., pyruvate, lactate, alanine).

DISCUSSION

The experimental animal model of sepsis used in the present work is considered to be a moderate form of sepsis compared with that of others,[16] and septic rats were not hypothermic or shocked. The microbial flora closely approximate that of human sepsis and therefore proved to be a suitable model of human disease.[16] The single puncture technique with a smaller needle gauge than that used previously[16] was chosen so that the peritonitis could develop at a slower rate allowing animals to starve and survive for 48 hours.[11] Moreover, it was decided to use starved animals since dietary nutrients can not be absorbed in this model, and this is likely also to be the case in patients with intraabdominal sepsis.[16]

Hyperglycemia and glucose intolerance are frequent manifestations of the metabolic response to sepsis.[1,3,5] The abnormalities in glucose homeostasis would be readily explained if an insulin deficiency actually existed. However, plasma insulin levels are normal or elevated.[33-35] In the present work no significant change in blood glucose was observed in spite of an increased level of plasma insulin in septic rats (Table I). The latter suggests that insulin resistance is present in septic rats of the present work. Moreover, several reports have shown increased plasma levels of stress hormones, which would cause increased plasma levels of fatty acids.[7,36,37] This, via the glucose-fatty acid cycle could cause inhibition of glucose utilization and pyruvate oxidation, which could be responsible for the insulin resistance.

The results of the present work have demonstrated that the liver of septic rats (as described herein) exhibited marked decreases and impairments in the rates of hepatic gluconeogenesis as compared with sham-operated control animals. These observations are consistent with the findings of Filkins and Cornell,[38] which showed impaired rates of gluconeogenesis from alanine in isolated hepatocytes from endotoxin-treated rats; from lactate in isolated perfused livers from guinea pigs previously injected with live *Escherichia coli* bacteria,[39] and in livers of similar septic rats.[11] Moreover, Long et al.[40] using a ^{14}C-glucose infusion, reported an increased glucose-turnover in a heterogeneous group of hypermetabolic septic patients. Other in vivo studies have further demonstrated that the rates of gluconeogenesis are accelerated under similar conditions.[6] These differences in the results obtained in such studies are influenced by several factors including the severity and point of time in the course of the infection when the observations were made and the method of induction of sepsis.[16]

Gluconeogenesis is subject to long-term and short-term control by hormones such as glucagon, insulin,

adrenaline, and noradrenaline. It is also controlled by the supply of gluconeogenic substrates and by its end product, glucose.[41,42] Changes in gluconeogenesis, which occur during starvation or in pathologic conditions such as diabetes, result most probably from complex and intricate control by different factors (including hormones; changes in regulatory mechanisms of key enzymes; changes in substrate(s) availability).[41,43] Sepsis as described in the present work exhibited changes in the concentrations of all hepatic gluconeogenic intermediates (Table III) and in vitro studies have shown that sepsis decreased the rate of hepatic gluconeogenesis from all the substrates used (see Table IV). The latter changes indicate that the net rates of various key steps in the pathway of hepatic gluconeogenesis have been affected by external regulation (i.e., by factors other than their pathway substrates[41]). The factors that may be responsible include (1) changes in maximal enzyme activities, (2) allosteric effectors, and (3) hormonal factors.

A decrease in the maximum activities of key gluconeogenic enzymes or an increase in those of the glycolytic enzymes or both (owing to changes in enzyme concentration via effects on hepatic protein synthesis and/or degradation) could account for the impaired rates of gluconeogenic flux. Indeed, there was a marked decrease in the maximal activities of glucose 6-phosphatase (32%, $p < 0.01$); fructose, 1,6-bisphosphatase (29%, $p < 0.01$), pyruvate carboxylase (17.5%; $p < 0.05$), and phosphoenolpyruvate carboxykinase (37%, $p < 0.01$), respectively. These findings are consistent with other observations: injection of endotoxin into mice decreased the activities of hepatic glucose 6-phosphatase, fructose 1,6-bisphosphatase, and phosphoenolpyruvate carboxykinase.[44-46] Thus, it is possible that changes in the maximal activity of key enzymes may play some role in the impaired rate of hepatic gluconeogenesis during sepsis.

The key hepatic gluconeogenesis and glycolytic enzymes can be regulated by changes in the concentration of allosteric regulators. Pyruvate kinase and 6-phosphofructokinase are activated in vitro by fructose 1,6-bisphosphate, so that the increased concentration of this metabolite (see Table III) in the liver of septic animals may increase the activities of these enzymes; this was not the case. However, it is possible that the in vivo concentration of fructose 1,6-bisphosphate may be too low to be effective.[47]

Fructose 2,6-bisphosphate is a potent stimulator of 6-phosphofructokinase and an inhibitor of fructose 1,6-bisphosphatase.[48,49] An increase in the level of fructose 2,6-bisphosphate will favor glycolysis, whereas a decrease will result in a stimulation of gluconeogenesis at the loci of 6-phosphofructokinase and glucose 6-phosphatase.

In the present work, sepsis induced a marked decrease in the level of hepatic fructose 2,6-bisphosphate (Table III) and accumulation of hepatic hexosemonophosphates and triose phosphates. These changes may indicate that one of the rate-limiting steps in hepatic gluconeogenesis (in septic rats) from lactate and pyruvate to glucose is at the level of glucose 6-phosphatase and causes the accumulation of glucose 6-phosphate and hence, the increase in the level of fructose 6-phosphate. This change in turn is expected to increase the hepatic concentration of fructose 2,6-bisphosphate, by a mechanism resulting from the increased levels of fructose 6-phosphate, as suggested previously.[41,50] However, this was not the case in livers of septic rats. The latter is probably related to factor(s) affecting 6-phosphofructose-2-kinase, which catalyzes the synthesis of fructose 2,6-bisphosphate from fructose 6-phosphate and ATP.[51]

Fructose 2,6-bisphosphate is found to be decreased in livers of diabetic rats when hyperglucagonaemia is present.[52] The elevated levels of hepatic fructose 2,6-bisphosphate in the obese mouse is also consistent with that in hyperinsulinaemia.[53,54] In the present work (Table I), hyperglucagonemia and hyperinsulinemia are observed in septic rats. Sepsis induced more than threefold increase in the level of glucagon and a 2.4-fold increase in the level of insulin. The relative hyperglucagonaemia of septic rats is consistent with the decreased levels of hepatic fructose 2,6-bisphosphate; however, the latter is not consistent with hyperinsulinemia. Therefore, insulin action was obviously inhibited by unexplained mechanism.

The adenine and guanine nucleotides are known to be allosteric affectors of several key enzymes of glycolysis and gluconeogenesis,[41,43] so if the concentration ratio ATP/ADP decreased, this would be expected to increase the activities of the key glycolytic enzymes and decrease those of gluconeogenesis: there was no significant change in the concentration ratio of ATP/ADP in livers of septic and sham-operated rats (Table III), and since hepatic glycolytic key enzyme did not show marked changes in the septic rats, it is possible that other regulators must play a role in the changes observed in hepatic gluconeogenesis.

Acetyl-CoA is an activator of pyruvate carboxylase,[41,42] and the decrease in acetyl-CoA with sepsis should decrease the enzyme activity. These changes in concentrations of allosteric regulators are consistent with decreased gluconeogenic flux, although no studies on the changes in the concentrations of these metabolites in the compartment of the cell in which the specific enzymes is located have been made.

The results of the present work suggest that the diminished rates of hepatic gluconeogenesis in septic rats could be the result of changes in the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, and glu-

cose 6-phosphatase with no change in that of 6-phosphofructokinase and pyruvate kinase. The elevated plasma insulin in septic rats of the present work is likely to be due to the stimulation of pancreatic secretion by bacterial product(s).[55] Insulin is known to decrease gluconeogenesis by its short-term actions via the activation state of key enzymes and by long-term regulation of the enzyme concentration. Although plasma glucagon is also increased (Table I) in septic rats, its stimulatory action on gluconeogenesis may be antagonized by the increase in plasma insulin. Several allosteric regulators could be involved, however, other factors are not excluded (e.g., toxins).

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