Nitric Oxide in Liver Diseases

Friend, Foe, or Just Passerby?

WEI MIN HON, KANG HOE LEE, AND HOON ENG KHOO

Departments of Medicine and Biochemistry, National University of Singapore, Singapore

ABSTRACT: **Research on the free radical gas, nitric oxide (NO), during the past twenty years is one of the most rapid growing areas in biology. NO seems to play a part in almost every organ and tissue. However, there is considerable controversy and confusion in understanding its role. The liver is one organ that is clearly influenced by NO. Acute versus chronic exposure to NO has been associated with distinct patterns of liver disease. In this paper we review and discuss the involvement of NO in various liver diseases collated from observations by various researchers. Overall, the important factors in determining the beneficial versus harmful effects of NO are the amount, duration, and site of NO production. A low dose of NO serves to maximize blood perfusion, prevent platelet aggregation and thrombosis, and neutralize toxic oxygen radicals in the liver during acute sepsis and reperfusion events. NO also demonstrates antimicrobial and antiapoptosis properties during acute hepatitis infection and other inflammatory processes. However, in the setting of chronic liver inflammation, when a large sustained amount of NO is present, NO might become genotoxic and lead to the development of liver cancer. Additionally, during prolonged ischemia, high levels of NO may have cytotoxic effects leading to severe liver injury. In view of the various possible roles that NO plays, the pharmacologic modulation of NO synthesis is promising in the future treatment of liver diseases, especially with the emergence of selective NO synthase inhibitors and cell-specific NO donors.**

KEYWORDS: **nitric oxide; liver diseases; friend or foe**

INTRODUCTION

During the past decade, particular attention has been paid to the small, diffusible, unique molecule—nitric oxide (NO). NO, the end product of the enzyme NO synthase (NOS), influences various physiological processes in essentially every organ and tissue. It has a remarkably broad spectrum of such functions as regulation of vascular tone, neurotransmission, antimicrobial defence mechanisms, and immunomodulation.[1] The role of NO in regulating an organ function is always complex, resulting in sometimes conflicting experimental data. In biological systems, NO has a very short half-life of less than five seconds and, therefore, direct measurement of NO is not easy and not commonly practiced. NO is rapidly inactivated in tissues by oxidation to its metabolite nitrites and nitrates, which readily diffuse into the

Address for correspondence: Wei Min Hon, Ph.D., Department of Medicine, Faculty of Medicine, National University of Singapore, 10 Kent Ridge Crescent, Singapore, 119260. Voice: (65) 779 5555 ext 2368; fax: (65) 779-4112.
mdchonwm@nus.edu.sg

Ann. N.Y. Acad. Sci. **962: 275–295 (2002).**

circulation. Thus, concentrations of nitrite and nitrate are often used as indicators of the presence of NO. NO elicits many of its actions by signal transduction through activation of the soluble guanylate cyclase, binding to the iron in the heme center, resulting in an intracellular increase of cGMP levels.[2] Hence, increase of NO normally leads to an increase in cGMP concentration. Thus, cGMP is also used as an indicator of the presence of NO. NO can bind to non-heme iron, particularly to the iron in the iron-sulfur centers of numerous enzymes, thus altering their biological activity.[3] These enzymes include those in the respiratory cycle, the tricarboxylic acid (TCA) cycle, and the DNA synthesis pathway. Indirectly, NO can combine with superoxide anion to form the highly reactive peroxynitrite that has been linked to lipid peroxidation and nitrosation of tyrosine residues in proteins.[4] At different redox states, NO may appear in various chemical forms such as NO^+, $\cdot NO$, and NO^-. These NO-related species have distinct chemical reactivities and they can all influence the fate of cells in response to various insults. This review focuses on the possible roles of NO in liver diseases where the basic chemistry or biology of NO has been extensively discussed and reviewed.[1,5,6]

The liver is one of the most important body organs in that it performs so many different functions at the same time. The liver makes proteins, eliminates waste material from the body, produces cholesterol, stores and releases glucose and metabolizes many drugs used in medicine. Liver diseases, such as viral hepatitis, liver cirrhosis, and liver cancer appear to be on the increase and each year more than 43,000 Americans die of liver disease. There are few effective treatments for most life-threatening liver diseases other than liver transplants. It has been postulated that NO is involved with the etiology or progression of liver diseases because NO plays a major role in homeostasis regulation as well as in immunology defence mechanisms. However, the exact role of NO in most of these hepatic diseases is still unknown. Some results indicate that NO is beneficial whereas others postulate a detrimental effect. At the same time, some results even suggest that the presence of NO in certain situations is merely a consequence of other more important disease mechanisms.

There are three isoforms of NOS all of which are expressed in the liver. Of these, the inducible NOS (iNOS) and endothelial NOS (eNOS) are the most important. Neuronal NOS (nNOS) appears to be restricted to nerve endings found in large blood vessels. Under physiologic conditions only the constitutive eNOS is believed to be present in the liver and the low level of NO produced regulates hepatic perfusion, preventing platelet adhesion, thrombosis, and polymorphoneuclear cell (PMN) accumulation.[7] iNOS has been found in almost every cell type of the liver, such as hepatocytes,[8,9] Kupffer cells,[10] hepatic endothelial cells,[11] and Ito cells.[12] However, the degree and duration of NO production in various situations are probably determined by factors such as the type of stimulus, as well as the cell types that are being stimulated to express iNOS. Although most human cell types require a combination of cytokines to activate iNOS expression, interleukin-1β (IL-1β) alone at high doses can induce iNOS mRNA in primary human hepatocytes.[13] Hence, iNOS seems to be more readily induced in hepatocytes than other human cell types. In pathologic conditions, such as endotoxaemia, hemorrhagic shock, ischemia-reperfusion, sepsis, infection, hepatitis, liver cancer, and liver regeneration, iNOS can be induced and produce a significant sustained amount of NO. This NO serves as an important regulator and effector. Study of the actions of NO has been facilitated by the availability of

various NOS inhibitors, the most common of which are N^G-nitro-L-arginine methyl ester (L-NAME), N^G-monomethyl-L-arginine (L-NMMA), and N^G-nitro-L-arginine (L-NNA).

The role of NO in various liver pathological conditions has yet to be clearly defined, which may be due to the differences among animal models used as well as the variations in experimental setup. Furthermore, the activity of NO produced by human cells is much lower than that produced by the rodent cells. Hence, the results observed in animal models may not be directly relevant to human pathophysiologic conditions. Therefore, in this article, out discussion is mainly based on the evidence observed in NOS knockout animal models and human patients.

LIVER DAMAGE DURING INFLAMMATION

Cytokines, such as tumor necrosis factor-α (TNF-α), are produced in the liver in endotoxaemia, systemic inflammation, and fulminant hepatic failure. They are responsible for the extensive hepatocellular injury under these conditions. Even though the mechanism of hepatocellular dysfunction has been widely studied, there are still many unanswered questions. Basically, the injured cells either survive or die through apoptosis or necrosis.[14] Because cytokines are well-known stimuli of iNOS, various experiments have been carried out to study the role of NO in these situations. However, results to date concerning the role of NO in liver damage during inflammatory conditions remains controversial. Both beneficial and detrimental NO-mediated properties have been described.

Non-specific inhibition of NOS in endotoxaemia models results in increased liver damage, suggesting a beneficial role of NO in liver during sepsis. Suppression of endogenous NO might aggravate hepatic injury, partly caused by decrease in hepatic blood flow accompanied with increase oxidative stress.[18,19] Recently, Takemura *et al.*[20] further demonstrated that a potent inhibitor of NOS markedly deteriorates the lipopolysacharride (LPS)-induced liver injury by disturbing hepatic microvascular blood flow. Zhu and Fung[21] showed that NO protects liver injury through scavenging lipid radical and, thus, inhibiting lipid peroxidation chain reaction.

Another possible protective mechanism of NO during liver injury might be through its antiapoptotic action. Increasing evidence shows that NO is a potent antiapoptotic molecule in hepatocytes *in vitro*[22] and *in vivo*[23] despite the fact that NO induces apoptosis in various other cell types.[24–26] The antiapoptotic actions of NO in hepatocytes were reviewed by Li and Billiar.[27]

NO is able to prevent TNF-mediated activation of the proapoptotic protease caspase 3 and to protect hepatocytes from cytokine-mediated death, conferring protection against TNF-induced liver injury.[23,28,29] NO acts via the *S*-nitrosylation of procaspases and active caspase enzymes.[30] Moreover, NO stimulation of the cGMP/protein kinase G pathway also appears to contribute to the protective effect of NO.[31] The antiapoptotic role of NO is further illustrated in two reports. First, Mojena *et al.*[32] studied the effect of preexistent hepatic NO synthesis on liver injury induced by LPS in animals carrying an iNOS transgene. These animals expressed iNOS in liver cells under fasting conditions. LPS-induced liver injury was impaired in animals expressing iNOS. Their results indicated that NO protects the liver by

inhibiting the synthesis of proinflammatory mediators, such as TNF-α, and prevents apoptosis of the injured cells by inhibiting caspase 3. In the second report, Morio *et al.* analyzed the hypothesis by using knockout mice lacking the gene for the TNF-α receptor, TNF-α cytokine or iNOS. They found that there is significantly less carbon tetrachloride (CCl_4)-induced hepatic injury in mice lacking TNF-α receptor-1 or the TNF-α cytokine. In contrast, liver injury is increased in knockout mice lacking the gene for iNOS. These data further confirm the hepatoprotective effects of NO, which may be due in part to inhibition of TNF-α.

In contrast to the documented protective effects of NO, its detrimental role in liver injury has also been reported. Administration of aminoguanidine (iNOS selective inhibitor) markedly diminished the severity of the liver injury induced by various methods of induction.[34–36] Apparently, hydroxyl radicals produced by the reaction of NO and superoxide anion via peroxynitrite may be involved in the pathogenesis of hepatotoxicity. Recently, Sass *et al.* demonstrated that iNOS knockout mice are protected from liver damage following concanavalin A (Con A) treatment. Furthermore, they found that the amount of plasma TNF-α and intrahepatic TNF-α mRNA and protein is significantly reduced in iNOS knockout mice. These data imply that iNOS-derived NO regulates proinflammatory genes *in vivo,* thereby contributing to inflammatory liver injury.

The contradictory findings about the roles of NO during liver injury might be due to the differences in the redox status of the liver and the dosage and type of the insult. At the beginning of hepatic injury, when only low amount of NO is being produced, NO might protect the liver through its vasodilation, antioxidative, and antiapoptotic effects. However, in the event of massive injury (high dosage of inducers and elevated oxidative stress) large amounts of NO produced might favor the cells to move to the non-returnable channel—necrosis and cell death. As hepatocellular injury is not the major determinant of survival for patients with multiorgan failure due to sepsis or other reasons, the role of NO in hepatic injury has not been well studied. Most of the time, NO in liver injury was studied in conjunction with other liver diseases, such as hepatitis, liver cirrhosis, and ischemic-reperfusion, which we discuss in detail in a later section.

VIRAL HEPATITIS INFECTION

Exposure to hepatitis viruses, such as hepatitis virus B (HBV) and hepatitis virus C (HCV), can give rise to several outcomes: no obvious clinical infection, fulminant hepatitis, a self-limiting acute hepatitis, or a chronic infection that may progress to cirrhosis and hepatocellular carcinoma. Infection with the hepatitis virus is very common worldwide especially in Asia and Africa. The factors that determine the development of chronic viral hepatitis have not been fully identified. It is characterized by a parenchymal infiltration of activated cytotoxic T lymphocytes—the main cause of hepatic injury.

Antiviral activity of NO is well documented.[38,39] It has been shown to be able to inhibit the growth of DNA and RNA viruses, such as herpes simplex virus,[40] Coxsackie B3 virus,[41] and Japanese encephalitis virus.[42] The exact role of NO in hepatitis viral infection is not yet known. However, increased hepatocellular iNOS

expression could be part of a nonspecific host response to viral infection inhibiting viral replication. This possible antiviral effect of NO may partly explain the increase in viral titers observed when corticosteroids, which are also iNOS inhibitors, were used in patients with chronic viral hepatitis during the 1980s.[43] Recently, the ability of NO to inhibit the replication of HBV virus was demonstrated in the livers of NO-knockout mice.[44] This result may indicate a role for NO as an antiviral agent for hepatitis infection since NO may reduce the expression of viral antigens in the cells, thereby diminishing the severity of the immune-mediated liver disease.

Initial experimental evidences for a connection between hepatitis infection and NO came from several reports of Liu *et al.*[45–47] First, these authors demonstrated that woodchucks chronically infected with woodchuck hepatitis virus produce high nitrate and *N*-nitroso carcinogenic compounds.[45] Subsequently, they showed that hepatocytes, isolated from woodchucks that were chronic carriers of woodchuck hepatitis virus, formed twice as much nitrite as hepatocytes from noninfected animals.[46] Moreover, woodchuck hepatitis virus surface antigen alone was sufficient to induce high levels of NO in culture hepatocytes.[47]

In 1997, Kane *et al.*[48] showed that 60% of liver biopsies of HCV-positive patients expressed iNOS by RT-PCR. Using immunohistochemistry, 100% of the HCV-positive patients expressed iNOS compared to 12.5% of controls. p53 was not detected in either group, but there was upregulation of p21 over baseline expression in a number of the HCV-positive patients. Kane *et al.* concluded that chronic expression of NO in HCV hepatitis might play a role in DNA mutagenesis and the development of HCC. In the same year, Mihm *et al.*[48] also demonstrated, using quantitative, competitive RT-PCR, that iNOS mRNA expression was increased in the liver tissue from chronically HCV-infected patients. Moreover, it was positively correlated with interferon-γ (IFN-γ) expression, as well as hepatic HCV RNA content in these patients. In contrast, other researchers demonstrated unchanged or decreased NO in patients with chronic hepatitis infection.[49–51] However, in these studies, the researchers measured only the plasma nitrite/nitrate level as an index of NO generation. The methods employed by these researchers for the measurement of serum nitrite/nitrate might not be sensitive enough to detect small changes. Besides this, other possible explanations for the discrepancy include differences in the duration of disease, severity of inflammation, and the degree of fibrosis of various patient groups chosen.

Subsequently, other researchers have found other links between NO and chronic hepatitis viral infection. Majano *et al.*[52] demonstrated intense expression of iNOS in liver biopsies of patients with chronic viral hepatitis by immunohistochemistry and *in situ* hybridization. Immunohistochemistry localized iNOS protein only to hepatocytes whereas some mononuclear cell infiltrate and vascular endothelium were also positive for the mRNA by *in situ* hybridization. Thereafter, they demonstrated that transfection with either the HBV genome or HBV X gene into HepG2 cells resulted in induction of iNOS mRNA expression. Maximal induction of this transcript and NO production was observed in cytokine-stimulated HBV-transfected cells. Subsequently, by applying a non-radioactive *in situ* hybridization method, Schweyer *et al.*[53] also found increased IFN-γ and iNOS gene expression in liver biopsy specimens from patients with chronic HCV infection. However, in contrast, IFN-γ and iNOS mRNA were observed in CD3+ lymphocytes infiltrating portal tracts and

hepatic lobules, but not in hepatocytes. The differences might be due to the use of different hybridization probes as well as the hybridization conditions. More work needs to be done to confirm these results.

Recently, Garcia-Monzon *et al.*[54] demonstrated, by means of double immunostaining, that NO-mediated nitration of hepatocellular proteins is markedly induced in the inflamed liver tissue from patients with chronic viral hepatitis, and appears to be associated with the histological severity of viral chronic liver disease. The capability of HBV to increase the transcription of human hepatic iNOS by transactivating its promoter has also been studied. Amaro *et al.*[55] demonstrated that HBV X protein transactivates the iNOS promoter.

All these results demonstrated that NO was indeed increased during viral hepatitis. Increased production of NO during viral hepatitis has two implications. First, NO may serve as an antiviral agent that aids the host to fight against the viral infection. Second, when considered in the light of the known genotoxicity of NO,[56] long-term elevated production of NO free radicals in chronic hepatitis may directly cause reactions with cellular DNA leading to mutagenesis, as well as the formation of hepatocarcinogenic *N*-nitroso compounds. The well known relationship between viral hepatitis infection with increased risk of liver cancer may be the result of increased production of NO in addition to the antiapoptotic properties (apoptosis represents the paramount mechanism for eliminating virus-infected hepatocytes) of certain hepatitis viral proteins.[57]

LIVER CIRRHOSIS

Persistent hepatic injury leads to cirrhosis—a scarring process in the liver that includes both increased fibrogenesis and wound contraction. Cirrhotic patients often present with several systemic hemodynamic disturbances, characterized by symptoms such as hypotension, low systemic vascular resistance, and a reduced sensitivity to vasoconstrictors. During the progression of cirrhosis, vascular resistance continues to decrease and the low arterial pressure may lead to secondary disturbances in renal and hepatic blood flow, ascites, and portal hypertension.

Since Vallance and Moncada[58] first proposed that NO could be responsible for the hyperdynamic circulation in cirrhosis, there has been growing evidence that implicates role of NO in the pathophysiology of liver cirrhosis. According to their hypothesis, NO overproduction may be due to increased incidence of endotoxemia during liver cirrhosis, and endotoxaemia may induce NO overproduction directly or indirectly through cytokines. Their hypothesis was supported by data provided by other experiments: (1) NOS is induced in the endothelium and smooth muscle when vascular tissue is exposed to endotoxin or cytokines *in vitro*;[59,60] (2) infusion of endotoxin in humans leads to the gradual appearance of peripheral vasodilatation;[61,62] (3) inhibitors of NOS increases blood pressure of patients with septic shock;[63] and (4) high circulating levels of endotoxin are found in cirrhotic patients with or without clinical evidence of infection.[64,65]

Evidence supporting a role for NO has been obtained from various experimental models of cirrhosis.[66,67] For example, NO-dependent vasodilation is increased in the aortic rings of cirrhotic rats, and administration of NOS inhibitors to cirrhotic rats

increases systemic vascular resistance, thus modulating hyperdynamic circulation.[68] Moreover, the production of NO is observed to increase in cirrhotic patients by using various parameters. One of the earliest reports was that urinary cGMP levels are elevated in cirrhotic patients, directly correlating with the severity of hemodynamic changes.[69] Subsequently, a case report described an event where a cirrhotic patient with severe hypotension and resistance to other pressor agent demonstrated an elevation in blood pressure in response to methylene blue infusion.[70] Methylene blue blocks the action of NO through the inhibition of guanylate cyclase. This provides clinical evidence that NO is responsible, at least in part, for the hypotension associated with liver failure. Subsequently, the increased production of endogenous NO, as shown by the increased plasma/serum, urine, and ascitic fluid nitrate levels, was demonstrated in patients with liver cirrhosis.[51,71–74] These results suggest that long-lasting increased local production of NO may contribute to maintenance of splachnic vasodilatation and, thus, worsen the hyperkinetic state in these patients. These reports also found that the degree of liver damage severity correlates with high serum nitrate levels. In addition, significantly higher peripheral and hepatic vein NO levels are observed in cirrhotic patients by the measurement of NO-hemoglobin complexes using electron paramagnetic resonance spectroscopy.[75] It has also been demonstrated that NO output is increased in the air exhaled by patients with cirrhosis, especially by patients with decompensated cirrhosis.[76] The NO might be produced by the vascular and bronchial tissues in the lungs of these patients instead of directly from the liver. Nevertheless, increases in the production of endogenous NO correspond to the progress of liver cirrhosis. At the cellular level, PMNs and monocytes isolated from cirrhotic patients have greater iNOS activity compared to that of healthy subjects,[77] and NOS activity in peripheral neutrophils of cirrhotic patients increases with increasing severity of liver dysfunction.[78] To provide further evidence that NO does play a role in the pathologic events of cirrhosis, Ryan *et al.*[79] and Campillo *et al.* demonstrated that infusion of NOS inhibitor, L-NMMA, improved the responsiveness to noradrenaline in forearm arteries of cirrhotic patients.[79]

According to the hypothesis of Vallance and Moncada,[58] as well as the results demonstrated by various researchers, one would expect that iNOS would be the NOS isoform responsible for the sustained production of large amounts of NO during liver cirrhosis. However, there is contradictory evidence. Thus, the exact role of iNOS in liver cirrhosis and portal hypertension remains in doubt. For example, Fernandez *et al.*[81] could not show any significant increase in iNOS activity in BDL rats since dexamethasone failed to modify the hyperdynamic circulation in the cirrhotic animal model. In addition, it seems that an increase of eNOS activity could be responsible for NO overproduction in animal models of portal hypertension.[82] Campillo *et al.*[83] reported that NO might be a consequence, rather than a cause, of hemodynamic abnormalities and that anemia might play a key role in NO production of patients with cirrhosis. Renal impairment and diminished urinary nitrate clearance might be another cause of increased NO observed since the major metabolic pathway of serum nitrate is through urinary excretion.[84]

Generally, all these observations still support a detrimental role of NO in the pathogenesis of liver cirrhosis, where increased NO leads to the vascular hyperdynamic state, and reduction of NO is beneficial. However, other findings do not support the Vallance and Moncada hypothesis.[58] Studies have shown that NO

production was reduced in the cirrhotic rat liver.[85] In humans, Sarela *et al.*[86] demonstrated that the activity of constitutive NOS is substantially lower in the liver of cirrhotic patients as compared to the histologically normal liver from metastatic colorectal adenocarcinoma patients who underwent liver resection. The activity of iNOS was unaltered. Reduced hepatic cNOS activity, with a resultant decrease in NO release, is likely to facilitate the counteracting influence of the potent vasoconstricting agent, endothelin 1, especially in the cirrhotic liver, and thereby bring about an increase in the sinusoidal resistance. The cause of diminished cNOS may reflect a response to hepatocellular damage. Portal hypertension is a major complication of liver cirrhosis. An increase in the intrahepatic resistance to portal venous flow is an important factor in the development and maintenance of portal hypertension. A decrease of NO may, therefore, promote portal hypertension through an increase in the hepatic sinusoidal resistance. Overall, alternation of NO synthesis at various times and sites is detrimental for the host and aggravates the pathogenesis of liver cirrhosis.

HEPATOCELLULAR CARCINOMA (HCC)

HCC is one of the most common solid tumor malignancies affecting humans. It is responsible for about one million deaths per year worldwide. A major epidemiologic association between either HBV or HCV infections and HCC is evident.[87] However, the underlying mechanisms that lead to the development of HCC are still not clear. Although integration of virus DNA sequences into liver cell genome could activate cellular protooncogenes, this integration is very infrequent and does not explain the majority of virus-induced HCCs. Increased NO formation by liver cells has been shown to occur in many hepatic diseases, such as that caused by the parasites *Opisthorchis viverrini* (liver fluke),[88] by hepatitis viruses,[52,89] and in cirrhosis.[76,76] Increased NO could be the common underlying mechanism for the increased risk of liver cancer associated with these various forms of chronic liver diseases, where liver tissues that are exposed to high concentrations of NO over long periods of time could accumulate mutations.

In a woodchuck hepatitis virus model, chronic infection with hepatitis virus seems to enhance NO production and formation of carcinogenic nitrosamines.[45–47] Viral hepatitis might increase the risk of liver cancer through a mechanism of increased NO production. In man, NO levels are elevated in patients with chronic hepatitis and this has been linked with the predisposition to develop liver cancer.[90] Other data showed that plasma nitrite/nitrate concentrations in patients with HCC are correlated with tumor volume as well as tumor surface area.[50] Recently, it has been suggested that plasma nitrite/nitrate concentrations could be used as a tumor marker for HCC in conjunction with serum α-fetoprotein (AFP, a well established biochemical parameter for HCC).[91] In agreement with this study, Moussa *et al.*[51] also found that plasma nitrite/nitrate levels in patients with HCC are elevated. A possible explanation for increased plasma NO levels in HCC is that NO is reactively induced by the hepatic tissue surrounding HCC by three independent mechanisms: (1) tumor cells directly stimulate macrophages and Kupffer cells to produce NO, (2) HCC produces a variety of cytokines that may stimulate hepatocytes to produce NO, and

(3) a marked deterioration of liver function in HCC patients may be associated with increased portosystemic shunting and further development of hyperdynamic circulation, leading to an increase in NO production. In addition, preliminary work in our laboratory has demonstrated increased iNOS and eNOS mRNA expression by RT-PCR in the tumor tissue compared to the tissue surrounding the tumor of HCC patients (unpublished data). These may lead to the observed increased NO level.

The role of NO in cancer formation is controversial. It has mutagenic effects as well as known antitumor effects.[92] It is well known that high levels of NO can cause nitrosative deamination or oxidation of DNA bases, leading to DNA damage, as well as mutation in human cells.[56,93,94] In addition, as tumor growth progresses, NO may mediate capillary leakiness, support angiogenesis, and limit leukocyte infiltration. On the other hand, NO possesses antitumor properties because of its long known cytotoxic and cytostatic[95] effects toward tumor cells, as well as its anti-apoptotic role.[27]

Several specific types of mutations would be expected from DNA deamination, including GC to AT, GC to TA, and AT to GC base pair substitutions.[96] Sequence data on precore and core gene mutations in hepatitis B virus isolated from chronic HBV-infected individuals show GC to AT mutations.[97] Similar patterns of mutations of the p53 gene have been found in HCC.[98,99] These suggest that chronic inflammation and NO produced by NOS may mutate these genes through deamination of DNA bases. The p53 tumor suppressor is a 393-amino acid nuclear transcription factor that plays a central role in cell cycle regulation and apoptosis.[100] Inactivation of p53 function has been reported in approximately 50% of all human cancers, including malignancies of the liver. It has also been shown that NO is able to induce conformational changes of p53, decrease its specific DNA binding activity, and thus, increasing the risk of malignant cell transformation.[101,102]

Recently, Morbidelli *et al.*[103] and Ziche *et al.*[104] reported that NO plays a central role in the angiogenic cascade by demonstrating that vascular endothelial growth factor (VEGF), released by tumor cells, requires a functioning NO/cGMP pathway within the endothelial compartment to promote neovascular growth. Other research groups have found that NO induces VEGF expression in human Hep G2 hepatocarcinoma cells.[105] VEGF plays an important role in tumor biology in at least two ways: as a vascular permeability factor and/or endothelial growth factor. Elevated expression of VEGF has also been reported for tumors of the gastrointestinal tract,[106] kidney,[107] and breast[108] in humans. However, there are few reports on the expression of VEGF in HCC, which is well known for its hypervascularity.[109,110] The promotion of tumor growth by NO may involve the induction of angiogenic factors such as VEGF.

In contrast, there is also increasing evidence indicating that NO might also play an important role in antitumor mechanisms. It was demonstrated that Kupffer cell-derived NO suppresses proliferation and induces apoptosis of hepatoma cells.[111] These results indicate that in the event of HCC, Kupffer cells surrounding the tumor can induce apoptosis of the tumor cells via the production of NO.[112,113] In conjunction with the well known cytotoxic effects of NO, through its effect of nitrosating important proteins in tumor cells, the hepatoprotective role of NO in HCC cannot be ignored.

The effects of NO in various stages of cancer are widespread and often self contradictory. Its effects can be better understood based on timing, location, and concentration. Here, we hypothesize that NO plays an important role in HCC formation and its progression. During the pre-HCC period, viral infection (hepatitis B and C) or other unforeseen circumstances may lead to uncontrolled, prolonged, and/or massive production of NO by iNOS in the liver. Genotoxic properties of NO may lead to the mutation of certain oncogenes or tumor suppressor genes, such as p53, that allow the cells to escape from the cell cycle growth arrest mechanism and thus contribute to hepatic carcinogenesis. At the same time, NO regulates angiogenesis. NO released by the tumor cells might enhance angiogenesis, which can lead to accelerated growth of the primary tumor, as well as facilitate the process of metastasis. Angiogenic effects of NO may be due to an upregulation of proangiogenic factors and/or downregulation of their natural inhibitors (antiangiogenic factors). On the other hand, other functional cells surrounding the tumor might possess certain tumoristatic and tumoricidal effects on the tumor cells through the action of NO and, hence, provide hepatoprotective action.

LIVER TRANSPLANTATION AND HEPATIC ISCHEMIA-REPERFUSION INJURY

Orthotopic liver transplantation is now widely used for the treatment of end-stage liver disease. Cellular rejection is one of the common complications following liver transplantation and remains a significant cause of graft loss. The basis of cellular rejection is an alloantigen activation and proliferation of inflammatory cells that infiltrate the donor organ and mediate graft damage. Experimental work suggests that NO as a cytotoxic molecule may play an important role in the *in vivo* response to allogenic tissue.

Increased endogenous NO production, represented by the rise in systemic nitrate levels, have been described during clinical hepatic allograft rejection.[114–116] It was found that serum[114] and urinary[115] nitrate levels correlate well with the grade of allograft rejection in human liver transplantation. These results agree with the data reported during allograft rejection in animal models.[117,118] Hence, it was suggested that plasma and urinary nitrate levels could offer a sensitive predictor of acute allograft rejection in human liver transplantation, as well as its severity, and the evaluation of its resolution. Moreover, Sugioka *et al.*[119] found that changes in graft tissue NO preceded any other indicators of acute rejection, thus providing more concrete proof that NO is a mediator of acute rejection, and not a consequence. Corticosteroid (a well known iNOS inhibitor) treatment during the rejection episode leads to significant decline of the serum nitrate levels, hence, further confirms the involvement of NO in allograft rejection. Direct evidence was demonstrated by Romero *et al.*[120] using an immunohistochemical method. They found marked expression of iNOS in hepatocytes from liver biopsies of patients with acute rejection when compared with patients without rejection. Positive hepatocytes presented strong cytoplasmic staining, whereas no detectable iNOS reactivity was detected in any other cell types. After treatment with intravenous corticosteroids, iNOS reactivity decreased significantly.

This provides evidence that NO plays a role in clinical hepatic allograft rejection. However, it remains controversial whether NO generated during acute rejection is beneficial or harmful to the transplanted recipients. A beneficial role of NO was supported by the following findings during allograft rejection or other forms of liver injuries: (1) NO inhibits lymphocyte proliferation and acquisition of cytolytic T lymphocytes during alloimmune response;[121] (2) NO scavenges free oxygen radicals during hepatocyte injury;[122,123] and (3) blocking of iNOS activity impairs hepatic microvascular blood and increases tissue damage.[15,124] Hence, NO may be an important mediator of the graft-versus-host reaction and may serve as a marker of this process. On the other hand, a sudden burst of NO locally may produce damage to cells during rejection.[125,126] Nitrosylation of key enzymes involved in the respiratory cycle and the synthesis of DNA in target cells could mediate the cytotoxicty of NO. The differences in the effects of NO in rejection may be related to the amount of NO produced. Additional studies are warranted for an understanding of the exact function of NO in hepatic allograft rejection.

Liver ischemia-reperfusion is an important syndrome encountered in a number of clinical scenarios, such as trauma, hemorrhagic shock, liver resection, and liver transplantation. In particular, for liver transplantation, ischemia-reperfusion injury is an important cause of primary nonfunction of the liver leading to urgent retransplantation. The generation of reactive oxygen intermediates and inflammatory cytokines, as well as microcirculatory disturbances during the reperfusion phase, underlies the pathophysiology of this syndrome. In view of the capability of sinusoidal endothelial cells in producing a basal level of NO from eNOS in response to flow, and the increased concentration of oxygen free radicals and cytokines in inducing the expression of iNOS in other cell types of the liver,[127,128] the role of NO in hepatic ischemia-reperfusion has aroused tremendous research interest in the field.

NO maintains hepatic perfusion under physiologic conditions in normal rat livers, inhibition of NO synthesis causes a marked increase in perfusion pressure.[7] Administration of both non-selective and selective inhibitors of NOS into ischemic rat models results in marked aggravation of postischemic liver injury.[124,129,130] By increasing NO availability, using either an NO precursor or NO donors, reperfusion injury in animal models of hepatic ischemia-reperfusion was markedly reduced.[130–134] Hence, the liver dysfunction associated with reperfusion injury was suggested to be linked to a decrease in the release of NO. The beneficial role of NO during reperfusion is believed to be related to its ability to counteract the effects of endothelin,[135] reduced inflammatory cell activity, and expression of cytokines and adhesion molecules.[136,137]

Notwithstanding, some researchers have found that NO producing toxic injuries during reperfusion[138.139] may act as a lethal messenger in cell-mediated cytotoxicity. It has been shown that hypoxia also favors the transcription of iNOS through the engagement of hypoxia response elements present in the iNOS promoter.[140] Therefore, it is possible that ischemic episodes might contribute to iNOS expression and in this way account for the obvious sustained NO synthesis posttransplantation.

In conclusion, it appears that multiple factors, such as NO-superoxide radical ratios, hepatic stores of reduced glutathione, and length of ischemia, all determine whether NO will act as a cytoprotective or cytotoxic agent. The constitutive form of NOS predominates in acute conditions of ischemia releasing a small amount of NO that is important in the preservation of homeostasis, regulating microvascular

permeability and neutrophil–endothelial cell interaction, and maintaining local perfusion. In severe chronic ischemia, the high levels of NO produced by iNOS, may be cytotoxic. NO may combine with the high levels of superoxides to form peroxynitrite, which becomes extremely toxic to cells.

In liver transplant recipients, L-arginine seems to be extremely deficient after reperfusion since high plasma arginase (an enzyme that hydrolyzes arginine to urea and ornithine) levels were observed following liver perfusion.[141,142] In view of the protective role of NO in ischemic-reperfusion, endogenous NO augmentation may be more effective in attenuating hepatic ischemia reperfusion than any other approach. Furthermore, therapeutic strategies that increase endogenous NO may be useful in improving the outcome of liver transplantation involving the use of suboptimal grafts, which are known to be more susceptible to ischemic-reperfusion injury and poorer outcomes. However, we need to bear in mind that NO also exerts a strong hypotensive effect and has the ability to produce toxic peroxynitrite radicals after reacting with superoxide radicals. Thus, treatment with high doses of NO can be hazardous. In the event of ischemia reperfusion, a combination of NO donors and endothelin blockers might provide an alternative intervention strategy since inhibition of enthothelin in animal ischemic models seems to improve microcirculation and circumvented reperfusion injury of the liver.[143,144] In view of the availability of the selective hepatic NO donor—V-PYRRO/NO (O^2-vinyl 1-[pyrrolodin-1-yl]diazen-1-ium-1,2-diolate), which selectively delivers NO directly to the hepatocyte by virtue of cytochrome P450 metabolism,[23] we are getting closer to the use of this molecule in a clinical setting.

LIVER REGENERATION

In adult vertebrates, the capacity for regeneration is limited to a few tissues, one of which is the liver. In the event of surgical resection of diseased livers, the ability of the remnant liver to initiate the regeneration process is a very important part of the recovery process for the patients. The mechanisms that permit the tissues to regenerate are not well understood. Initiation of liver regeneration requires injury-related cytokines, such as TNF-α[145] and IL-6,[146] and it involves the activation of cytokine-regulated transcription factors, such as NF-$\kappa\beta$[147] and STAT3.[148] During regeneration, these cytokines promote hepatocyte viability, as well as proliferation.[149] These observations suggest that the cytokines induce hepatoprotective factors in the regenerating liver. iNOS is a well known cytokine-inducible enzyme; thus, the product, NO might be involved in one way or the other for the action of cytokines accumulated during tissue damage.

Among the multiple changes in the immediate hours following partial hepatectomy, an induction of iNOS and the release of NO have been reported in rats.[150–152] The role of NO in liver regeneration was further tested by estimating the amount of nitrite accumulated during 24 hours in the culture media of hepatocytes, Kupffer cells, and sinusoidal endothelial cells isolated at various times following partial hepatectomy.[153,154] The time course of NO production was compared with the course of the proliferating activity of the same cells. During the time interval when liver cells passed through their first cell cycles, hepatocytes were the main producers

of NO in the liver. The time-dependent changes of their NO production corresponded to those obtained with the whole liver and were inversely correlated with DNA-synthesizing activity. NO production by Kupffer and endothelial cells followed that by hepatocytes in this order; the time displacement between them corresponded to the schedule of their proliferating activity. Díaz-Guerra *et al.*[155] further confirmed the role of NO in partially hepatectomized mice and they postulated that NF-κβ was required for the transcriptional control of iNOS in the regenerating liver.

Subsequently, Wang and Lautt[156] hypothesized that the hemodynamic consequence of partial hepatectomy triggered the cascade of events that leads to liver regeneration. After partial hepatectomy, all the portal flow must go through the remaining vascular bed, thus producing increased shear stress and release of NO, which then initiates the next stages of the regeneration process. Their results in rat models demonstrated that the vascular shear stress-induced release of NO following partial hepatectomy serves as a primary trigger to initiate the regeneration process.

Recently, more significant results were demonstrated by Rai *et al.*[157] in iNOS knockout mice. These iNOS knockout animals exhibit a significant increase in hepatocyte apoptosis 24 hours after partial hepatectomy, indicating that NO is probably involved in preventing apoptosis and serves as an important hepatoprotective factor in the regenerating liver. With further advances in research, someday, NO or its analogues may be delivered directly to the liver to limit hepatic injury and facilitate liver cell regeneration.

CONCLUSIONS

The significance of NO in various diseases, whether it is a cause or merely a consequence, has not been fully determined. Depending on the experimental conditions, NO can attenuate or enhance certain pathophysiologic responses that reflect the complex behavior of this small molecule in the body. Most detrimental effects of NO are based on the concept that NO serves as a precursor, yielding potent toxic factors, such as peroxynitrite and hydroxyl radical, as a result of the interaction with the superoxide anion. The beneficial role of NO is normally based on its ability to maintain the integrity of microvascular function, its ability to inhibit platelet aggregation and neutrophil infiltration, as well as its unique ability to prevent apoptosis in hepatocytes and, hence, prevent further liver injury and promote liver regeneration.

Conflicting results may be due to concentration-dependent effects of NO, the variety of *in vivo* and *in vitro* experimental models and differences in cell sensitivity to NO. The effects of NO may also vary depending on acute or chronic inflammatory states and the animal species used in experiments. Posttranslational modifications may also modulate the amount of NO released. It should be borne in mind that studies evaluating mRNA or protein levels alone are insufficient for determination of true NOS-dependent NO production. This might also explain the contradictory results from various research groups, as they look at various regulation levels under different settings and at different times. A study that involves the direct measurement of NOS activity in the organ involved will give a clearer picture of what NO does in a particular pathologic event than those studies that only demonstrate the effects of NO donors or NOS inhibitors in the entire animal. Because NO has diverse

physiologic functions, the overall effects of the donors and inhibitors might not represent the specific action of NO in the particular organ involved. Drugs that can modulate NO levels have been used as therapeutic agents for a long time. Nitrovasodilators, such as nitroprusside, that act by donating NO spontaneously, or glyceryl trinitrite and isosorbide dinitrate, that release NO after metabolic conversions are able to activate guanylate cyclase and elevate cGMP in the vasculature, thus providing a favorable adjustment of the vasculature environment. In view of the existence of more specific NOS inhibitors, as well as cell-directed NO donors, the use of NO donors or NOS inhibitors in the treatment of liver diseases may soon become reality.

REFERENCES

1. MONCADA, S., R.M. PALMER & E.A. HIGGS. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. **43:** 109–142.
2. SCHMIDT, H.H., S.M. LOHMANN & U. WALTER. 1993. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. Biochim. Biophys. Acta **1178:** 153–175.
3. NATHAN, C. 1992. Nitric oxide as a secretory product of mammalian cells. FASEB J. **6:** 3051–3064.
4. BARTOSZ, G. 1996. Peroxynitrite: mediator of the toxic action of nitric oxide. Acta Biochim. Pol. **43:** 645–659.
5. DAVIES, M.G., G.J. FULTON & P.O. HAGEN. 1995. Clinical biology of nitric oxide. Br. J. Surg. **82:** 1598–1610.
6. MONCADA, S. & A. HIGGS. 1993. The L-arginine-nitric oxide pathway. N. Engl. J. Med. **329:** 2002–2012.
7. MITTAL, M.K., T.K. GUPTA, F.Y. LEE, *et al.* 1994. Nitric oxide modulates hepatic vascular tone in normal rat liver. Am. J. Physiol. **267:** G416–G422.
8. GELLER, D.A., S.M. DI, A.K. NUSSLER, *et al.* 1993. Nitric oxide synthase expression is induced in hepatocytes *in vivo* during hepatic inflammation. J. Surg. Res. **55:** 427–432.
9. GELLER, D.A., P.D. FREESWICK, D. NGUYEN, *et al.* 1994. Differential induction of nitric oxide synthase in hepatocytes during endotoxemia and the acute-phase response. Arch. Surg. **129:** 165–171.
10. HARBRECHT, B.G., E.A. MCCLURE, R.L. SIMMONS, *et al.* 1995. Prostanoids inhibit Kupffer cell nitric oxide synthesis. J. Surg. Res. **58:** 625–629.
11. ROCKEY, D.C. & J.J. CHUNG. 1996. Regulation of inducible nitric oxide synthase in hepatic sinusoidal endothelial cells. Am. J. Physiol. **271:** G260–G267.
12. HELYAR, L., D.S. BUNDSCHUH, J.D. LASKIN, *et al.* 1994. Induction of hepatic Ito cell nitric oxide production after acute endotoxemia. Hepatology **20:** 1509–1515.
13. KITADE, H., K. SAKITANI, K. INOUE, *et al.* 1996. Interleukin 1β markedly stimulates nitric oxide formation in the absence of other cytokines or lipopolysaccharide in primary cultured rat hepatocytes but not in Kupffer cells. Hepatology **23:** 797–802.
14. KAPLOWITZ, N. 2000. Cell death at the millennium. Implications for liver diseases. Clin. Liver Dis. **4:** 1–23.
15. HARBRECHT, B.G., T.R. BILLIAR, J. STADLER, *et al.* 1992. Nitric oxide synthesis serves to reduce hepatic damage during acute murine endotoxemia. Crit. Care Med. **20:** 1568–1574.
16. SMETS, D., H. SPAPEN, M. DILTOER, *et al.* 1999. Liver perfusion and hepatocellular inflammatory response in sepsis. Acta Clin. Belg. **54:** 201–206.
17. VOS, T.A., A.S. GOUW, P.A. KLOK, *et al.* 1997. Differential effects of nitric oxide synthase inhibitors on endotoxin-induced liver damage in rats. Gastroenterology **113:** 1323–1333.

18. HARBRECHT, B.G., T.R. BILLIAR, J. STADLER, *et al.* 1992. Inhibition of nitric oxide synthesis during endotoxemia promotes intrahepatic thrombosis and an oxygen radical-mediated hepatic injury. J. Leukocyte Biol. **52:** 390–394.
19. HARBRECHT, B.G., B. WU, S.C. WATKINS, *et al.* 1995. Inhibition of nitric oxide synthase during hemorrhagic shock increases hepatic injury. Shock **4:** 332–337.
20. TAKEMURA, S., Y. MINAMIYAMA, M. INOUE, *et al.* 2000. Nitric oxide synthase inhibitor increases hepatic injury with formation of oxidative DNA damage and microcirculatory disturbance in endotoxemic rats. Hepatogastroenterology **47:** 1364–1370.
21. ZHU, W. & P.C. FUNG. 2000. The roles played by crucial free radicals like lipid free radicals, nitric oxide, and enzymes NOS and NADPH in CCl_4-induced acute liver injury of mice. Free Radical Biol. Med. **29:** 870–880.
22. KIM, Y.M., M.E. DE VERA, S.C. WATKINS, *et al.* 1997. Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor-alpha-induced apoptosis by inducing heat shock protein 70 expression. J. Biol. Chem. **272:** 1402–1411.
23. SAAVEDRA, J.E., T.R. BILLIAR, D.L. WILLIAMS, *et al.* 1997. Targeting nitric oxide (NO) delivery in vivo. Design of a liver-selective NO donor prodrug that blocks tumor necrosis factor-alpha-induced apoptosis and toxicity in the liver. J. Med. Chem. **40:** 1947–1954.
24. ALBINA, J.E., S. CUI, R.B. MATEO, *et al.* 1993. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. J. Immunol. **150:** 5080–5085.
25. FEHSEL, K., K.D. KRONCKE, K.L. MEYER, *et al.* 1995. Nitric oxide induces apoptosis in mouse thymocytes. J. Immunol. **155:** 2858–2865.
26. MCDANIEL, M.L., J.A. CORBETT, G. KWON, *et al.* 1997. A role for nitric oxide and other inflammatory mediators in cytokine-induced pancreatic beta-cell dysfunction and destruction. Adv. Exp. Med. Biol. **426:** 313–319.
27. LI, J. & T.R. BILLIAR. 1999. The anti-apoptotic actions of nitric oxide in hepatocytes. Cell Death Differ. **6:** 952–955.
28. BOHLINGER, I., M. LEIST, J. BARSIG, *et al.* 1995. Interleukin-1 and nitric oxide protect against tumor necrosis factor alpha-induced liver injury through distinct pathways. Hepatology **22:** 829–1837.
29. OU, J., T.M. CARLOS, S.C. WATKINS, *et al.* 1997. Differential effects of nonselective nitric oxide synthase (NOS) and selective inducible NOS inhibition on hepatic necrosis, apoptosis, ICAM-1 expression, and neutrophil accumulation during endotoxemia. Nitric Oxide **1:** 404–416.
30. KIM, Y.M., R.V. TALANIAN & T.R. BILLIAR. 1997. Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. J. Biol. Chem. **272:** 31138–31148.
31. FARINELLI, S.E., D.S. PARK & L.A. GREENE. 1996. Nitric oxide delays the death of trophic factor-deprived PC12 cells and sympathetic neurons by a cGMP-mediated mechanism. J. Neurosci. **16:** 2325–2334.
32. MOJENA, M., S. HORTELANO, A. CASTRILLO, *et al.* 2001. Protection by nitric oxide against liver inflammatory injury in animals carrying a nitric oxide synthase-2 transgene. FASEB J. **15:** 583–585.
33. MORIO, L.A., H. CHIU, K.A. SPROWLES, *et al.* 2001. Distinct roles of tumor necrosis factor-alpha and nitric oxide in acute liver injury induced by carbon tetrachloride in mice. Toxicol. Appl. Pharmacol. **172:** 44–51.
34. AOKI, K., M. OHMORI, M. TAKIMOTO, *et al.* 1997. Cocaine-induced liver injury in mice is mediated by nitric oxide and reactive oxygen species. Eur. J. Pharmacol. **336:** 43–49.
35. DIEZ-FERNANDEZ, C., N. SANZ, A.M. ALVAREZ, *et al.* 1998. Influence of aminoguanidine on parameters of liver injury and regeneration induced in rats by a necrogenic dose of thioacetamide. Br. J. Pharmacol. **125:** 102–108.
36. GARDNER, C.R., D.E. HECK, C.S. YANG, *et al.* 1998. Role of nitric oxide in acetaminophen-induced hepatotoxicity in the rat. Hepatology **27:** 748–754.
37. SASS, G., K. KOERBER, R. BANG, *et al.* 2001. Inducible nitric oxide synthase is critical for immune-mediated liver injury in mice. J. Clin. Invest. **107:** 439–447.
38. MANNICK, J.B. 1995. The antiviral role of nitric oxide. Res. Immunol. **146:** 693–697.
39. POWELL, K.L. & S.A. BAYLIS. 1995. The antiviral effects of nitric oxide. Trends Microbiol. **3:** 81–82.

40. CROEN, K.D. 1993. Evidence for antiviral effect of nitric oxide. Inhibition of herpes simplex virus type 1 replication. J. Clin. Invest. **91:** 2446–2452.
41. LOWENSTEIN, C.J., S.L. HILL, A. LAFOND-WALKER, *et al.* 1996. Nitric oxide inhibits viral replication in murine myocarditis. J. Clin. Invest. **97:** 1837–1843.
42. LIN, Y.L., Y.L. HUANG, S.H. MA, *et al.* 1997. Inhibition of Japanese encephalitis virus infection by nitric oxide: antiviral effect of nitric oxide on RNA virus replication. J. Virol. **71:** 5227–5235.
43. SCULLARD, G.H., C.I. SMITH, T.C. MERIGAN, *et al.* 1981. Effects of immunosuppressive therapy on viral markers in chronic active hepatitis B. Gastroenterology **81:** 987–991.
44. GUIDOTTI, L.G., H. MCCLARY, J.M. LOUDIS, *et al.* 2000. Nitric oxide inhibits hepatitis B virus replication in the livers of transgenic mice. J. Exp. Med. **191:** 1247–1252.
45. LIU, R.H., B. BALDWIN, B.C. TENNANT, *et al.* 1991. Elevated formation of nitrate and *N*-nitrosodimethylamine in woodchucks (*Marmota monax*) associated with chronic woodchuck hepatitis virus infection. Cancer Res. **51:** 3925–3929.
46. LIU, R.H., J.R. JACOB, B.C. TENNANT, *et al.* 1992. Nitrite and nitrosamine synthesis by hepatocytes isolated from normal woodchucks (*Marmota monax*) and woodchucks chronically infected with woodchuck hepatitis virus. Cancer Res. **52:** 4139–4143.
47. LIU, R.H., J.R. JACOB, J.H. HOTCHKISS, *et al.* 1994. Woodchuck hepatitis virus surface antigen induces nitric oxide synthesis in hepatocytes: possible role in hepatocarcinogenesis. Carcinogenesis **15:** 2875–2877.
48. MIHM, S., A. FAYYAZI & G. RAMADORI. 1997. Hepatic expression of inducible nitric oxide synthase transcripts in chronic hepatitis C virus infection: relation to hepatic viral load and liver injury. Hepatology **26:** 451–458.
49. AMARO, M.J., J. BARTOLOME, M. PARDO, *et al.* 1997. Decreased nitric oxide production in chronic viral hepatitis B and C. J. Med. Virol. **51:** 326–331.
50. MORIYAMA, A., A. MASUMOTO, H. NANRI, *et al.* 1997. High plasma concentrations of nitrite/nitrate in patients with hepatocellular carcinoma. Am. J. Gastroenterol. **92:** 1520–1523.
51. MOUSSA, Y.I., J.N. PLEVRIS & P.C. HAYES. 2000. Plasma nitrites/nitrates in HCV infection and hepatocellular carcinoma. Eur. J. Gastroenterol. Hepatol. **12:** 159–163.
52. MAJANO, P.L., C. GARCIA-MONZON, M. LOPEZ-CABRERA, *et al.* 1998. Inducible nitric oxide synthase expression in chronic viral hepatitis. Evidence for a virus-induced gene upregulation. J. Clin. Invest. **101:** 1343–1352.
53. SCHWEYER, S., S. MIHM, H.J. RADZUN, *et al.* 2000. Liver infiltrating T lymphocytes express interferon gamma and inducible nitric oxide synthase in chronic hepatitis C virus infection. Gut **46:** 255–259.
54. GARCIA-MONZON, C., P.L. MAJANO, I. ZUBIA, *et al.* 2000. Intrahepatic accumulation of nitrotyrosine in chronic viral hepatitis is associated with histological severity of liver disease. J. Hepatol. **32:** 331–338.
55. AMARO, M.J., J. BARTOLOME & V. CARRENO. 1999. Hepatitis B virus X protein transactivates the inducible nitric oxide synthase promoter. Hepatology **29:** 915–923.
56. BURNEY, S., J.L. CAULFIELD, J.C. NILES, *et al.* 1999. The chemistry of DNA damage from nitric oxide and peroxynitrite. Mutat. Res. **424:** 37–49.
57. PAN, J., L.X. DUAN, B.S. SUN, *et al.* 2001. Hepatitis B virus X protein protects against anti-Fas-mediated apoptosis in human liver cells by inducing NF-κ B. J. Gen. Virol. **82:** 171–182.
58. VALLANCE, P. & S. MONCADA. 1991. Hyperdynamic circulation in cirrhosis: a role for nitric oxide? Lancet **337:** 776–778.
59. BUSSE, R. & A. MULSCH. 1990. Induction of nitric oxide synthase by cytokines in vascular smooth muscle cells. FEBS Lett. **275:** 87–90.
60. REES, D.D., S. CELLEK, R.M. PALMER, *et al.* 1990. Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone: an insight into endotoxin shock. Biochem. Biophys. Res. Commun. **173:** 541–547.
61. FONG, Y.M., M.A. MARANO, L.L. MOLDAWER, *et al.* 1990. The acute splanchnic and peripheral tissue metabolic response to endotoxin in humans. J. Clin. Invest. **85:** 1896–1904.

62. SUFFREDINI, A.F., R.E. FROMM, M.M. PARKER, *et al.* 1989. The cardiovascular response of normal humans to the administration of endotoxin. N. Engl. J. Med. **321:** 280–287.
63. PETROS, A., G. LAMB, A. LEONE, *et al.* 1994. Effects of a nitric oxide synthase inhibitor in humans with septic shock. Cardiovasc. Res. **28:** 34–39.
64. LUMSDEN, A.B., J.M. HENDERSON & M.H. KUTNER. 1988. Endotoxin levels measured by a chromogenic assay in portal, hepatic and peripheral venous blood in patients with cirrhosis. Hepatology **8:** 232–236.
65. YOKOTA, M., J. KAMBAYASHI, T. TANAKA, *et al.* 1989. A simple turbidimetric time assay of the endotoxin in plasma. J. Biochem. Biophys. Meth. **18:** 97–104.
66. SIEBER, C.C., J.C. LOPEZ-TALAVERA & R.J. GROSZMANN. 1993. Role of nitric oxide in the in vitro splanchnic vascular hyporeactivity in ascitic cirrhotic rats. Gastroenterology **104:** 1750–1754.
67. PIZCUETA, P., J.M. PIQUE, M. FERNANDEZ, *et al.* 1992. Modulation of the hyperdynamic circulation of cirrhotic rats by nitric oxide inhibition. Gastroenterology **103:** 1909–1915.
68. CLARIA, J., W. JIMENEZ, J. ROS, *et al.* 1994. Increased nitric oxide-dependent vasorelaxation in aortic rings of cirrhotic rats with ascites. Hepatology **20:** 1615–1621.
69. MIYASE, S., S. FUJIYAMA, H. CHIKAZAWA, *et al.* 1990. Atrial natriuretic peptide in liver cirrhosis with mild ascites. Gastroenterol. Jpn. **25:** 356–362.
70. MIDGLEY, S., I.S. GRANT, W.G. HAYNES, *et al.* 1991. Nitric oxide in liver failure. Lancet **338:** 1590.
71. GUARNER, C., G. SORIANO, A. TOMAS, *et al.* 1993. Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. Hepatology **18:** 1139–1143.
72. HORI, N., T. OKANOUE, T. MORI, *et al.* 1996. Endogenous nitric oxide production is augmented as the severity advances in patients with liver cirrhosis. Clin. Exp. Pharmacol. Physiol. **23:** 30–35.
73. BORIES, P.N., B. CAMPILLO, L. AZAOU, *et al.* 1997. Long-lasting NO overproduction in cirrhotic patients with spontaneous bacterial peritonitis. Hepatology **25:** 1328–1333.
74. COSKUN, U., S. OZENIRLER, B. SANCAK, *et al.* 2001. Serum and ascitic fluid nitrate levels in patients with cirrhosis. Clin. Chim. Acta **306:** 127–132.
75. BATTISTA, S., F. BAR, G. MENGOZZI, *et al.* 1997. Hyperdynamic circulation in patients with cirrhosis: direct measurement of nitric oxide levels in hepatic and portal veins. J. Hepatol. **26:** 75–80.
76. MATSUMOTO, A., K. OGURA, Y. HIRATA, *et al.* 1995. Increased nitric oxide in the exhaled air of patients with decompensated liver cirrhosis. Ann. Intern. Med. **123:** 110–113.
77. LAFFI, G., M. FOSCHI, E. MASINI, *et al.* 1995. Increased production of nitric oxide by neutrophils and monocytes from cirrhotic patients with ascites and hyperdynamic circulation. Hepatology **22:** 1666–1673.
78. GALLEY, H.F., D. COOMANSINGH, N.R. WEBSTER, *et al.* 1998. Nitric oxide synthase activity is increased in relation to the severity of liver dysfunction. Clin. Sci. **95:** 355–359.
79. RYAN, J., G. JENNINGS, F. DUDLEY, *et al.* 1996. Smooth muscle-derived nitric oxide is elevated in isolated forearm veins in human alcoholic cirrhosis. Clin. Sci. **91:** 23–28.
80. CAMPILLO, B., P.E. CHABRIER, G. PELLE, *et al.* 1995. Inhibition of nitric oxide synthesis in the forearm arterial bed of patients with advanced cirrhosis. Hepatology **22:** 1423–1429.
81. FERNANDEZ, M., J.C. GARCIA-PAGAN, M. CASADEVALL, *et al.* 1995. Evidence against a role for inducible nitric oxide synthase in the hyperdynamic circulation of portal-hypertensive rats. Gastroenterology **108:** 1487–1495.
82. GADANO, A.C., P. SOGNI, J. HELLER, *et al.* 1999. Vascular nitric oxide production during the development of two experimental models of portal hypertension. J. Hepatol. **30:** 896–903.
83. CAMPILLO, B., P.N. BORIES, C. BENVENUTI, *et al.* 1996. Serum and urinary nitrate levels in liver cirrhosis: endotoxemia, renal function and hyperdynamic circulation. J. Hepatol. **25:** 707–714.

84. WENNMALM, A., G. BENTHIN, A. EDLUND, *et al.* 1993. Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. Circ. Res. **73:** 1121–1127.
85. ROCKEY, D.C. & J.J. CHUNG. 1998. Reduced nitric oxide production by endothelial cells in cirrhotic rat liver: endothelial dysfunction in portal hypertension. Gastroenterology **114:** 344–351.
86. SARELA, A.I., F.M. MIHAIMEED, J.J. BATTEN, *et al.* 1999. Hepatic and splanchnic nitric oxide activity in patients with cirrhosis. Gut **44:** 749–753.
87. SCHAFER, D.F. & M.F. SORRELL. 1999. Hepatocellular carcinoma. Lancet **353:** 1253–1257.
88. OHSHIMA, H., T.Y. BANDALETOVA, I. BROUET, *et al.* 1994. Increased nitrosamine and nitrate biosynthesis mediated by nitric oxide synthase induced in hamsters infected with liver fluke (*Opisthorchis viverrini*). Carcinogenesis **15:** 271–275.
89. KANE, J.M., L.L. SHEARS, C. HIERHOLZER, *et al.* 1997. Chronic hepatitis C virus infection in humans: induction of hepatic nitric oxide synthase and proposed mechanisms for carcinogenesis. J. Surg. Res. **69:** 321–324.
90. OHSHIMA, H. & H. BARTSCH. 1994. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. Mutat. Res. **305:** 253–264.
91. MORIYAMA, A., A. TABARU, H. UNOKI, *et al.* 2000. Plasma nitrite/nitrate concentrations as a tumor marker for hepatocellular carcinoma. Clin. Chim. Acta **296:** 181–191.
92. WINK, D.A., Y. VODOVOTZ, J. LAVAL, *et al.* 1998. The multifaceted roles of nitric oxide in cancer. Carcinogenesis **19:** 711–721.
93. NGUYEN, T., D. BRUNSON, C.L. CRESPI, *et al.* 1992. DNA damage and mutation in human cells exposed to nitric oxide *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **89:** 3030–3034.
94. WINK, D.A., K.S. KASPRZAK, C.M. MARAGOS, *et al.* 1991. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. Science **254:** 1001–1003.
95. HIBBS, J.B., JR., R.R. TAINTOR, Z. VAVRIN, *et al.* 1988. Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem. Biophys. Res. Commun. **157:** 87–94.
96. FELLEY-BOSCO, E. 1998. Role of nitric oxide in genotoxicity: implication for carcinogenesis. Cancer Metastasis Rev. **17:** 25–37.
97. CHUANG, W.L., M. OMATA, T. EHATA, *et al.* 1993. Precore mutations and core clustering mutations in chronic hepatitis B virus infection. Gastroenterology **104:** 263–271.
98. HOLLSTEIN, M., D. SIDRANSKY, B. VOGELSTEIN, *et al.* 1991. p53 mutations in human cancers. Science **253:** 49–53.
99. SHI, C.Y., T.W. PHANG, A. WEE, *et al.* 1995. Mutations of the tumour suppressor gene p53 in colorectal and hepatocellular carcinomas. Ann. Acad. Med. Singapore **24:** 204–210.
100. LEVINE, A.J., J. MOMAND & C.A. FINLAY. 1991. The p53 tumour suppressor gene. Nature **351:** 453–456.
101. CALMELS, S., P. HAINAUT & H. OHSHIMA. 1997. Nitric oxide induces conformational and functional modifications of wild-type p53 tumor suppressor protein. Cancer Res. **57:** 3365–3369.
102. FORRESTER, K., S. AMBS, S.E. LUPOLD, *et al.* 1996. Nitric oxide-induced p53 accumulation and regulation of inducible nitric oxide synthase expression by wild-type p53. Proc. Natl. Acad. Sci. U.S.A. **93:** 2442–2447.
103. MORBIDELLI, L., C.H. CHANG, J.G. DOUGLAS, *et al.* 1996. Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. Am. J. Physiol. **270:** H411–H415.
104. ZICHE, M., L. MORBIDELLI, R. CHOUDHURI, *et al.* 1997. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. J. Clin. Invest. **99:** 2625–2634.
105. CHIN, K., Y. KURASHIMA, T. OGURA, *et al.* 1997. Induction of vascular endothelial growth factor by nitric oxide in human glioblastoma and hepatocellular carcinoma cells. Oncogene **15:** 437–442.

106. BROWN, L.F., B. BERSE, R.W. JACKMAN, *et al.* 1993. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. Cancer Res. **53:** 4727–4735.
107. BROWN, L.F., B. BERSE, R.W. JACKMAN, *et al.* 1993. Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. Am. J. Pathol. **143:** 1255–1262.
108. BROWN, L.F., B. BERSE, R.W. JACKMAN, *et al.* 1995. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer. Hum. Pathol. **26:** 86–91.
109. MISE, M., S. ARII, H. HIGASHITUJI, *et al.* 1996. Clinical significance of vascular endothelial growth factor and basic fibroblast growth factor gene expression in liver tumor. Hepatology **23:** 455–464.
110. SUZUKI, K., N. HAYASHI, Y. MIYAMOTO, *et al.* 1996. Expression of vascular permeability factor/vascular endothelial growth factor in human hepatocellular carcinoma. Cancer Res. **56:** 3004–3009.
111. KUROSE, I., H. HIGUCHI, Y. YONEI, *et al.* 1996. Rat Kupffer cell-derived nitric oxide suppresses proliferation and induces apoptosis of syngeneic hepatoma cells. Gastroenterology **111:** 1058–1070.
112. SAITO, H., I. KUROSE, H. EBINUMA, *et al.* 1996. Kupffer cell-mediated cytotoxicity against hepatoma cells occurs through production of nitric oxide and adhesion via ICAM-1/CD18. Int. Immunol. **8:** 1165-1172.
113. FUKUMURA, D., Y. YONEI, I. KUROSE, *et al.* 1996. Role in nitric oxide in Kupffer cell-mediated hepatoma cell cytotoxicity *in vitro* and *ex vivo.* Hepatology **24:** 141–149.
114. DEVLIN, J., R.M. PALMER, C.E. GONDE, *et al.* 1994. Nitric oxide generation. A predictive parameter of acute allograft rejection. Transplantation **58:** 592–595.
115. UCHIYAMA, H., K. YANAGA, K. KISHIHARA, *et al.* 2000. Increased urinary nitrate excretion associated with hepatic allograft rejection in experimental rat models and clinical cases. Transpl. Int. **13:** 260–265.
116. FABREGA, E., F. CASAFONT, J. DE LA PENA, *et al.* 1997. Nitric oxide production in hepatic cell rejection of liver transplant patients. Transplant. Proc. **29:** 505–506.
117. OHDAN, H., Y. FUKUDA, S. SUZUKI, *et al.* 1995. Simultaneous evaluation of nitric oxide synthesis and tissue oxygenation in rat liver allograft rejection using near-infrared spectroscopy. Transplantation **60:** 530–535.
118. KUO, P.C., E.J. ALFREY, K.Y. ABE, *et al.* 1996. Cellular localization and effect of nitric oxide synthesis in a rat model of orthotopic liver transplantation. Transplantation **61:** 305–312.
119. SUGIOKA, A., M. MORITA, T. ESAKI, *et al.* 1999. Kinetics and significance of graft tissue nitric oxide after rat and mouse liver transplantation. Transplant. Proc. **31:** 860–862.
120. ROMERO, M., C. GARCIA-MONZON, G. CLEMENTE, *et al.* 2001. Intrahepatic expression of inducible nitric oxide synthase in acute liver allograft rejection: evidence of modulation by corticosteroids. Liver Transpl. **7:** 16–21.
121. LANGREHR, J.M., R.A. HOFFMAN, T.R. BILLIAR, *et al.* 1991. Nitric oxide synthesis in the *in vivo* allograft response: a possible regulatory mechanism. Surgery **110:** 335–342.
122. KUO, P.C. & A. SLIVKA. 1994. Nitric oxide decreases oxidant-mediated hepatocyte injury. J. Surg. Res. **56:** 594–600.
123. KOKEN, T. & M. INAL. 1999. The effect of nitric oxide on ischemia-reperfusion injury in rat liver. Clin. Chim. Acta **288:** 55–62.
124. KOEPPEL, T.A., J.C. THIES, P. SCHEMMER, *et al.* 1997. Inhibition of nitric oxide synthesis in ischemia/reperfusion of the rat liver is followed by impairment of hepatic microvascular blood flow. J. Hepatol. **27:** 163–169.
125. AKIZUKI, E., T. AKAIKE, S. OKAMOTO, *et al.* 2000. Role of nitric oxide and superoxide in acute cardiac allograft rejection in rats. Proc. Soc. Exp. Biol. Med. **225:** 151–159.
126. ROZA, A.M., M. COOPER, G. PIEPER, *et al.* 2000. NOX 100, a nitric oxide scavenger, enhances cardiac allograft survival and promotes long-term graft acceptance. Transplantation **69:** 227–231.

127. KUO, P.C., K. ABE & R.A. SCHROEDER. 2000. Superoxide enhances interleukin 1β-mediated transcription of the hepatocyte-inducible nitric oxide synthase gene. Gastroenterology **118:** 608–618.
128. SHAH, V., F.G. HADDAD, G. GARCIA-CARDENA, *et al.* 1997. Liver sinusoidal endothelial cells are responsible for nitric oxide modulation of resistance in the hepatic sinusoids. J. Clin. Invest. **100:** 2923–2930.
129. WANG, Y., W.R. MATHEWS, D.M. GUIDO, *et al.* 1995. Inhibition of nitric oxide synthesis aggravates reperfusion injury after hepatic ischemia and endotoxemia. Shock **4:** 282–288.
130. COTTART, C.H., L. DO, M.C. BLANC, *et al.* 1999. Hepatoprotective effect of endogenous nitric oxide during ischemia-reperfusion in the rat. Hepatology **29:** 809–813.
131. KOBAYASHI, H., T. NONAMI, T. KUROKAWA, *et al.* 1995. Role of endogenous nitric oxide in ischemia-reperfusion injury in rat liver. J. Surg. Res. **59:** 772–779.
132. OHMORI, H., D.K. DHAR, Y. NAKASHIMA, *et al.* 1998. Beneficial effects of FK409, a novel nitric oxide donor, on reperfusion injury of rat liver. Transplantation **66:** 579–585.
133. SHIMAMURA, T., Y. ZHU, S. ZHANG, *et al.* 1999. Protective role of nitric oxide in ischemia and reperfusion injury of the liver. J. Am. Coll. Surg. **188:** 43–52.
134. RICCIARDI, R., D.P. FOLEY, S.H. QUARFORDT, *et al.* 2001. V-PYRRO/NO: an hepatoselective nitric oxide donor improves porcine liver hemodynamics and function after ischemia reperfusion. Transplantation **71:** 193–198.
135. PANNEN, B.H., F. AL ADILI, M. BAUER, *et al.* 1998. Role of endothelins and nitric oxide in hepatic reperfusion injury in the rat. Hepatology **27:** 755–764.
136. LIU, P., B. XU, C.E. HOCK, *et al.* 1998. NO modulates P-selectin and ICAM-1 mRNA expression and hemodynamic alterations in hepatic I/R. Am. J. Physiol. **275:** H2191–H2198.
137. DE CATERINA, R., P. LIBBY, H.B. PENG, *et al.* 1995. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. J. Clin. Invest. **96:** 60–68.
138. MA, T.T., H. ISCHIROPOULOS & C.A. BRASS. 1995. Endotoxin-stimulated nitric oxide production increases injury and reduces rat liver chemiluminescence during reperfusion. Gastroenterology **108:** 463–469.
139. ISOBE, M., T. KATSURAMAKI, K. HIRATA, *et al.* 1999. Beneficial effects of inducible nitric oxide synthase inhibitor on reperfusion injury in the pig liver. Transplantation **68:** 803–813.
140. MELILLO, G., L.S. TAYLOR, A. BROOKS, *et al.* 1997. Functional requirement of the hypoxia-responsive element in the activation of the inducible nitric oxide synthase promoter by the iron chelator desferrioxamine. J. Biol. Chem. **272:** 12236–12243.
141. IKEMOTO, M., S. TSUNEKAWA, K. TANAKA, *et al.* 1998. Liver-type arginase in serum during and after liver transplantation: a novel index in monitoring conditions of the liver graft and its clinical significance. Clin. Chim. Acta **271:** 11–23.
142. LANGLE, F., E. ROTH, R. STEININGER, *et al.* 1995. Arginase release following liver reperfusion. Evidence of hemodynamic action of arginase infusions. Transplantation **59:** 1542–1549.
143. GOTO, M., Y. TAKEI, S. KAWANO, *et al.* 1994. Endothelin-1 is involved in the pathogenesis of ischemia/reperfusion liver injury by hepatic microcirculatory disturbances. Hepatology **19:** 675–681.
144. DHAR, D.K., A. YAMANOI, H. OHMORI, *et al.* 1998. Modulation of endothelin and nitric oxide: a rational approach to improve canine hepatic microcirculation. Hepatology **28:** 782–788.
145. YAMADA, Y., I. KIRILLOVA, J.J. PESCHON, *et al.* 1997. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc. Natl. Acad. Sci. U.S.A. **94:** 1441–1446.
146. CRESSMAN, D.E., L.E. GREENBAUM, R.A. DEANGELIS, *et al.* 1996. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science **274:** 1379–1383.

147. CRESSMAN, D.E., L.E. GREENBAUM, B.A. HABER, *et al.* 1994. Rapid activation of post-hepatectomy factor/nuclear factor kappa B in hepatocytes, a primary response in the regenerating liver. J. Biol. Chem. **269:** 30429–30435.
148. CRESSMAN, D.E., R.H. DIAMOND & R. TAUB. 1995. Rapid activation of the Stat3 transcription complex in liver regeneration. Hepatology **21:** 1443–1449.
149. FAUSTO, N. 2000. Liver regeneration. J. Hepatol. **32:** 19–31.
150. OBOLENSKAYA, M.Y., A.F. VANIN, P.I. MORDVINTCEV, *et al.* 1994. Epr evidence of nitric oxide production by the regenerating rat liver. Biochem. Biophys. Res. Commun. **202:** 571–576.
151. HORTELANO, S., B. DEWEZ, A.M. GENARO, *et al.* 1995. Nitric oxide is released in regenerating liver after partial hepatectomy. Hepatology **21:** 776–786.
152. CARNOVALE, C.E., C. SCAPINI, M.L. ALVAREZ, *et al.* 2000. Nitric oxide release and enhancement of lipid peroxidation in regenerating rat liver. J. Hepatol. **32:** 798–804.
153. OBOLENSKAYA, M., A. SCHULZE-SPECKING, B. PLAUMANN, *et al.* 1994. Nitric oxide production by cells isolated from regenerating rat liver. Biochem. Biophys. Res. Commun. **204:** 1305–1311.
154. DECKER, K.F. & M.Y. OBOLENSKAYA. 1995. Cytokines, nitric oxide synthesis and liver regeneration. J. Gastroenterol. Hepatol. **10**(Suppl. 1): S12–S17.
155. DIAZ-GUERRA, M.J., M. VELASCO, P. MARTIN-SANZ, *et al.* 1997. Nuclear factor κB is required for the transcriptional control of type II NO synthase in regenerating liver. Biochem. J. **326**(3): 791–797.
156. WANG, H.H. & W.W. LAUTT. 1998. Evidence of nitric oxide, a flow-dependent factor, being a trigger of liver regeneration in rats. Can. J. Physiol. Pharmacol. **76:** 1072–1079.
157. RAI, R.M., F.Y. LEE, A. ROSEN, *et al.* 1998. Impaired liver regeneration in inducible nitric oxide synthase deficient mice. Proc. Natl. Acad. Sci. U.S.A. **95:** 13829–13834.