1986

Endotoxicosis During Liver Injury and Regeneration

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ENDOTOXICOSIS DURING LIVER INJURY AND REGENERATION

by

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A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Master of Science

May

1986

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. James P. Filkins for his invaluable guidance during my graduate education. My warmest gratitude is also extended to Loretta Dalaskey, Elaine McLaughlin, Randy Powell, Gail Kummer and Kathleen Goelz for their expertise and assistance in the preparation of this thesis.

Lastly, I wish to acknowledge the members of my committee, Dr. Mohammed M. Sayeed, Dr. Stephen B. Jones and Dr. Robert P. Cornell.

VITA

James M. Harig was born 9 October 1955 in Chicago, Illinois, the son of John and Anne Harig.

He received his elementary education from Immaculate Conception School and his secondary education from St. Patrick High School, both located in Chicago.

In September of 1973, he entered the University of Notre Dame, Notre Dame, Indiana and was awarded the degree of Bachelor of Science in May of 1977.

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INTRODUCTION

The syndrome of endotoxicosis is an extremely complex and poorly understood organismic response to gram negative bacterial cell wall components. Mammalian responses to endotoxin occur along a continuum ranging from the physiologic (the normal percolation of gut-derived endotoxins in the portal circulation) to the extreme pathophysiologic (septic shock and death). Virtually all organ systems are affected either directly or indirectly (via mediators) in endotoxicosis (31); thus it becomes an extremely difficult task to define the mechanisms responsible for the often dramatic derangements of the organism's homeostasis. Therefore, most investigators focus on individual phenomena observed during endotoxicosis and sepsis such as cardiovascular, hormonal, immunological, hematologic or metabolic effects.

A key metabolic change involves the profound alterations in glucose metabolism in the endotoxic state. Early hyperglycemia followed by late severe hypoglycemia and hyperlactacidemia characterize the time course of endotoxic shock. The early hyperglycemia has been attributed to glycogenolysis and increased gluconeogenesis by the liver. Conversely, the failure to maintain adequate plasma glucose levels terminally is due to increased peripheral glucose oxidation, exhaustion of liver glycogen stores, and depression of hepatic gluconeogenesis.

As in normal metabolism, the liver plays a central and critical role in the perversions of glucose metabolism observed in endotoxicosis (57,58). The liver is comprised mainly of parenchymal cells (hepatocytes) and endothelial cells (mostly Kupffer cells). Parenchymal cells carry out the synthesis, storage

and release of glucose, while the Kupffer cells perform an immunodefensive function by clearing the portal circulation of bacteria, viruses, endotoxin and senescent erythrocytes (46). These two cell types and their respective functions appear to be closely interrelated in the metabolic pathophysiology of endotoxicosis. Indeed, endotoxin appears to be initially taken up by Kupffer cells as well as other macrophages of the mononuclear phagocyte system (MPS). Following this uptake, the MPS synthesizes and liberates various mediators which may initiate a multitude of effects in all organ systems. With their anatomical proximity to the blood in the hepatic sinusoids and to the hepatocytes, Kupffer cells and their mediators represent important effectors of altered glucose metabolism in endotoxicosis.

One method to study the role of the liver in endotoxicosis is to perturb the delicate balance of the liver and its cellular components in the environment of the total organism. Resection of a large percentage of the liver and its subsequent regeneration provides just such a perturbation. By observing the effects of endotoxin during various stages of liver injury and regeneration, possible mechanisms of the liver's role in the development of the metabolic derangements of endotoxic shock can be defined. Also, because the different hepatic cellular components regenerate to differing degrees and time frames, we can investigate the mechanisms and interrelationships of the separate cell types, namely the hepatocytes and the Kupffer cells.

LITERATURE REVIEW

ENDOTOXIN - ORGANISM INTERACTIONS

The interactions between an organism and bacterial endotoxins are poorly understood phenomena. There are many reasons for this confusion. Firstly, endotoxins comprise a variety of bacterial cell wall components consisting mainly of lipopolysaccharides. These lipopolysaccharides are complex molecules which vary in their structure depending on such factors as the species, method of isolation, intraspecies mutations, etc. Thus, scientifically, it is a difficult task to study such a heterogenous compound (60).

Secondly, responses to endotoxins by the mammalian organism include virtually all organ systems at all levels of organization. These responses may range from subtle scientific observations such as alterations of plasma membrane fluidity (44,74) to the full blown picture of septic shock and multiple organ system failure. These responses may vary depending on species differences, fed and fasted state, season and a multitude of other variables. Therefore, a myriad of reactions by the organism forms a second source of difficulty.

Another problem in studying endotoxin host interactions lies in the fact that not all effects of endotoxin are truly toxic (as the name implies) to the organism. Certainly, there are instances when endotoxin is relatively innocuous. An obvious case in point occurs in the normal gastrointestinal tract lumen where large numbers of bacteria and their cell wall components reside without apparent ill effects. Likewise, endotoxin can be detected using the Limulus lysate test in the portal circulation of perfectly healthy humans. Bacteremia following a

dental procedure, a proctoscopy or even a hard bowel movement usually occurs without consequence. Besides being innocuous, there are instances where endotoxin is felt to be beneficial to the organism. Cornell has provided data demonstrating a possible beneficial role for gut derived endotoxin in liver regeneration (21). In addition, endotoxin percolating down the intestine may be necessary for normal colonic function as evidenced by the development of "exclusion colitis" which occurs when the fecal stream is surgically diverted from a segment of colon. The ability of endotoxins to act as immunomodulators is well known; they have been shown to increase general host resistance (37), stimulate interferon production (43) and possibly attenuate the graft vs. host reaction following bone marrow transplantation (48). Endotoxin effects run the gamut from the fulminant deadly scenario of shock to the symbiotic existence of bacteria in the intestinal lumen.

Finally, most of the actions of endotoxin are not direct or primary in nature. That is, the observed phenomena (e.g. galactosamine-induced hepatitis) are mediated through a number of different intermediate steps of which little is known (5,14,75). For example, the fever produced by administration of endotoxin is felt to be caused by the elaboration of a thermoregulatory monokine termed endogenous pyrogen which is secreted by macrophages and acts on the hypothalamus (35). The hypothalamus then orchestrates the production of the fever initiated by phagocytosis of endotoxin by the macrophage. As in any cause and effect relationship, it becomes necessary to unravel these individual steps before one can understand the entire process.

THE LIVER

The liver occupies a central role in endotoxin-organism interrelationships. Because it performs many functions in the normal physiologic state, it seems logical that the liver will contribute greatly to the pathophysiology of endotoxic shock. To demonstrate this point: 1) the liver synthesizes blood coagulation factors which are consumed in the syndrome of disseminated intravascular coagulation (DIC) observed in sepsis. 2) vasoactive substances such as serotonin are normally removed from the bloodstream by the liver. During endotoxemia, these substances escape and contribute to the altered hemodynamics. 3) Gluconeogenesis and glycogenolysis help to maintain a normal blood glucose. These metabolic processes fail during late sepsis causing profound hypoglycemia. Also, the failure of the liver to remove lactate produced during endotoxic shock aggravates the already existing acidosis and hypotension. 4) Bilirubin is cleared normally by the hepatocytes. Cholestasis and jaundice can result from endotoxemia (72,73). 5) Normal clearance of gut derived endotoxins and bacteria is depressed in sepsis. This defect is felt to enhance and perpetuate the syndrome of endotoxins. Also, the secretion of various immune mediators elaborated by Kupffer cells in the normal state is deranged in the septic state, leading to exaggerated host responses to these mediators.

For the purposes of this study, only the metabolic and immunologic functions of the liver will be addressed. The liver has been traditionally divided into two parts: hepatocytes (parenchymal cells) and endothelial cells (predominantly Kupffer cells). The hepatocytes perform most of the metabolic and synthetic functions of the liver, while the Kupffer cells participate in the organism's immune responses as part of the mononuclear phagocyte system

(MPS). It will become clear from this review and study that these two cell types interact in an indivisible manner during the metabolic dyshomeostasis (19,30,32,34) of endotoxic shock. For the sake of clarity, these two hepatic components will be initially discussed as separate entities.

HEPATOCYTES-METABOLIC FUNCTION

As stated previously, the hepatocytes engage in a number of metabolic, synthetic and detoxifying functions. However, the most important metabolic responsibility is the maintenance of a normal blood glucose level. This is accomplished by the liver through glycogenolysis, gluconeogenesis and degradation of insulin which all tend to increase blood glucose. Conversly, the liver also engages in glycogenesis, clearance of glucose and receptor binding of insulin to decrease blood sugar.

Gluconeogenesis and subsequent delivery of glucose to other tissues occurs only in the liver and kidney, due partially to the fact that glucose 6-phosphatase is found only in these organs. The kidney, however, only contributes significantly to glucose homeostasis in times of prolonged starvation. Thus, the liver is for practical purposes the only gluconeogenic organ. The liver utilizes mainly pyruvate, lactate, glycerol and alanine (derived from catabolism of other amino acids) as gluconeogenic precursors. Gluconeogenesis therefore, serves to synthesize and deliver glucose back to the body by clearing metabolic "byproducts" (especially lactate) from the bloodstream. This clearance and synthesis phenomenon becomes quite important in endotoxic shock where there is a failure of gluconeogenesis.

Another important hepatic modulator of blood glucose involves the synthesis and breakdown of glycogen. Glycogenesis and glycogenolysis also take place in skeletal and cardiac muscle, but these organs utilize this glycogen locally with little or no systemic contribution (27). This source for blood sugar obviously depends on the temporal sequence to the fed state - early on it is a significant source, but its importance wanes as the organism progresses further into a fasted state. Like gluconeogenesis, it is dependent on the blood glucose level, fed or fasted state and hormonal milieu. Release of this hepatic glucose is quite sensitive to the "counter insulin" hormones, especially the catecholamines. Glycogen sources can be rapidly depleted during times of extreme stress, such as is seen in septic shock.

The liver can also regulate blood sugar by clearance of hormones. Probably, the best example of this phenemenon is the clearance or "detoxification" of insulin. The effect of insulin on the liver is to lower the blood sugar via an increase in glycogenesis and an inhibition of gluconeogenesis. However, the liver can also "degrade" insulin so that it cannot exert its hypoglycemic effects on peripheral tissues. This poorly understood degradation of insulin has a net effect of maintaining blood glucose.

This normal physiological maintenance of blood glucose by the liver is complexly altered in the time course of endotoxic shock (54). As stated before, the early stages of sepsis and endotoxemia is characterized by hyperglycemia, especially in the fed state (33). This hyperglycemia is felt to be due to a number of factors: 1) absorption of dietary carbohydrates (important in the fed state). 2) increased glycogenolysis due to effect of stress/counterinsulin hormones on the liver (27). 3) increased gluconeogenesis due to a generalized

catabolic state which sends large quantities of gluconeogenic substrates to the liver. 4) relative insulin resistance of peripheral tissues and liver. This insulin resistance has been inferred by the presence of exagerrated insulin levels for the existing glucose levels. More profound hyperglycemia in the fed state leads to more deranged insulin levels which probably contribute to the eventual increased lethality of endotoxin in fed animals. Following the hyperglycemia is a transition phase of euglycemia.

More dramatic alterations of glucoregulation occur in the later hypoglycemic phase of endotoxicosis. This metabolic derangement precedes and then lasts through the terminal stages of coma, hypotension and death observed in septic shock. The causes of this hypoglycemia include: 1) anorexia and cessation of feeding probably due to alterations of gastrointestinal motility (76) and effects of monokines on the hypothalamus 2) exhaustion of glycogen stores resulting from prolonged sympathetic drive, hepatic insulin resistance and decreased delivery of glucose from the intestine 3) increasing glucose oxidation occurring in the periphery because of inappropriate elevations of immunoreactive insulin as well as nonsuppressible insulin like activity (13,28). 4) depression of gluconeogenesis which is not related to decreased delivery of gluconeogenic substrate (15,25,41). Rather, it is a failure of the parenchymal cell gluconeogenic enzyme system. This failure is due to the effect of various immunometabolic regulators elaborated by the mononuclear phagocyte system. This concept of glucoregulatory monokines will be discussed in more detail later in this treatise.

The terminal stages of endotoxic shock are characterized metabolically by the appearance of profound hypoglycemia and hyperlactacidemia. The hypoglycemia results from the continued influence of the aforementioned factors.

The hyperlactacidemia occurs because of increased peripheral lactate production (because of hypoperfusion and tissue hypoxia) and decreased lactate clearance by malfunctioning hepatocytes. This hyperlactacidemia aggravates existing acidosis, perpetuating a vicious cycle of acidosis, hypotension and circulatory collapse. At this point, the shock scenario is irreversible and death of the animal soon follows (68).

KUPFFER CELLS-IMMUNOLOGIC FUNCTION

The Kupffer cells are resident macrophages which line the hepatic sinusoids. These cells are of bone marrow origin, but they also are capable of cell division. Along with blood monocytes and other resident macrophages (spleen, lung, etc.) they collectively comprise the mononuclear phagocyte system. This system interacts with all organ systems and classically its role has been ascribed to host defense. Indeed, its wide range of immunodefensive functions have been known for some time. In addition, numerous other functions such as tumor defense, metabolism and endocrinological control have been related to the MPS. For this discussion, focus will be placed on Kupffer cell-endotoxin interactions.

The MPS is responsible for the removal of bacterial endotoxin. An injected dose of intravenous endotoxin or colloidal carbon will be cleared by the MPS and be distributed roughly in the following manner: 70% liver, 20% spleen, 10% lung (8,10). Some endotoxin may be primarily taken up by hepatocytes (50). Gut derived endotoxin, however, is normally cleared entirely by the Kupffer cells. Evidence for this is the fact that in a healthy animal systemic blood rarely tests positive for endotoxin by the Limulus assay. However,

portal blood commonly gives a positive test. When there is liver disease or portasystemic shunting (as in alcoholic cirrhosis), then the systemic circulation will show evidence of endotoxin.

This ongoing release of endotoxin into the portal circulation appears to be a quite healthy and physiological situation (39). Dead gram negative (and some gram positive) bacteria, residing primarily in the colon provide the source of this gut derived endotoxin. It appears that this continuing challenge to the Kupffer cells may act as a "groomer" to the MPS and the liver. Reductions in the endotoxin "load" in the portal circulation will alter RES function. Animals on antibiotics (4) as well as germfree rats (3) will demonstrate decreased phagocytic activity. In addition, maintenance of liver size, alteration of certain hepatic enzyme activities, regulation of hepatic regeneration and secretion of pancreatic hormones all appear to be somewhat dependent on this continual delivery of lipopolysaccharides from the intestine to the liver (20). There may indeed be numerous other physiological functions for endotoxin in the portal circulation. Thus, the symbiotic relationship between an animal and its gut flora is much more complex than merely the provision to the animal of vitamin K precursors by the bacteria.

What then causes Kupffer cells to react so violently to endotoxin in the syndrome of endotoxic shock? This question is difficult to answer in light of the previous discussion, since these cells are continually phagocytosing endotoxin without any ill effects to the animal. Certainly, it is a dose related phenomenon and the presence of endotoxin in the systemic circulation also is an important factor (61,67). Regardless of amount and location in the bloodstream, the endotoxin is cleared initially by the MPS, mostly by the hepatic macrophages.

This ingestion of lipopolysaccharide by the Kupffer cells causes the release of mediators which initiate the various sequelae of endotoxicosis (35). These mediators may consist of substances which are not normally liberated, or they may merely be "physiological" substances released in exagerrated amounts. Interleukin-1 (IL-1) is one such mediator (56). IL-1 is present in the normal and endotoxic animal. This protein substance has been demonstrated to induce lymphocyte proliferation in vitro and is also similar to the endogenous pyrogen. Many more possible functions of IL-1 are currently under study. Most probably IL-1 will indeed be shown to mediate a wide variety of immunologic and metabolic functions in a way which is analogous to the many actions of insulin. Whether or not IL-1 is the only monokine responsible for endotoxic shock remains to be shown pending further study (45).

One final aspect of endotoxin-Kupffer cell interactions which should be addressed is the phenomenon of tolerance. Whereas lipopolysaccharide is usually lethal in relatively small quantities, an animal can be made virtually invincible to its effects by repeated incremental subcutaneous injections (65,70). A single endotoxin injection can protect against mixed flora septic mortality (71). There is also a C3H/HeJ strain of mice which is extremely resistant to endotoxin while the syngeneic strain (C3HeB/FeJ) is exquisitely sensitive (52,53). This phenomenon of tolerance may be in part humorally mediated (55), as demonstrated in Braude and Ziegler's work with the J5 mutant E. coli antiserum and its attenuation of septic shock in humans (79) and animals (11). An actual macrophage tolerance probably is also an important factor. This "indifference" of the macrophage to endotoxin is poorly understood as it relates to other macrophage functions such as phagocytosis. Repeated doses of endotoxin will

eventually produce a hyperphagocytic MPS. However, the resistant C3H/HeJ strain actually demonstrates a defect in phagocytosis.

Manipulations which alter various functions of the MPS have inconsistent results on endotoxic sensitivity. Administration of lead salts will increase lethality of endotoxin by a factor of 10,000 (24,29), yet it has also been shown to stimulate hepatic proliferation (16). In contrast, glucan also stimulates proliferation yet was shown to protect against Staphylococcus aureus septicemia in normal and leukemic mice (22). In contrast, glucan sensitized mice to endotoxin in other studies (35,36). Colloidal carbon "blockades" the MPS in regard to further phagocyte activity, but also sensitizes the animal to shock. Perhaps this blockade is in reality an activation of the MPS system in regard to mediator release. There are numerous other examples of MPS perturbations which also give inconsistent results in regard to alterations in endotoxin response. Thus, there exist some critical unexplained factors in the endotoxin-phagocytes-Kupffer cell response which will ultimately determine the organism's response along the continuum which ranges from resistance to death (23).

KUPFFER CELL - PARENCHYMAL CELL INTERACTIONS

Despite the preceding discussion, it is more correct to envision the Kupffer cells and hepatocytes as a single functional unit. Since all of the hepatic arterial and portal venous blood must percolate through the hepatic sinusoids, the Kupffer cells will oversee and process any circulatory contents before these substances reach the hepatocytes (49,59,78). Therefore, the perisinusoidal space (between the sinusoidal endothelial lining and the hepatocytes) will contain: 1) substances which the Kupffer cells allow to pass

unchanged. 2) substances which have been modified by the Kupffer cells. 3) substances (mediators or monokines) synthesized and released by the Kupffer cells in response to circulatory signals. The understanding of this "social behavior" (as Popper states it) of liver cells is still in its infancy (63). It is without question, however, that these cell to cell communications exist and certainly play a significant role in the metabolic dyshomeostasis of septic shock.

As stated earlier, the hypoglycemia observed in sepsis is a well known phenomenon. One of the reasons for this failure to maintain adequate blood glucose is a decrease in hepatic gluconeogenesis. Strong evidence exists that this hepatocyte failure is effected through Kupffer cell monokines. Filkins and Cornell showed that hepatocytes from an endotoxic animal demonstrated depressed gluconeogenesis, but that hepatocytes from a non-toxic fasted animal synthesized glucose normally, even when endotoxin was added to the cells directly. Mc Callum (52) investigated PEPCK enyzme activity in endotoxic mice and found it to be less than half of those values found in control mice. Again, endotoxin added directly to hepatocytes in vitro did not depress PEPCK activity. In another study, McCallum (52) measured PEPCK activity using combinations of hepatocytes and Kupffer cells from C3 HeB/FeJ (sensitive) and C3H/HeJ (resistant) mice. He found that the critical factor for depression of PEPCK activity was the presence of the endotoxic "sensitive" Kupffer cells. These studies all seem to lend credence to the fact that the Kupffer cells are causing a hepatocytic gluconeogenic failure.

Certain activities and factors have been studied relating to this depression of gluconeogenesis. Berry (80) and his group have extensively studied a macrophage mediator termed glucocorticoid antagonizing factor (GAF). This

product of endotoxic macrophages inhibits the induction of gluconeogenic enzymes by corticosteroids. Insulin has been shown to contribute significantly to endotoxic shock (12). Filkins described a macrophage insulin-like activity (MILA) derived from peritoneal exudate macrophages which increases peripheral tissue glucose oxidation. Filkins and Yelich investigated another macrophage product which stimulates pancreatic insulin secretion. This monokine was termed macrophage insulin-releasing activity (MIRA) and appears to play a role in the hyperinsulinism of sepsis. Leukocyte endogenous mediator (LEM), now known as interleukin-1 has been shown to mobilize hepatic glycogen and elevate plasma insulin and glucagon levels. As these factors and activities are purified and studied further, they may all be of similar structure. Perhaps just one monokine such as interleukin-1 will demonstrate all of these actions on glucose regulation.

HEPATIC REGENERATION MODEL

This thesis studied liver and endotoxin interactions using the rat partial hepatectomy and regeneration model. The following description of the pertinent hepatic events occurring temporally during this experimental model will aid in understanding both the rationale and advantages of this system for our study.

This partial hepatectomy model was first described in 1921 by Higgins and Anderson (42). It involves resection of the left lateral and median lobes of the rat liver resulting in a 70% reduction of liver mass. No special postoperative care is needed and the operation results in roughly a 95% survival rate (See Methods section for further details of the operative procedure). Since 1921, a large volume of literature has accumulated on the process of liver injury and regeneration utilizing this model. The model has also been used

extensively to study areas such as cell division, malignancy growth factors, DNA and RNA synthesis and endocrine control (17,51).

The rat liver possesses both an enormous functional reserve and a large capacity for regeneration. Vic (77), using testosterone and Gaub (40) have been able to induce full regeneration with a 50% survival after 90% hepatectomy. Various biochemical indices of liver functions such as serum bilirubin, prothrombin time and serum albumin are only minimally perturbed immediately following 70% resection (2,69). Plasma glucose undergoes only a slight decline following 70% partial hepatectomy. Inducement of gluconeogenesis and early glycogen depletion maintain this plasma glucose level, indeed, little if any hypoglycemia occurs in fasted rats following as much as a 70% hepatectomy (17). This prevention of early hypoglycemia seems to be a critical hepatocytic function as the vast functional reserve or "metabolic machinery" of the hepatocyte shifts to ensure the maintenance of plasma glucose (62). Hypoglycemia occurring in the early post hepatectomy period results in delayed liver regeneration (18).

Once this maintenance of blood glucose is assured, the liver begins its process of regeneration. The hepatocytes are the first to regenerate, followed by the sinusoidal cells and the ductal cells. Incorporation of radiolabelled thymidine into hepatocyte DNA begins after twelve hours with peak labelling occurring at twenty to twenty four hours post hepatectomy (66). Histological evidence of mitosis follows DNA labelling in 6-8 hours. During the first twenty four hours, a massive infiltration of fat vacuoles located in the hepatocytes occurs, disappearing by forty eight hours. The liver doubles in size after two days and usually approaches original weight by seven days. The entire hepatocyte

mass regenerates locally; existing hepatocytes undergo mitosis to entirely replace the resected parenchymal cell mass.

Regeneration of Kupffer cells occurs in a somewhat different manner than regeneration of hepatocytes. The new Kupffer cells can come from two sources, either local proliferation or recruitment of bone marrow monocytes which transform into resident macrophages (Kupffer cells). Probably both sources contribute to the repopulation of the liver, but controversy exists as to the relative importance of each source (9,38,64). A bone marrow source for Kupffer cells has been established in humans showing that the karyotype of Kupffer cells following liver or bone marrow transplant resembles that of the marrow whether it be transplanted or native (38,64). Bouwens has shown, however, that local proliferation is responsible for most of the early replenishment following partial hepatectomy (9). Kupffer cells lag behind parenchymal cells in their regeneration with peak thymidine labeling occurring at roughly forty two hours for sinusoidal cells versus the aforementioned twenty hours for hepatocytes. Also, the Kupffer cell population will double its original complement following resection, whereas the hepatocytes will only approach the prehepatectomy cell mass. Coincident with this exaggerated replenishment of Kupffer cells is an increase in phagocytic activity (6,7,47). A hyperphagocytic state exists as early as three days post hepatectomy and can persist for as long as six months.

THE STUDY

From the above discussion it is clear that the process of liver injury and repair involves a dynamic orchestration of intracellular processes and cellular balances. Studying the organismic response to endotoxin during various times

in the injury and subsequent regenerative process may provide information regarding the roles of the hepatic cellular components and their interactions in the development of endotoxic shock. The liver injury/regeneration model can be used to alter the delicate homeostatic balance between hepatocyte and Kupffer cell. By studying alterations in the development of endotoxic shock and glucoregulation failure during regeneration, a clearer assessment may be made of the functions in septic shock of the hepatocyte, Kupffer cell and most important, the hepatocyte-Kupffer cell unit.

Specifically, this study addresses the following questions:

1) Is susceptibility to endotoxic shock altered during acute liver injury and subsequent regeneration?

2) Is the glucose and lactate dyshomeostasis observed in endotoxic shock also observed in this experimental model? Is it accelerated or attenuated?

3) If alterations of endotoxic shock sensitivity are found, are these changes due to alterations of hepatocyte function, Kupffer cell function, or both?

MATERIALS AND METHODS

ANIMALS

Healthy male rats of the Holtzman strain (Holtzman Company, Madison, Wisconsin) weighing 275-400 grams were used for all experiments. The rats were acclimated for a minimum of five days in the animal care facility of Loyola University Medical Center. They were maintained on a 12 hour light-dark cycle (7:00 a.m. - 7:00 p.m., CST) and kept at an ambient temperature of 22°C (71.6°F). The rats were fed Purina Rat Chow (Ralston Purina, St. Louis, Missouri) and water was provided ad libitum. Animals were fasted at 4:00 p.m. on the day prior to the in vivo gluconeogenesis studies. For all other studies, rats were allowed free access to food.

PARTIAL HEPATECTOMY AND HEPATIC REGENERATION

Partial hepatectomy to induce liver regeneration was performed in a manner similar to the procedure described by Higgins and Anderson (42). All surgery occurred between 8:00 a.m. and 12 noon. Fed rats were placed under ether anesthesia, and a midline laparotomy was carried out from the xiphoid process caudad for a length of 2-3 centimeters. The left lateral and median lobes of the liver were delivered through the laparotomy by applying simultaneous pressure on the abdomen and thorax. Two ligatures of 2-0 silk were tightly placed around the vascular pedicles of these lobes. The lobes were then removed with a scissors as close to the ligature as possible. The abdominal musculature was closed with 3-0 silk, and the skin was closed using 3 or 4 wound clips. There was no special postoperative care. The rats were returned to the animal

care facility and given food and water ad libitum until the time of the subsequent experiments. Left lateral and medial lobectomy resulted in approximately a 70% removal of liver mass.

Sham partial hepatectomies were also carried out on rats age matched to the hepatectomized rats. These rats also underwent a midline laparotomy. The same lobes were delivered outside of the abdominal cavity, manipulated and replaced along with a 1 cm length of 2-0 silk ligature to control for any foreign body reaction. The abdomen was closed in an identical fashion. The sham rats received the same post op care. A third group of age matched controls received no operation. Subsequent experiments were performed on all three groups on the same postoperative days.

ASSESSMENT OF LIVER REGENERATION

The ratio of liver mass to total body mass was used as an assessment of hepatic regeneration following partial hepatectomy. Rats were weighed on the day of all experiments. After the studies were completed, the animals were autopsied and the livers were removed and blotted dry. The blotted livers were weighed, and these weights were then recorded as a percentage of total body mass.

IN VIVO ENDOTOXIN SENSITIVITY

The effect of liver injury (70% hepatectomy) and subsequent hepatic regeneration on endotoxic sensitivity was studied by utilizing intravenous bolus injections of endotoxin as a model for endotoxicosis.

The three groups of animals (partial hepatectomy, sham hepatectomy and control) were allowed food and water until 8:00 a.m. on the morning of the experiments. Between 8:00 a.m. and 10:00 a.m., the rats were lightly anesthetized, weighed and injected with 10 mg/kg of Salmonella enteritidis lipopolysaccharide (Difco Co., Lot No. 719776 and 706110). The rats were then allowed free access to water and observed for a twelve hour study period. These in vivo studies were performed on operated and control rats on days 1, 4, 9, and 16 following partial hepatectomy.

LETHALITY

During the twelve hour study period, rats were sacrificed by decapitation when their righting reflex was judged to be lost (the rat would not attempt to regain normal posture after being placed in the supine position). The loss of this reflex was taken to be indicative of a preterminal state of septic shock. These rats were tallied as deaths and the time to death (TTD) was recorded in minutes from time of injection to decapitation. Any rats surviving for twelve hours were tallied as "survivors" and assigned a TTD of 720 minutes. The survivors were then sacrificed and blood was collected for plasma glucose and lactate analyses.

PLASMA GLUCOSE AND LACTATE

All rats had blood specimens collected in 250 ul heparinized microcentrifuge tubes (Beckman Company, Lincolnwood, Illinois) at the time of decapitation. All specimens were promptly placed on ice. These samples were then centrifuged and assayed for plasma glucose and plasma lactate levels.

Plasma glucose measurements in mg/dl were obtained directly from a glucose analyzer (Yellow Springs Instruments, Model 23A, Yellow Springs, Ohio). Likewise, plasma lactate levels expressed in mmol/liter were obtained directly from a lactate analyzer (YSI Model 23L, Yellow Springs, Ohio). All samples were analyzed twice if plasma quantity was sufficient, and the recorded value was the mean of the two measurements.

ASSESSMENT OF THE IN VIVO GLUCONEOGENESIS

An assessment of the gluconeogenic capability of the newly regenerated liver was performed using a modification of the intravenous alanine load test (26). Rats which were 4 days post hepatectomy and control rats were fasted for 18 hours prior to the experiment. Between 8:00 a.m. and 10:00 a.m. rats lightly anesthetized with intraperitoneal pentobarbital were injected with 100 mg of mannoheptulose through the penile dorsal vein to block insulin release. Fifteen minutes later, baseline blood samples for glucose were collected from tail snips and the rats were immediately injected with 100 mg of alanine intravenously to provide substrate for gluconeogenesis. Blood samples were then collected at 15, 30, 45 and 60 minutes following alanine injection and analyzed for glucose content.

ASSESSMENT OF PHAGOCYTIC FUNCTION

It has been previously demonstrated that Kupffer cell phagocytic function is increased in the regenerating rat liver (6,7,47). In order to reconfirm this finding in this study, a check of phagocytic activity was performed by assaying the blood clearance of colloidal carbon. Rats 4 days post hepatectomy and

control rats were lightly anesthetized with intraperitoneal pentobarbital. Following anesthesia, the rats were injected with 1 cc of heparinzed saline into the dorsal penile vein to facilitate subsequent blood sampling via tail snips. The tail snips were then performed on both groups of rats. Baseline blood samples were collected from the tail snips into heparinized porcelain spot plates. Immediately following the baseline blood collection, the rats were injected with a solution of colloidal carbon (Gunther Wagner Pelikan Ink, 96 mg carbon/cc, West Germany). This stock ink was diluted with normal saline to a concentration of 48 mg/cc. Each rat received 16 mg colloidal carbon/100 g body weight for a total injected volume of roughly 1 cc. Taking the injection time as time zero, blood samples were then collected every three minutes for twenty one minutes from the tail snips and placed in heparinzed spot plates. Twenty five ul of each of these blood samples was then mixed in a glass 13 X 100 mm cuvette with 5 cc of a 0.1% Na_2CO_3 solution. The cuvettes were then shaken vigorously to ensure a homogenous mixture.

The samples were then measured on a Klett Colorimeter for clearance of colloidal carbon. Since measurement involved whole blood, a red filter was used in the colorimeter to correct for the presence of hemoglobin. After zeroing the instrument for each rat with its individual baseline "blood blanks," the light absorbance of subsequent samples for each rat was measured in "Klett units" (arbitrary units, which connote absorbance). For each rat sample, the Klett units were plotted against time on semilog paper and the best fit line was drawn through the points. Since this clearance is known to follow first order kinetics, the half time for clearance of the colloidal carbon was then determined from this line as an assessment of phagocytic function of the rat.

DATA ANALYSIS

Differences in lethality between the hepatectomy, sham hepatectomy and control groups was analyzed via a chi square analysis. Differences in plasma glucose, plasma lactate, liver wet weights, in vivo gluconeogenesis and the time to death in minutes were analyzed using a two-tailed t-test. Analysis of increase in plasma glucose during the intravenous alanine load test was performed with a one tailed t-test since the glucose parameter will only behave in one manner or increase.

RESULTS

ASSESSMENT OF LIVER REGENERATION

After the technique of partial hepatectomy was established in our laboratory, recovery from the operation (until the day of experimentation) approximated 95%. Recovery of the laparotomy rats approached 100%. The assessment of liver regeneration in all experimental rats was measured against the intact liver weights of control and sham rats. Removed livers were blotted dry and weighed, and each individual liver was expressed as a percentage of the body weight of the respective rat. As Table 1 shows, liver regeneration by this parameter is nearly complete by 96 hours following resection. The regeneration percentage tends to drop off at 9 and 16 days after operation. This tendency is due to continued weight gain by the rats. Also, there is little difference between the liver percentages of control and sham laparotomy rats, indicating that both groups, as expected, are interchangeable in this study.

LETHALITY

Rats were sacrificed by decapitation when there was loss of the righting reflex (see Methods). Rats surviving twelve hours were then decapitated and termed survivors. All nonsurviving rats regardless of group appeared to undergo similar deaths; the rats displayed hyperventilation, piloerection, lethargy, irritability and diarrhea. Terminally, some rats displayed hypoglycemic convulsions. At autopsy, the rats showed varying degrees of intestinal hemorrhage.

TABLE 1

ASSESSMENT OF LIVER REGENERATION

FOLLOWING 70% HEPATECTOMY

GROUPS	N	LIVER WEIGHT[b]
CONTROL	23	3.45 ± .09
SHAM	39	3.16 ± .06
HEPATECTOMY[a]		
1	14	1.64 ± .08[c]
4	20	3.22 ± .13
9	21	2.78 ± .10
16	18	2.65 ± .09

[a] Numbers refer to days following hepatectomy

[b] Liver weight expressed as grams liver (wet weight)/grams total rat weight in percent ± S.E.M. for each individual rat.

[c] p less than .05 versus control on sham group.

Previous studies in our laboratory had demonstrated that the LD_{50} of S. enteritidis injected intravenously for fed rats was roughly 10 mg/kg. Table 2 shows that this held true for this investigation. The twelve hour survival for control and sham rats was 43% and 44% respectively. This also indicates that laparotomy alone did not increase endotoxic lethality and thus the sham hepatectomy rat behaves as a suitable control. There is a significant sensitization to endotoxic shock at four days post hepatectomy, which is abolished by nine days. The four day rats are more sensitive (with their regenerated livers) than the one day rats (without significant regeneration) when the TTD is used as a measurement of sensitivity. Note also that the sixteen day rats are somewhat protected, although this difference did not reach statistical significance.

TERMINAL PLASMA GLUCOSE AND LACTATE DURING ENDOTOXICOSIS

In order to assess the failure of glucoregulation terminally, blood samples were collected at the time of sacrifice. These samples were analyzed for plasma glucose and lactate levels. The table values for each group include blood levels for both survivors and nonsurvivors. There exists an obvious trend between increasing lethality and failure of glucoregulation, manifested by hypoglycemia and failure of the liver to clear lactate. However, nearly all of the lethalities, regardless of grouping, exhibited profound hypoglycemia and hyperlactacidemia. Taken together with the lethality figures, these data indicate an enhanced and earlier glucoregulatory failure in the four day hepatectomy group. It should be noted that previous studies established that all groups have normal plasma glucose and lactate levels prior to the injection of endotoxin. Thus, glucoregulation appears to be normal in all groups in the baseline state. Most striking about the data from Tables 2 and 3 is the fact that there is

earlier lethality and failure of glucoregulation in the four day group even when compared to the one day hepatectomy group. Thus, although the liver mass has been restored to nearly normal, its ability to maintain blood glucose during endotoxicosis is inferior to a liver which has roughly one half of normal weight.

IN VIVO GLUCONEOGENESIS

Because of the accentuated glucose dyshomeostasis observed in the four day hepatectomized rat, this study was carried out to assess any alteration in the gluconeogenic capacity of the regenerated liver. This was tested by using an alanine tolerance test. The animal's ability to undergo gluconeogenesis was optimized by performing the study in the fasted state, by injecting an excess of substrate (alanine) and by administering mannoheptulose to block any insulin response to increasing blood sugar. The data of Table 4 demonstrate that there is indeed a significant defect in the gluconeogenic capability of the newly regenerated liver. Note that fasting blood sugar levels are not different between the two groups. However, the maximal gluconeogenic capacity of the four day hepatectomy rat is suboptimal; this maximal gluconeogenic drive would be of critical importance during the development of hypoglycemia in the glycogen depleted, anorectic septic animal. Since it was done in vivo, this experiment does not differentiate this failure of gluconeogenesis between a primary hepatocytic defect or a secondary effects such as the influence of monokines or other mediators. The test also makes certain assumptions which will be addressed in the Discussion section.

TABLE 2

ENDOTOXIC LETHALITY FOLLOWING

70% PARTIAL HEPATECTOMY

GROUPS	N	PERCENT SURVIVAL AT 12 HOURS	TIME TO DEATH[b] (MINUTES)
CONTROL	23	43	500 ± 46
SHAM	39	44	522 ± 34
HEPATECTOMY[a]			
1	14	14	341 ± 57
4	20	5[c]	199 ± 32[c]
9	21	43	525 ± 49
16	18	67	576 ± 55

[a] Numbers refer to days following hepatectomy.

[b] Time to death expressed in minutes ± S.E.M. after injection of endotoxin. Rats surviving for twelve hours were assigned a value of 720 minutes.

[c] p less than .05 versus control or sham group.

TABLE 3

TERMINAL PLASMA GLUCOSE AND LACTATE LEVELS

FOLLOWING ENDOTOXICOSIS

GROUPS	N	PLASMA GLUCOSE mg/dl[b]	PLASMA LACTATE mmol/L[b]
CONTROL	23	56 ± 9	5.2 ± .7
SHAM	39	35 ± 10	5.8 ± .7
HEPATECTOMY[a]			
1	13	10 ± 3	6.8 ± .9
4	20	10 ± 4	7.7 ± 1.0
9	21	48 ± 9	6.3 ± .6
16	18	65 ± 8	3.4 ± .7

Deaths and survivors are taken together for each group.

[a] Numbers refer to days following hepatectomy

[b] Values are ± S.E.M.

TABLE 4

EVALUATION OF IN VIVO GLUCONEOGENESIS

FOLLOWING HEPATECTOMY

GROUP	N	GLUCOSE VALUES (mg/dl)[b]			
		TIME[a]			
		0	30	45	60
CONTROL	12	112 ± 6	164 ± 6	178 ± 5	174 ±7
(PERCENT CHANGE)		-	46	58	55
4 Days Post Hepatectomy	23	110 ± 4	148 ± 5[c]	157 ± 5[d]	161 ± 5[c]
(PERCENT CHANGE)		-	35	43	46

[a] Time in minutes following injection of I.V. Alanine.

[b] Numbers are ± S.E.M.

[c] p less than .01 when compared to control.

[d] p less than .001 when compared to control.

ASSESSMENT OF PHAGOCYTIC FUNCTION

Previous studies have shown the regenerating rat liver to have an increased number of Kupffer cells beginning roughly two days after hepatectomy. This increase in Kupffer cell number is parallelled by an increase in phagocytic capacity. Therefore, this phase of the study was implemented to assess the phagocytic activity of the four day hepatectomy rat in an attempt to relate this aspect of Kupffer cell function to the increased sensitivity to endotoxin. The rats with regenerated livers cleared the injection of colloidal carbon much faster than the control group (Table 5). The T½ was 6.8 minutes for the hepatectomy group vs. 14.0 minutes for the control group. This large increase in phagocytic capacity is in agreement with other studies which demonstrated increased clearance for colloidal gold, and labelled endotoxin. At autopsy, the distribution of carbon in the viscera was similar between experimental and control groups. The livers and spleens grossly were quite blackened with minimal blackening of the lungs. One interesting autopsy finding revealed that the spleens of the previously hepatectomized animals were often enlarged; in some cases the spleens had doubled in size. Other autopsies revealed that this enlargement was not present in laparotomy only rats.

TABLE 5

EFFECT OF HEPATECTOMY ON

COLLOIDAL CARBON CLEARANCE

GROUP	N	T½[a]
CONTROL	6	14.0 ± 1.3
4 DAY POST HEPATECTOMY	5	6.8 ± .3[b]

[a] T½ expressed in minutes ±.S.E.M. for clearance of one half of original whole blood colloidal carbon concentration.

[b] p less than .001 when compared to control group.

DISCUSSION

The rat partial hepatectomy operation provides a quick and reproducible model for studying liver injury and regeneration. Once the technical aspects were mastered in our laboratory, nearly all of the rats survived for later study. The reproducibility of hepatic regeneration at various stages was fairly uniform as evidenced by the small standard errors in Table 1. Thus, this model proved quite suitable to help answer some of the scientific questions which prompted this study.

Table 1 demonstrates that rat liver regeneration is, as expected, quite rapid and is nearly complete by ninety six hours after surgery. The apparent increase in hepatic mass (assuming a 70% hepatectomy would leave 3.45x.3=1.04% liver) after only twenty four hours is probably due to the well described phenomenon of fatty infiltration for the first day following surgery. Although peak mitotic activity occurs at twenty hours, the major increase in the true hepatocytic mass occurs in postoperative days two to four. The slight decrease of liver weight percentage observed at days nine and sixteen probably reflects increased total rat mass as all rats continued to gain weight throughout the postoperative period. The remnants were nearly completely regenerated by day four and subsequently remained at a fairly constant weight. Although no histology was performed in our study, it has been previously shown that other than the early fatty deposition, the regenerating rat liver has nearly normal histology by light microscopy (except for an increased mitotic index).

For the lethality studies, we attempted to utilize an endotoxin dose which approximated an LD_{50} for these rats. The rationale for this dosage lies in the

fact that we were unsure as to whether liver regeneration would sensitize or protect the rat in endotoxic shock. Table 2 shows that the survivals for the control and sham groups were as close to 50% as one might expect considering the somewhat erratic organismic response to intravenous endotoxin.

The most striking finding in the lethality study is the profound sensitivity of the 4-day rat to endotoxic shock, even with its nearly normal complement of the liver. In contrast, the 1-day rat demonstrates better survival data despite the fact that its liver mass is markedly reduced and it also has undergone the stress of recent surgery. Therefore, this "newly regenerated" liver may react differently to endotoxin or merely decompensate quicker than either a nonregenerated liver or a recently resected liver. Other than the overabundance of Kupffer cells, the regenerated rat liver is felt to be the functional equivalent of an intact liver; the usual biochemical parameters are not altered in the non-stressed state. This study demonstrates that there are indeed profound differences in the response of the newly regenerated liver when stressed with endotoxin.

In contrast to the 1 and 4 day rats, the 9 day rats show no difference in lethality or time to death, and the 16 day rats seem to enjoy somewhat of a protective effect. Thus, whatever alteration in the 4-day rat enhances the sensitivity to endotoxin disappears by day 9 following hepatectomy. What causes the relative protection observed in the sixteen day rat also is unclear; possible explanations for these data will be addressed later in this discussion.

The well known relationship of hypoglycemia and hyperlactacidemia observed in terminal sepsis and endotoxic shock was again shown in this study.

Virtually all nonsurvivors, regardless of group, exhibited a profound failure of glucoregulation (and gluconeogenesis) as characterized by the low plasma glucose and high lactate levels (Table 3). As expected, these abnormalities correlated closely with the lethality and survival times of the animals. These data indicate that all groups succumb to similar metabolic deaths. The stage of liver injury and regeneration appears to alter mainly the temporal susceptibility to gluconeogenic failure and subsequent shock.

What is the reason for this early metabolic failure? Certainly it would seem logical to implicate a hepatocyte malfunction, since the maintenance of euglycemia is a direct function of hepatocyte gluconeogenesis. The observed failure of hepatocyte glucoregulation may be either primary, secondary or most likely a combination of defects.

Direct endotoxin effect on hepatocyte gluconeogenesis may indeed occur, but no convincing evidence to date has demonstrated this phenomenon. Primary suppression of gluconeogenic enzymes could result from endotoxin. McCallum, however, showed that there was no effect on hepatic phosphoenolpyruvate carboxykinase (PEPCK) activity when endotoxin was added directly to liver homogenates or hepatocyte cultures (52). Since the lipid A portion of the endotoxin molecule is obviously quite lipophilic and direct membrane effects have been demonstrated, then a primary effect of endotoxin on the heptocyte remains a possibility. Secondary effects of endotoxin mediated through Kupffer cells is probably playing the major role in the gluconeogenic failure in this study. McCallum demonstrated profound decreases in PEPCK activity when media from endotoxin incubated Kupffer cells was added to normal hepatocytes (52). In our study, the liver (day 4) with the largest complement of new Kupffer

cells show the most profound depression of gluconeogenesis, suggesting that monokines liberated from these Kupffer cells contributed significantly to the metabolic failure observed in the 4 day rats.

This effect of Kupffer cells on hepatocyte gluconeogenesis certainly was observed in the endotoxic state. However, many phenomena observed in the endotoxic state may be merely an exaggeration of the so called normal interactions between Kupffer cells and hepatocytes. The data from Table 4 on in vivo gluconeogenesis demonstrates that this exaggeration may indeed be the case. With conditions maximized for gluconeogenic production of new glucose, the 4 day rats demonstrated a definite inferior capacity to elevate the blood glucose in response to gluconeogenic challenge. Since this is an in vivo study, however, no comment can be made whether or not this represents a primary hepatocyte dysfunction or whether this is due to the hepatocytes existing in a milieu of Kupffer cells producing monokines which may act to suppress gluconeogenesis (26). Other studies have shown that virtually all functions of the intact liver are normal in the newly regenerated rat liver. Thus, it is unlikely that this particular enzyme system would be defective in the hepatocytes without extracellular influences such as monokines. This theory could be tested most easily by an isolated hepatocyte short term culture which would be Kupffer cell free. Another consideration which could not be addressed in this study is the extrahepatic glucose consumption which may affect a blood glucose level. Measuring only plasma glucose levels does not afford one to comment on either rates of appearance or disappearance of glucose into the plasma. It is possible that the 4 day rat was exhibiting a more profound peripheral utilization of glucose and therefore could not elevate his glucose as high as the control rat. However, if we assume that the mannoheptulose injection prior to the test

blocked insulin effects on all insulin sensitive tissues, then perhaps this argument is minimized.

As others have previously shown, we have demonstrated that the 4 day rats indeed had hyperphagocytic livers indicative of increased of Kupffer cell mass and activity, therefore it is highly unlikely that the increased endotoxin sensitivity of these rats is due to a failure of the mononuclear phagocyte system to clear and process endotoxin. Rather, this speaks for an exaggeration of the normal host response in dealing with endotoxin. Grossly, most of the colloidal carbon appeared to be taken up in the newly regenerated liver as similar to that seen in control livers, reinforcing the fact that the Kupffer cells in the new liver are more than adequately performing their phagocytic function. We may assume then, that these new Kupffer cells are also performing their function in clearing the injected endotoxin dose. At this point I would like to summarize the experimental findings and then present a unifying hypothesis to explain the experimental results. Because of the limited studies performed, obviously no firm conclusions can or should be drawn from these studies. However, a unifying hypothesis is offered to provide a direction for future studies in this area.

These studies demonstrated that the liver generation model is, indeed, a useful tool to study Kupffer cell hepatocyte interactions, specifically in the area of endotoxic shock. A rat with a newly regenerated liver exhibits profound sensitivity to intravenous endotoxin in regards to lethality and glucoregulation. Qualitatively the lethality imposed on this 4 day rat by endotoxin does not appear to be any different from a control rat, a recently operated rat, or one further along in the regenerative process. However, the temporal sequence to the development of irreversible shock seems to be hastened in the 4 day rat.

Metabolically, the rat undergoes a much quicker and more profound process of hyperlactacidemia and hypoglycemia which appears to be due to a failure of mainly gluconeogenesis. In the nonendotoxic state, the 4 day rat appears to have inferior gluconeogenesis when compared to control rats, thus the organismic milieu which these hepatocytes reside in may already predispose to a dysfunction of gluconeogenesis even prior to induction of endotoxicosis. The 4 day rats' mononuclear phagocyte system is significantly more functional in regards to phagocytosis of colloidal carbon than is control rats. Thus the MPS appears to be hyperfunctioning rather than malfunctioning.

The following unifying hypothesis is offered to fit the experimental data from this study as well as previous studies performed which have investigated endotoxin liver interactions. In the physiologic state, the Kupffer cells normally phagocytize and process endotoxin in a constant fashion over the long run. They probably adapt to this continuous flow of endotoxin through the portal venous system as the organism will not go into septic shock every time there is a seeding of endotoxin into the portal system. Therefore, there is theoretically a continual baseline release of mediators in response to endotoxin. These mediators are released in an exaggerated fashion when the load of endotoxin is overwhelming as is seen in septic shock. These mediators such as MILA, MIRA and interleukin 1, exhibit profound influences on the metabolic behaviour of the neighboring hepatocytes. The injured liver prioritizes the maintenance of the gluconeogenic pathway to avoid dangerous hypoglycemia in the immediate postinjury period. Possibly, this hepatocyte "machinery" then shifts into cell

division processes as regeneration continues rather than maintaining gluconeogenic processes. This shift of intracellular priorities, along with the increased Kupffer cell responses, combine to produce the profound sensitivity to endotoxin observed in the day 4 rats.

The MPS cells repopulate the regenerated liver and spleen either by local division and/or bone marrow repopulation. They begin a slow process of degrading endotoxin in the portal circulation while becoming somewhat tolerant of endotoxin. However, in the 4 day liver, when the MPS cells are quite new in their environment, they may react in a profoundly exaggerated manner to endotoxin, both because they have no tolerance and their sheer numbers are increased. Indeed, splenectomized animals exhibit relative resistance to endotoxin (1). As the Kupffer cells reside in the hepatic sinusoids over time, this tolerance to endotoxin is reflected in the fact that the 9 day rats have now lost their profound sensitivity exhibited in the 4 day rats and by 16 days, this tolerance may actually connote a protective effect because of the increased ability of the phagocyte to degrade endotoxin. Previous studies have shown that this hyperphagocytic activity persists for up to 6 months in the rat liver. This development of tolerance and the process of desensitization would also explain why innoculations of endotoxin can make a rat markedly insensitive to otherwise lethal doses of endotoxin. This phenomenon may also help to explain the so called process of RES blockade which may actually be a sensitization of the Kupffer cells to hypersecrete physiologic mediators which may become pathophysiologic in these amounts and the amounts released during septic shock.

SUMMARY OF CONCLUSIONS

1. Partial hepatectomy (70%) with subsequent regeneration provides an appropriate method to study the role of hepatocyte-Kupffer cell interactions in the development of endotoxic shock.

2. Regardless of the timing of endotoxin injection following hepatectomy, rats develop profound hypoglycemia and hyperlactacidemia during late endotoxic shock.

3. Rats which are four days post hepatectomy are profoundly sensitized to endotoxic shock, while rats which are sixteen days post-hepatectomy appear to experience relative protection from the lethal effects of intravenous endotoxin.

4. In vivo gluconeogenesis is impaired in the four day post-hepatectomy rat.

5. The four day post-hepatectomy rat liver is hyperphagocytic as demonstrated by the accelerated clearance of intravenous colloidal carbon.

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