



Title	EFFECT OF PORTAL HEMODYNAMICS ON LIVER REGENERATION STUDIED IN A NOVEL PORTOHEPATIC SHUNT RAT MODEL
Author(s)	丸橋, 繁
Citation	大阪大学, 2004, 博士論文
Version Type	VoR
URL	https://hdl.handle.net/11094/46036
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

<https://ir.library.osaka-u.ac.jp/>

The University of Osaka



EFFECT OF PORTAL HEMODYNAMICS ON LIVER REGENERATION STUDIED IN A NOVEL PORTOHEPATIC SHUNT RAT MODEL

Shigeru Marubashi, Masato Sakon, Hiroaki Nagano, Kunihiro Gotoh, Kazuhiko Hashimoto, Masaru Kubota, Shogo Kobayashi, Shinji Yamamoto, Atsushi Miyamoto, Keizo Dono, Shoji Nakamori, Koji Umeshita, Morito Monden

Department of Surgery and Clinical Oncology, Osaka University,
Graduate School of Medicine, Suita, Osaka, JAPAN

Short title: Effect of portal hemodynamics on liver regeneration

All correspondence should be addressed to

M. Sakon, MD,

Department of Surgery and Clinical Oncology,
Osaka University Graduate School of Medicine,

2-2 YAMADAOKA, SUITA City,

Osaka 565-0871, JAPAN

TEL +81-6-6879-3251

FAX +81-6-6879-3259

Email: msakon@surg2.med.osaka-u.ac.jp

ABSTRACT

Background. The role of portal hemodynamics on liver regeneration after partial hepatectomy is not fully understood. The aim of our study was to characterize the effects of portal hemodynamics using a novel rat model.

Methods. We established a rat model of portohepatic shunt with 70% hepatectomy (PHS model), in which the portal pressure remained stable during and after 70% hepatectomy. To assess the effect of portal hemodynamics on liver injury and regeneration in the first 24 hours, we compared PHS rats with those with simple 70% hepatectomy.

Results. Biochemical and histopathological changes were similar between the two groups. Liver weight increased in the control, whereas it did not in PHS group ($P=0.0021$). Hepatocytes were enlarged in the control but not in PHS, although DNA synthesis was similar in both groups. Apoptotic hepatocytes increased markedly in PHS at 24 hours, whereas minimal apoptosis was noted throughout the course of the study in the control group. Hepatocyte growth factor increased similarly, except it was not activated in PHS.

Conclusions. Our results suggested that portal hyper-dynamic state early after 70% hepatectomy was necessary for liver regeneration through activation of HGF, promoting hepatocyte hypertrophy and avoiding apoptosis, while DNA synthesis in hepatocytes was independent of portal hemodynamics.

Key words: hepatectomy, rat, portal vein pressure, hepatocyte growth factor, cytokine

List of abbreviations:

BrdU, 5-bromo-2'-deoxyuridine;

HGF, hepatocyte growth factor;

IL-6, Interleukin-6;

PCNA, proliferative cell nuclear antigen;

PHS, portohepatic shunt;

TNF- α , tumor necrosis factor- α ;

TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling

Introduction

It is well known that the liver regenerates after partial hepatectomy, but the mechanism underlying this process has not been fully identified yet. At present, several growth factors are thought to control liver regeneration. These include hepatocyte growth factor (HGF), tumor necrosis factor (TNF)- α , and interleukin (IL)-6 (1-6), which increase during liver regeneration and promote the orchestrated cascade of hepatocyte regeneration.

Portal vein hemodynamics plays an important role in liver regeneration and injury after partial hepatectomy. In this condition, both portal vein pressure and liver tissue perfusion increase, and the remnant liver starts regenerating. In living donor liver transplantation, small-for-size graft, which needs enhanced regeneration, is considered to be a risk factor for delayed-graft function or graft failure, partly because small-for-size graft generates portal hyper-hemodynamics, which causes damage of hepatocytes (7-9). On the other hand, portal vein ligation in animal models resulted in the atrophy of the ligated part of the liver. (10) To explain these phenomena, several groups have reported that shear stress, which could be due to portal hyper-dynamic state, can induce various changes in endothelial cell function *in vitro* (11-15). However, the relation between these responses produced by hyper-hemodynamic change and the activation cascades of hepatotrophic factors after hepatectomy are not well understood. Thus, it is not clear how changes in portal vein hemodynamics alter liver regeneration in partially hepatectomized liver *in vivo*.

Several groups have described surgical models of portosystemic shunt (16, 17), but it was not appropriate to discuss liver regeneration in these models because there is no portal vein inflow to the liver and massive hepatectomy is fatal in such

animal models. To our knowledge, there is no animal model at present that can be used to evaluate both liver regeneration after hepatectomy and portal vein hemodynamics.

After several trials and technical improvements, we established a novel model of portosystemic shunt with 70% hepatectomy (PHS model), consisting of anastomosis of a portal vein branch with a hepatic vein branch. In our PHS model, the portal vein pressure remains almost unchanged during and after 70% hepatectomy. In the present study, we investigated the effects of changes in portal vein hemodynamics on liver injury and regeneration in the first 24 hours after 70% hepatectomy, using our novel PHS model.

Materials and Methods

Rats

All rats received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals. The Institutional Animal Use and Care Committee of Osaka University approved all housing arrangements and experimental procedures. All experiments were performed in WS rats (RT₁^k), which were kindly provided by Shionogi & Co., (Osaka, Japan). Rats were provided with standard chow and water *ad libitum* and maintained on a 12-12 h light-dark schedule in an air-conditioned room, and were used at the age of 9-11 weeks, weighing 300-330 g.

Surgical models

Rats were operated on under pentobarbiturate anesthesia.

Portohepatic shunt group (PHS group) (Fig. 1).

A midline incision was made to expose the liver. The left-central branch of the portal vein was ligated with 5-0 silk followed by injection of heparin sodium (50 unit) via the penile vein. Two minutes later, the left-lateral portal vein was ligated. A vascular clip was placed on the left portal vein, and the vein was divided. The left liver lobe was then freed up from the retroperitoneum, and the left hepatic vein was clamped using another vessel clip and the rest of left lobe peduncle was ligated with 5-0 silk and removed. A cuff made from 16-gauge catheter was placed on the stump of the left portal vein. This “portal vein cuff” was inserted into the end of the left hepatic vein, and 7-0 proline suture was wrapped around it. The vascular clip on the left portal vein was then removed and the vein recanalized. The middle lobe was

ligated at the basal level taking care not to occlude the outflow from the left hepatic vein, and 70% hepatectomy was completed.

Control group.

Similar to the PHS group, heparin sodium (50 unit/body) was injected via the penile vein after laparotomy. Two minutes later, the left portal vein and artery were ligated with 5-0 silk. The left and middle lobes of the liver were removed (70% hepatectomy).

Body weight, liver weight, portal vein pressure measurement, blood samples, and liver extraction

Body weight was measured just before surgery (Pre-OP). Liver weight was measured immediately after removal, and the growth of residual liver was assessed using liver weight index, which represented the ratio of liver weight divided by body weight. Portal vein pressure was measured using an electronic manometer (Nihon Kohden, Tokyo, Japan), which was directly placed into the portal vein after puncturing the vein by a 27-gauge needle. Inferior vena caval (IVC) pressure was measured in the same manner. Portal pressure gradient was defined as portal vein pressure minus IVC pressure. Pressures were measured immediately after laparotomy (Post-OP), immediately after 70% hepatectomy, 6, 12, 18, and 24 hours after hepatectomy in each group (n=3 or more). Furthermore, any change in portal vein pressure during PHS procedure was also recorded. At 6, 12, and 18 hours after surgery, rats (n=4 or more for each group) received intraperitoneal injection of 100 mg/kg body weight of 5-bromo-2'-deoxyuridine (BrdU) (Roche Diagnostics, Mannheim, Germany) one hour prior to liver extraction.

The abdomen was opened along the same incision under anesthesia at 6, 12, 18, 24 hours after the procedure in each group. Rats without hepatectomy were used as preoperative controls. After checking the patency of PHS, a blood sample (2 ml) was obtained from IVC, and followed immediately by liver extraction. Liver weight was measured and divided into two parts; one was placed in liquid nitrogen, then stored at -80°C , and the other was kept in 20% buffered formalin. Liver weight index was defined as the ratio of the weight of the extracted liver divided by pre-op body weight. Furthermore, after measuring the weight of the liver samples stored at -80°C , the dry weight of these samples were measured by freeze drying as reported previously (18), and the ratio of the wet to dry weight of the livers were determined. Blood samples were mixed with EDTA2Na, centrifuged, and the levels of AST, ALT, and LDH were measured in the sera using Auto Dry Chemistry Analyzer SP-4410 (Arkray, Kyoto, Japan).

Histology, immunohistochemistry, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL), and BrdU

Liver tissue sections were cut at 4 μm thickness and stained with H&E using standard technique. Cell volume was calculated as 1000 divided by numbers of nuclei in 10 randomly selected high-power fields in the parenchyma, assuming that the thickness of each section was constant. The numbers of nuclei were calculated manually in TUNEL, and automatically using Mac SCOPE ver. 2.5 (MITANI CORPORATION, Tokyo) in cell volume.

For immunohistochemistry, sections were incubated for 2 hours at room temperature with anti-PCNA monoclonal antibody (Novocastra, Newcastle, UK) and anti-BrdU monoclonal antibody (Dako, Glostrup, Denmark). Apoptotic cells were

visualized by TUNEL, using the Apop Tag In situ Apoptosis Detection Kit (Oncor, Gaithersburg, MD). Positive cells were counted in 10 randomly selected high-power fields each in the portal and central areas and distributed over at least three independent sections.

Tissue homogenization

The tissue mixed with the buffer as described previously (19) was homogenized on ice for 15 seconds with Ultrasonic homogenizer UD-201 (TOMY Digital Biology Co., Tokyo). The samples were then spun for 15 minutes at $20,000 \times g$ at 4°C in a KUBOTA-1910 centrifuge (Kubota Corporation, Tokyo). The supernatant was promptly aliquoted and stored at -80°C.

Western-blot analysis

Protein was extracted from homogenized livers. Western-blot analysis was performed with samples normalized for protein concentrations. The membrane was probed with anti-rat hepatocyte growth factor (HGF) (Institute of Immunology, Tokyo) as described previously (19). The exposed films were scanned in a flatbed scanner and the intensity of each band was quantified using NIH Image analysis software ver. 1.61 (National Institute of Health, MD).

Serum and tissue HGF concentration

HGF concentration was determined using rat HGF EIA Kit (Institute of Immunology, Tokyo). Liver tissues stored at -80°C were homogenized as described for Western blotting. HGF concentrations in liver samples and serums were

determined according to the instructions provided by the manufacturer. Briefly, microplates coated with mouse anti-rat HGF monoclonal antibody were incubated for 20 hours at room temperature with 100 μ l of each diluted sample. Wells were washed five times with washing buffer and incubated for 2 hours at room temperature in 100 μ l of rabbit anti-rat HGF polyclonal antibody. After washing, wells were again incubated for 2 hours at room temperature with peroxidase-conjugated anti-rabbit IgG and then with 100 μ l of enzyme substrate solution. The reaction was terminated with 50 μ l of 6N H₂SO₄. Absorbance at 492 nm was measured with an ELISA spectrometer (model 3550 reader, Bio-Rad).

RT-PCR

To assess the expression of TNF- α , IL-6, and cyclin B mRNAs, total RNA was subjected to real-time RT-PCR using the LightCycler PCR and detection system (Roche Diagnostics) as described previously by our group (20, 21) using the following primers. TNF- α : 5'-GTAGCCCACGTCGTAGCAAA-3' and 5'-CCCTTCTCCAGCTGGAAGAC-3', IL-6: 5'-AGCCAGACTCATTCAAGAGCA-3' and 5'-AGATACCCATCGACACGATA-3 (22). Primers for β 2-microglobulin as an internal control were designed as 5'-CTGGTCTTTCTACATCCTGG-3' and 5'-GGTGTGCTCATTGCTATTCT-3'.

Statistical analysis

All data were expressed as mean $\pm$ SD. Differences between groups were examined for statistical significance using the Mann-Whitney U-test (nonparametric) and two-way analysis of variance (ANOVA). A *P* value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Animal model

After initial learning period, we performed PHS procedure on over 60 rats. The success rate of PHS procedure was more than 95%. Survival study using 6 rats in each group showed that five rats (83%) in PHS group survived more than 24 hours. On the other hand, the survival rate at 24 hours of the control group was 100%. No thrombosis in hepatic vein or portal vein was noted, and lung, heart and other organs were macroscopically and microscopically intact in each rat as determined at 24 hour or postmortem examination.

Changes in portal pressure gradient

Minimal changes in portal pressure gradient were noted in PHS group during the surgical procedure and postoperatively. In contrast, the pressure increased immediately after 70% hepatectomy in the control group and returned to the pre-OP value after 12 to 18 hours (Fig. 2A, Intra-operative data not shown).

Liver injury and histopathological changes

The serum levels of AST, ALT, and LDH were elevated to similar levels in both PHS and control (Fig. 2B, AST, LDH; data not shown). H&E staining of the harvested liver samples from these two groups showed sinusoidal narrowing and increased fatty degeneration, which commenced around the portal area 12 hours after hepatectomy and spread to the central vein area by 24 hours after hepatectomy (Fig. 3A-C). There was no necrosis or bleeding in both groups, and no histopathological differences were noted between two groups.

Kinetics of liver regeneration

Liver weight index was almost unchanged at 6 hours after the procedure, and increased thereafter in the control group, while it was almost unchanged by 12 hours and slightly elevated toward 18 hours in the PHS group. There were significant differences in 12 and 18 hours after hepatectomy between the two groups (Fig. 4A). The ratio between wet and dry weight of the liver was almost unchanged throughout the experiment (Fig. 4B). The volume of hepatocytes remained unchanged in the PHS group up to 18 hours, but increased significantly in the control group. There was a significant difference between the two groups at 18 hours ($P=0.007$, Fig. 4C). However, proliferative cell nuclear antigen (PCNA) index (23) and 5'-bromo-2-deoxyuridine (BrdU) incorporation (24) changed in a similar fashion in both groups. PCNA index progressively increased after hepatectomy in both groups (Fig. 4D) and PCNA-positive nuclei were found diffusely in the liver parenchyma and no predominance was noted in the portal or central area. BrdU incorporation started after 12 hours in both groups, and at 18 hours after hepatectomy, substantial BrdU incorporation was noted in both groups (PHS group 14.4 ± 15.6 positive cells/1000 nuclei, Control 19.7 ± 22.2 positive cells / 1000 nuclei, p , not significant). BrdU-positive nuclei were diffusely present in the liver parenchyma. To further investigate cell proliferation, we examined the expression of cyclin B mRNA, representing one of the earliest markers for G2/M phase in cell cycle (25). The expression reached a peak level at 6 hours but no difference was observed between the two groups (Data not shown).

Apoptotic cells and expression of hepatotrophic factors in the remnant liver

TUNEL staining (26) showed very few positive hepatocytes throughout the course of experiment in both the control and PHS groups, until a substantial increase of apoptotic cells was noted in PHS group at 24 hours after the procedure (Fig. 5A). Both IL-6 and TNF- α were strongly upregulated in PHS group from 12 to 24 hours, while they were mildly upregulated in the control. There were significant differences in the mRNA expression of these two cytokines between the two groups (Fig. 5B, C).

HGF concentration and activated form of HGF

HGF concentrations in the serum and in liver tissue increased markedly after hepatectomy in both groups and no significant difference was noted between the two groups in either study (Fig. 6A and B). To identify the activation of single chain form of HGF (scHGF, 92kDa) in the liver, western-blot analysis was performed to detect the activated form of HGF (hcHGF, 62kD) (27). A marked increase in activated HGF was noted after 12 hours in control rats while no activation was detected in PHS rats (Fig. 6C). The ratio of hcHGF to scHGF was significantly different between the two groups (Fig. 6D).

DISCUSSION

In experimental animals with 70% partial hepatectomy, hepatic regeneration occurs most evidently following increase in portal blood pressure and flow (28). Although several groups reported that the hyper-dynamic state of the portal system causes sinusoidal epithelial injury (9, 29), or may also induce liver regeneration (30), the exact mechanisms of liver regeneration after partial hepatectomy have not been elucidated yet.

In this study, we established a novel animal model of 70% hepatectomy with porto-hepatic shunt, in which the portal blood pressure during and after 70% hepatectomy remained almost unchanged compared to the preoperative value (Fig. 2A). In this animal model, hepatic artery flow in the remnant liver was not disturbed, and portal vein flow was maintained by the cuff generating pressure gradient between the shunt. Portocaval shunt with 70% hepatectomy in rats is associated with a high mortality rate in rats (16), causing high ammonia (NH_3) level in peripheral blood (17). However, our PHS rats survived more than 24 hours and had normal NH_3 level similar to the control rats (data not shown). Based on these features, our animal model should allow assessment of the effects of portal hemodynamics on liver regeneration after partial hepatectomy.

Prior to the experiment, we postulated that the PHS liver should suffer less damage due to unchanged portal flow compared with the control liver with elevated portal flow. However, the results showed that the biochemical changes after 70% hepatectomy were similar in both groups (Fig. 2B). Histological examination of the remnant liver in the PHS group showed no ischemic or necrotic changes until 24 hours as in the control group. Furthermore, similar extent of lipogenesis was noted in

both groups as confirmed by the time course studies (Fig. 3A-C), as reported in previous studies (31).

Despite the similar biochemical and histopathological findings, the liver weight index increased after 12 hours in the control group but not in PHS group (Fig 4A). Since the ratio of wet to dry weight of the liver was unchanged (Fig 4B), the increased remnant liver mass early after 70% hepatectomy is considered as a result of liver regeneration rather than a simple weight gain due to fluid retention or edema (31, 32). Liver regeneration after partial hepatectomy can be characterized by three different stages; hepatocyte enlargement, cell proliferation with DNA synthesis and apoptosis (33, 34). The hypertrophy of the remnant liver was more evident in control rats than PHS rats (Fig. 4C), while cell proliferation and DNA synthesis were similar between the two groups, and apoptosis of hepatocytes was similar until it increased in PHS rats at 24 hours after 70% hepatectomy. These findings suggested that neither cell proliferation with DNA synthesis nor apoptosis of hepatocytes, but cell hypertrophy could explain liver regeneration in this study.

All markers of the cell cycle and DNA synthesis (BrdU labeling index, PCNA index, and the expression of cyclin B mRNA) were elevated to similar levels in both groups (Fig. 4D), suggesting that cell proliferation with DNA synthesis after partial hepatectomy was not different between the two groups. Cell proliferation with DNA synthesis is regulated by various cytokines such as HGF, IL-6, and TNF- α . HGF is a multifunctional cytokine and one of the most potent mitogens for hepatocytes (35, 36). It is produced as an inactive, single chain form (scHGF) by various cells including hepatic nonparenchymal cells (37, 38). scHGF bound to the liver matrix can be degraded by plasmin and then activated by HGF activator (HGFA) (39) or uPA (40-42), forming two-chain type of HGF (tcHGF), which binds its receptor on

hepatocytes and induce intracellular signal transduction leading to cell proliferation (43). In the present study, serum HGF level and concentration of HGF in liver tissue were not different between PHS group and control group (Fig. 6A and B). However, the hcHGF/scHGF ratio was markedly increased in the control group at 12 and 18 hrs after partial hepatectomy while no such increase was noted in the PHS group (Fig. 6C and D). These results indicate that portal hyper-hemodynamics may be a critical trigger for HGF activation in the remnant liver. In fact, recent studies demonstrated that uPA activation was initiated after 70% hepatectomy (19, 42), and uPA degrades scHGF, forming an active form of HGF, hcHGF (19). HGFA can also splice scHGF to its active form. Therefore, it is possible that HGF activation occurs following partial hepatectomy by portal hyper-hemodynamics-mediated uPA or HGFA activation. However, cell proliferation with DNA synthesis in both groups cannot be explained by HGF only.

Transcription of both IL-6 and TNF- α in liver tissue was more strongly upregulated in PHS group from 12 to 18 hours, than in control group (Fig. 5B and C). Recent studies have highlighted IL-6 (4) and TNF- α (6) as major and initial cytokines promoting DNA synthesis in hepatocytes after partial hepatectomy. They can be downregulated, for example, by suppressors of cytokine signaling (SOCS)-3 as well (44). Considering the similarity of DNA synthesis in hepatocytes in both groups and upregulation of transcription of IL-6 and TNF- α in PHS rats, IL-6 and TNF- α may play an important role in hepatocyte proliferation in PHS group, compensating for the suppression of activation of HGF.

In contrast to the almost identical results of the regenerative markers of DNA synthesis, apoptotic properties were different between the two groups. Apoptotic indexes were low in the two groups until it was significantly elevated at 24 hours after

the procedure in PHS rats (Fig. 5A). Previous reports, which showed reduced portal vein hemodynamics resulted in increased apoptosis and liver atrophy (10, 45), add support to these findings, suggesting that the hyper-dynamic state of portal vein might be necessary for protection of hepatocytes against apoptosis.

The volume of hepatocytes was increased in the control group but not in PHS group (Fig. 4C). Several agents are known to promote the enlargement of hepatocytes (33). HGF is one such agent; activated HGF has been shown to cause enlargement of hepatocytes in rats (33, 46). Therefore, activation of HGF, which occurred only in control rats, can explain the difference in cellular enlargement and liver mass recovery between the two groups. It is conceivable that the hyper-dynamic state of portal vein was necessary for cellular enlargement.

The characteristics of the PHS model are the combination of partial hepatectomy and shunting of portal venous flow. Portal venous pressure can be decreased with agents such as beta-blocker or octreotide. The use of such agents combined with partial hepatectomy may result in the interference of hepatocyte regeneration as in PHS model. Several authors reported that octreotide administration inhibited liver regeneration after 70% hepatectomy in rats (47, 48). Their results may be explained by the mechanism of our HGF theory. On the other hand, portal venous pressure increases in cirrhotic liver. The application of our HGF theory on cirrhotic liver is quite interesting, although the situation is rather complicated with intrahepatic shunts and capillarization of sinusoids due to cell necrosis and deposition of new collagen, making the interpretation of experiments difficult.

In conclusion, our comparative study between partially hepatectomized rats with and without portohepatic shunt revealed that portal hyper-dynamic state early after 70% hepatectomy was necessary for liver regeneration through activation of HGF, promoting hepatocyte hypertrophy and avoiding apoptosis. However, DNA synthesis of the remnant liver did not correlate with portal hemodynamics.

REFERENCES

1. Michalopoulos GK, DeFrances MC. Liver regeneration. *Science* 1997;276:60-66.
2. Webber EM, Bruix J, Pierce RH, Fausto N. Tumor necrosis factor primes hepatocytes for DNA replication in the rat. *Hepatology* 1998;28:1226-1234.
3. Fausto N. Liver regeneration. *J Hepatol* 2000;32 Suppl 1:19-31.
4. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, et al. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science* 1996;274:1379-1383.
5. Court FG, Wemyss-Holden SA, Dennison AR, Maddern GJ. The mystery of liver regeneration. *Br J Surg* 2002;89:1089-1095.
6. Yamada Y, Kirillova I, Peschon JJ, Fausto N. 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* 1997;94:1441-1446.
7. Ben-Haim M, Emre S, Fishbein TM, Sheiner PA, Bodian CA, Kim-Schluger L, et al. Critical graft size in adult-to-adult living donor liver transplantation: impact of the recipient's disease. *Liver Transpl* 2001;7:948-953.
8. Nishizaki T, Ikegami T, Hiroshige S, Hashimoto K, Uchiyama H, Yoshizumi T, et al. Small graft for living donor liver transplantation. *Ann Surg* 2001;233:575-580.
9. Fraser R, Bowler LM, Day WA, Dobbs B, Johnson HD, Lee D. High perfusion pressure damages the sieving ability of sinusoidal endothelium in rat livers. *Br J Exp Pathol* 1980;61:222-228.
10. Bilodeau M, Aubry MC, Houle R, Burnes PN, Ethier C. Evaluation of hepatocyte injury following partial ligation of the left portal vein. *J Hepatol*

1999;30:29-37.

11. Reich KM, Gay CV, Frangos JA. Fluid shear stress as a mediator of osteoblast cyclic adenosine monophosphate production. *J Cell Physiol* 1990;143:100-104.

12. Bhagyalakshmi A, Berthiaume F, Reich KM, Frangos JA. Fluid shear stress stimulates membrane phospholipid metabolism in cultured human endothelial cells. *J Vasc Res* 1992;29:443-449.

13. Redmond EM, Cahill PA, Hirsch M, Wang YN, Sitzmann JV, Okada SS. Effect of pulse pressure on vascular smooth muscle cell migration: the role of urokinase and matrix metalloproteinase. *Thromb Haemost* 1999;81:293-300.

14. Nishizuka Y. Studies and perspectives of protein kinase C. *Science* 1986;233:305-312.

15. Hsieh HJ, Li NQ, Frangos JA. Pulsatile and steady flow induces c-fos expression in human endothelial cells. *J Cell Physiol* 1993;154:143-151.

16. Rokicki M, Rokicki W. Liver regeneration in rats after complete and partial occlusion of the portal blood influx. *Res Exp Med (Berl)* 1993;193:305-313.

17. Castaing D, Beaubernard C, Ariogul O, Gigou M, Franco D, Bismuth H. Liver atrophy and encephalopathy after portacaval shunt in the rat. *Eur Surg Res* 1982;14:192-202.

18. Wimmer M, Wilmering B, Sasse D. The relation of rat liver wet weight to dry weight. *Histochemistry* 1985;83:571-572.

19. Pediaditakis P, Lopez-Talavera JC, Petersen B, Monga SP, Michalopoulos GK. The processing and utilization of hepatocyte growth factor/scatter factor following partial hepatectomy in the rat. *Hepatology* 2001;34:688-693.

20. Miyake Y, Fujiwara Y, Ohue M, Yamamoto H, Sugita Y, Tomita N, et al. Quantification of micrometastases in lymph nodes of colorectal cancer using real-time

fluorescence polymerase chain reaction. *Int J Oncol* 2000;16:289-293.

21. Miyamoto A, Nagano H, Sakon M, Fujiwara Y, Sugita Y, Eguchi H, et al. Clinical application of quantitative analysis for detection of hematogenous spread of hepatocellular carcinoma by real-time PCR. *Int J Oncol* 2001;18:527-532.

22. Scotte M, Masson S, Lyoumi S, Hiron M, Teniere P, Lebreton JP, et al. Cytokine gene expression in liver following minor or major hepatectomy in rat. *Cytokine* 1997;9:859-867.

23. Tanno M, Taguchi T. Proliferating cell nuclear antigen in normal and regenerating rat livers. *Exp Mol Pathol* 1999;67:192-200.

24. Vitale M, Rizzoli R, Mazzotti G, Zamai L, Galanzi A, Rizzi E, et al. Characterization and cell cycle kinetics of hepatocytes during rat liver regeneration: in vivo BrdUrd incorporation analysed by flow cytometry and electron microscopy. *Cell Prolif* 1991;24:331-338.

25. Masson S, Scotte M, Francois A, Coeffier M, Provot F, Hiron M, et al. Changes in growth factor and cytokine mRNA levels after hepatectomy in rat with CCl₄-induced cirrhosis. *Am J Physiol* 1999;277:G838-846.

26. Motoyama S, Minamiya Y, Saito S, Saito R, Matsuzaki I, Abo S, et al. Hydrogen peroxide derived from hepatocytes induces sinusoidal endothelial cell apoptosis in perfused hypoxic rat liver. *Gastroenterology* 1998;114:153-163.

27. Kaibori M, Inoue T, Sakakura Y, Oda M, Nagahama T, Kwon AH, et al. Impairment of activation of hepatocyte growth factor precursor into its mature form in rats with liver cirrhosis. *J Surg Res* 2002;106:108-114.

28. Morsiani E, Aleotti A, Ricci D. Haemodynamic and ultrastructural observations on the rat liver after two-thirds partial hepatectomy. *J Anat* 1998;192 (Pt 4):507-515.

29. McCormick SM, Eskin SG, McIntire LV, Teng CL, Lu CM, Russell CG, et al.

DNA microarray reveals changes in gene expression of shear stressed human umbilical vein endothelial cells. *Proc Natl Acad Sci U S A* 2001;98:8955-8960.

30. Niiya T, Murakami M, Aoki T, Murai N, Shimizu Y, Kusano M. Immediate increase of portal pressure, reflecting sinusoidal shear stress, induced liver regeneration after partial hepatectomy. *J Hepatobiliary Pancreat Surg* 1999;6:275-280.

31. Murray AB, Strecker W, Silz S. Ultrastructural changes in rat hepatocytes after partial hepatectomy, and comparison with biochemical results. *J Cell Sci* 1981;50:433-448.

32. Ling YQ, Shibamoto T, Honda T, Kamikado C, Hironaka E, Hongo M, et al. Increased sinusoidal pressure is associated with early liver weight gain in ischemia-reperfusion injury in isolated perfused rat liver. *J Surg Res* 2000;88:70-77.

33. Nagy P, Teramoto T, Factor VM, Sanchez A, Schnur J, Paku S, et al. Reconstitution of liver mass via cellular hypertrophy in the rat. *Hepatology* 2001;33:339-345.

34. Minamishima YA, Nakayama K. Recovery of liver mass without proliferation of hepatocytes after partial hepatectomy in Skp2-deficient mice. *Cancer Res* 2002;62:995-999.

35. Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, et al. Molecular cloning and expression of human hepatocyte growth factor. *Nature* 1989;342:440-443.

36. Michalopoulos GK, Zarnegar R. Hepatocyte growth factor. *Hepatology* 1992;15:149-155.

37. Appasamy R, Tanabe M, Murase N, Zarnegar R, Venkataramanan R, Van Thiel DH, et al. Hepatocyte growth factor, blood clearance, organ uptake, and biliary

excretion in normal and partially hepatectomized rats. *Lab Invest* 1993;68:270-276.

38. Zioncheck TF, Richardson L, DeGuzman GG, Modi NB, Hansen SE, Godowski PJ. The pharmacokinetics, tissue localization, and metabolic processing of recombinant human hepatocyte growth factor after intravenous administration in rats. *Endocrinology* 1994;134:1879-1887.

39. Mizuno K, Tanoue Y, Okano I, Harano T, Takada K, Nakamura T. Purification and characterization of hepatocyte growth factor (HGF)-converting enzyme: activation of pro-HGF. *Biochem Biophys Res Commun* 1994;198:1161-1169.

40. Kwaan HC. The plasminogen-plasmin system in malignancy. *Cancer Metastasis Rev* 1992;11:291-311.

41. Naldini L, Vigna E, Bardelli A, Follenzi A, Galimi F, Comoglio PM. Biological activation of pro-HGF (hepatocyte growth factor) by urokinase is controlled by a stoichiometric reaction. *J Biol Chem* 1995;270:603-611.

42. Stolz DB, Mars WM, Petersen BE, Kim TH, Michalopoulos GK. Growth factor signal transduction immediately after two-thirds partial hepatectomy in the rat. *Cancer Res* 1999;59:3954-3960.

43. Recio JA, Merlino G. Hepatocyte growth factor/scatter factor activates proliferation in melanoma cells through p38 MAPK, ATF-2 and cyclin D1. *Oncogene* 2002;21:1000-1008.

44. Campbell JS, Prichard L, Schaper F, Schmitz J, Stephenson-Famy A, Rosenfeld ME, et al. Expression of suppressors of cytokine signaling during liver regeneration. *J Clin Invest* 2001;107:1285-1292.

45. Weinbren K, Washington SL, Smith CY. The response of the rat liver to alterations in total portal blood flow. *Br J Exp Pathol* 1975;56:148-156.

46. Ueno S, Aikou T, Tanabe G, Kobayashi Y, Hamanoue M, Mitsue S, et al.

Exogenous hepatocyte growth factor markedly stimulates liver regeneration following portal branch ligation in dogs. *Cancer Chemother Pharmacol* 1996;38:233-237.

47. Pruthi RS, Farouk M, Tsai WH, Michalopoulos G, Meyers WC. The effect of octreotide on hepatic regeneration in rats. *Surgery* 1993;113:84-89.

48. Papalampros E, Felekouras ES, Filis K, Angelopoulou R, Kourelis T, Tsamandas AC, et al. Liver pathology and cell proliferation after octreotide administration following partial hepatectomy in rats: an experimental study. *Dig Dis Sci* 2002;47:1953-1958.

FIGURE LEGENDS

Fig. 1. **Rat model of portohepatic shunt (PHS) model.** The left portal vein was anastomosed with left hepatic vein using 16-gauge catheter cuff, after systemic administration of heparin sodium (50 unit). 70% partial hepatectomy was completed, preserving the anastomosis of portohepatic shunt.

Fig. 2. **Time course of portal vein pressure and liver injury.** A, Portal vein pressure increased immediately after 70% hepatectomy from 5.7 ± 1.0 mmHg to 10.0 ± 1.2 mmHg, then gradually decreased to pre-OP value by 18 hours in the control group ($\circ$, $n = 6$), whereas it remained almost unchanged in PHS group ($\bullet$, $n = 6$). $*P < 0.0001$; $\dagger P < 0.05$. B, serum level of ALT reached a peak level at 12 hours in both groups. Data are mean $\pm$ SD. There was no statistical difference between the two groups ($\bullet$, PHS group, $n = 4$, $\circ$, control, $n = 4$).

Fig. 3. **Livers after 70% hepatectomy in PHS and control rats.** A-C, Left panel, control rats, right panel, PHS rats. A, H&E stain of liver section at portal area at 6 hours after 70% hepatectomy showing narrowing of sinusoids (arrowhead). B and C, livers at 18 hours after 70% hepatectomy showing narrowing of sinusoids (B, arrowhead) and marked balloon like hepatocytes containing fatty deposits (C, arrowhead) in both groups. These histopathological changes expanded from peri-portal area to the central area. Note the lack of ischemic changes or necrosis in both groups. P, Portal vein, C, Central vein, Original magnification, A and B, $\times 200$, C, $\times 400$.

Fig. 4. Liver regeneration and hepatocyte apoptosis. A, Liver weight index after 70% hepatectomy showing liver weight index did not increase until 18 hours in PHS group (●, n = 6), whereas it increased by 12 hours in the control (○, n = 6). Significant difference was noted between the two groups using ANOVA ($P = 0.0021$). * $P = 0.005$; † $P = 0.0183$. B, The ratio of wet and dry weight of the livers. The ratio almost did not change throughout the experiment in both groups. C, Cell volume in PHS group (●, n = 6) was almost unchanged until 18 hours, while it increased continuously until 18 hours in the control (○, n = 6). ‡ $P = 0.007$. D, Time course of PCNA index showing marked increase after 70% hepatectomy in both groups. There was no difference between two groups. (●, PHS group, n = 4, ○, control, n = 4).

Fig. 5. mRNA Expression in liver after 70% hepatectomy. A, TUNEL staining showed few hepatocytes were apoptotic in both groups throughout the experiment (■, PHS group, n = 3, ▨, control, n = 3), with the exception of marked apoptosis in PHS liver at 24 hours. * $P < 0.01$. Data are mean $\pm$ SD. B and C, marked expression of IL-6 and TNF- α in PHS group. (■, PHS group, n = 3, ▨, control, n = 3). Data are mean $\pm$ SD. † $P < 0.05$.

Fig. 6. Serum and tissue HGF concentrations, and detection of activated HGF in liver. A, serum concentration of HGF showing no remarkable increase in both groups. B, HGF concentration in homogenized liver showing remarkable increase in both groups. The pattern of increase was similar in both groups (●, PHS group, n = 6, ○, control, n = 6). C and D, Western blot of HGF demonstrating kinetics of induction of activated HGF. Single chain HGF (scHGF) was spliced into heavy chain HGF (hcHGF, 62 kDa) by limited proteolysis. C, control, hcHGF was markedly increased

after 12 hours. PHS group, No increase in hc HGF was observed. D, Time course of HGF activation. The ratio of hcHGF intensity divided by scHGF intensity represented activation of HGF (19). The intensities of hcHGF and scHGF were calculated using densitometry. There was no increase in PHS group, whereas a 5-fold increase was noted in the control group. Data are mean $\pm$ SD. There was a significant difference between the two groups by ANOVA ($P < 0.001$). * $P = 0.025$.

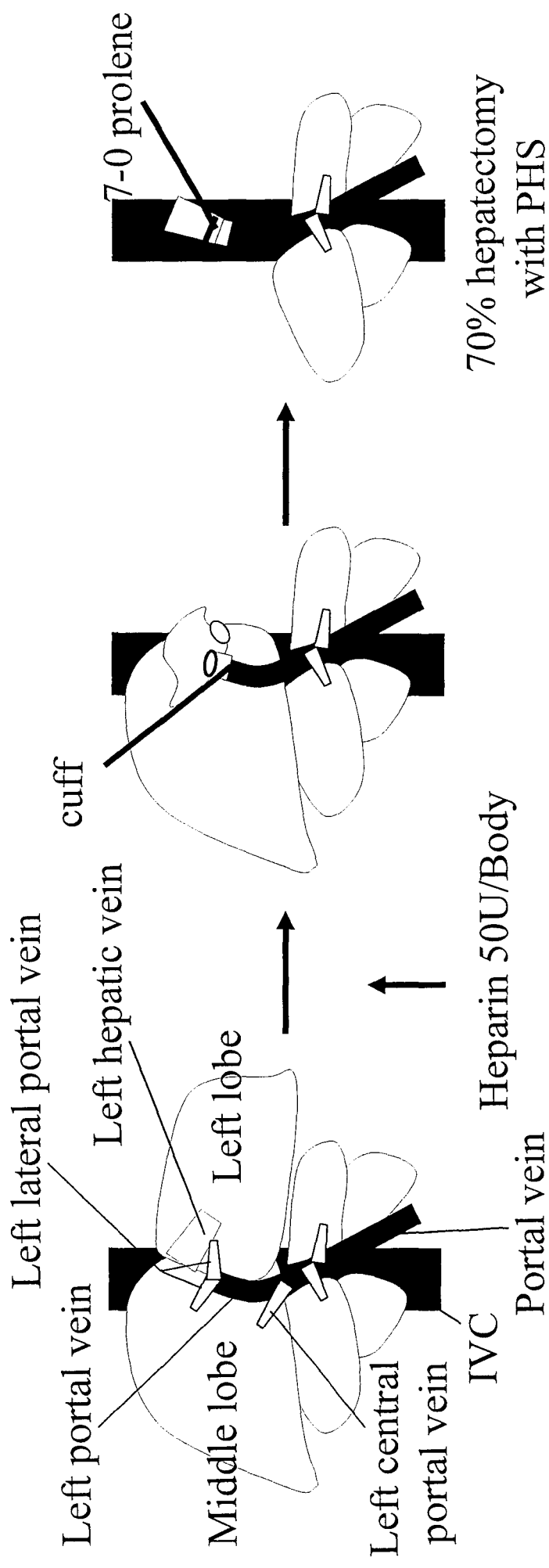
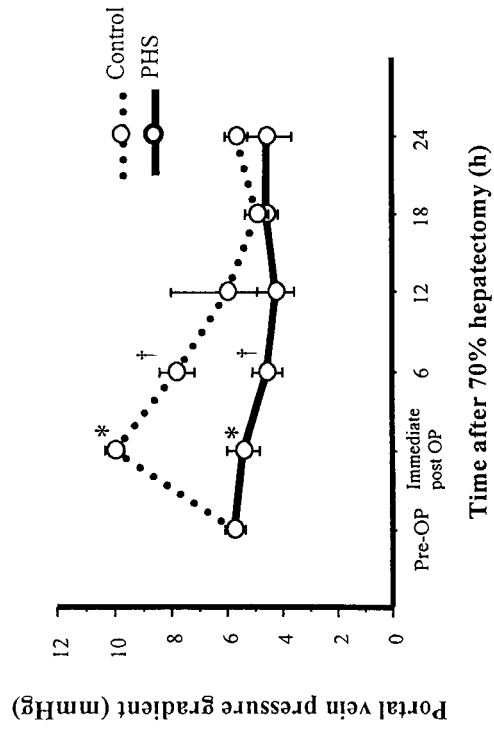


Fig. 1

A



B

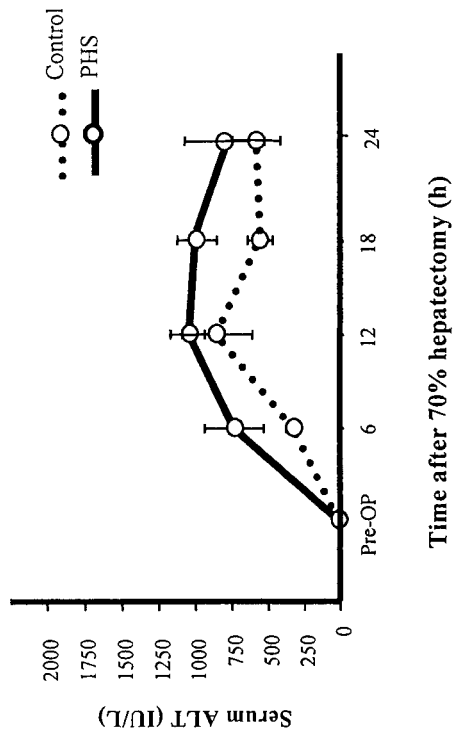
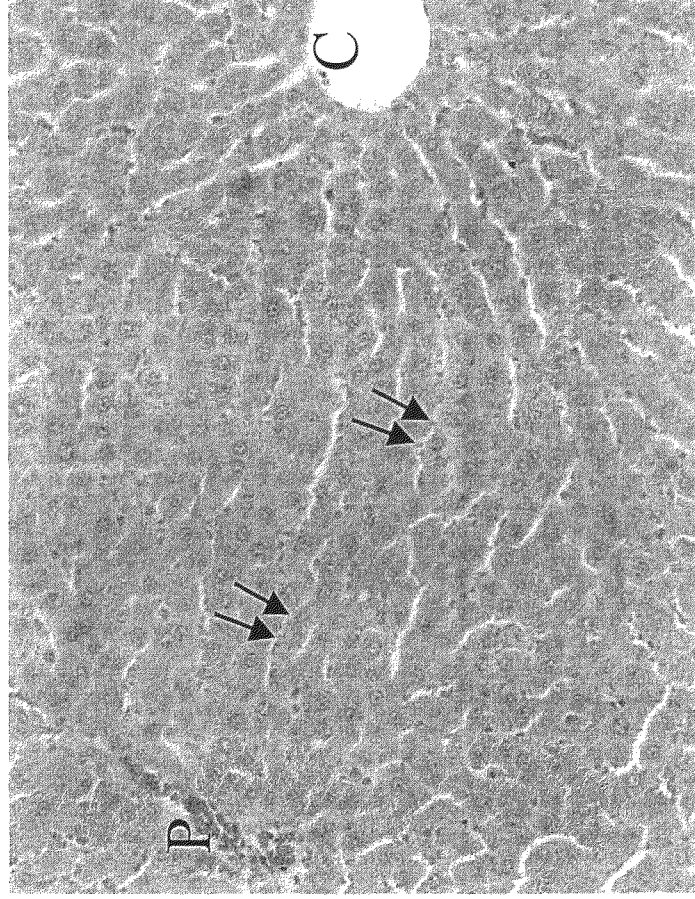
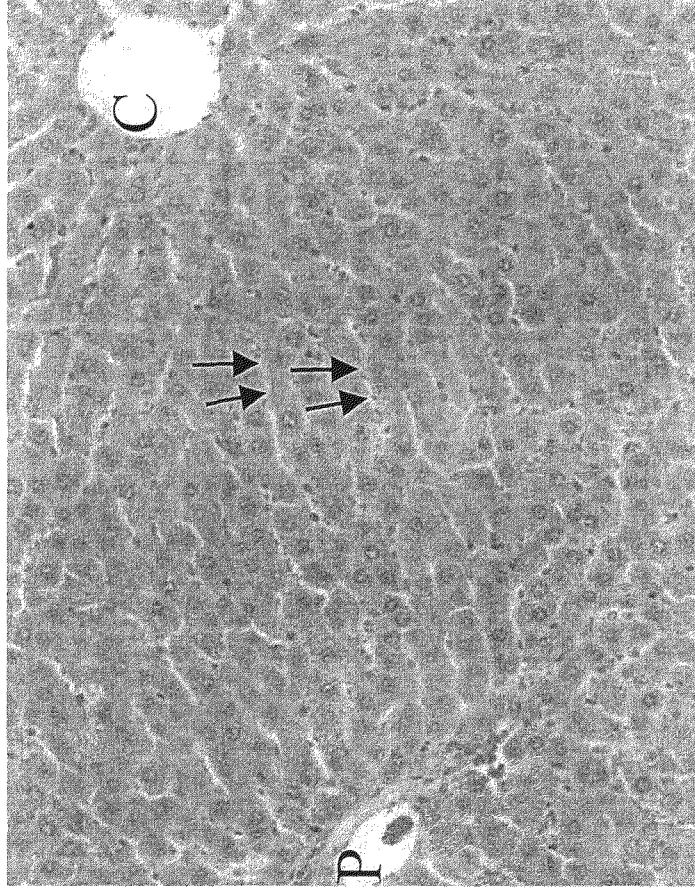


Fig. 2

A 6 h



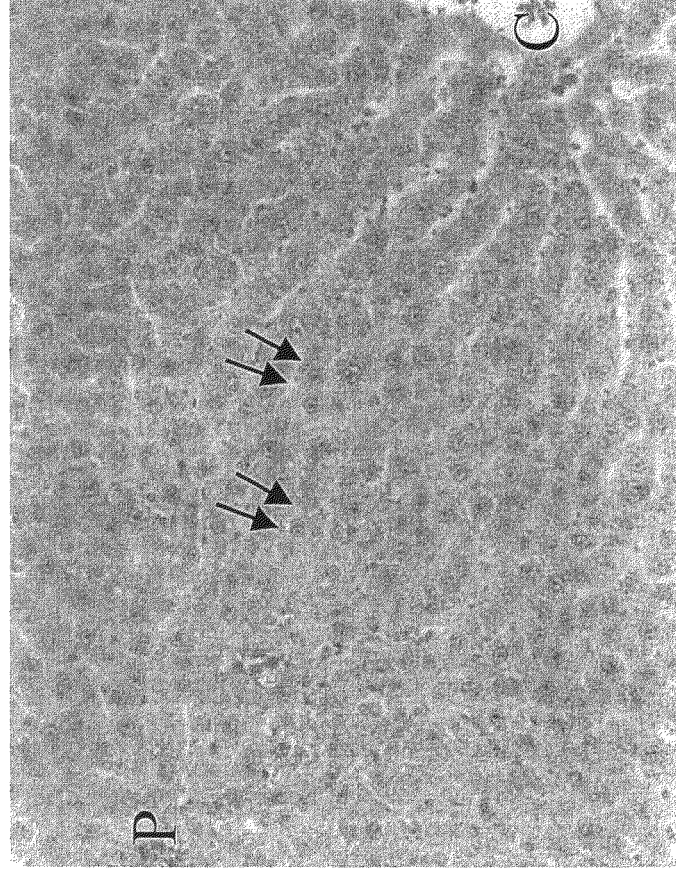
Control



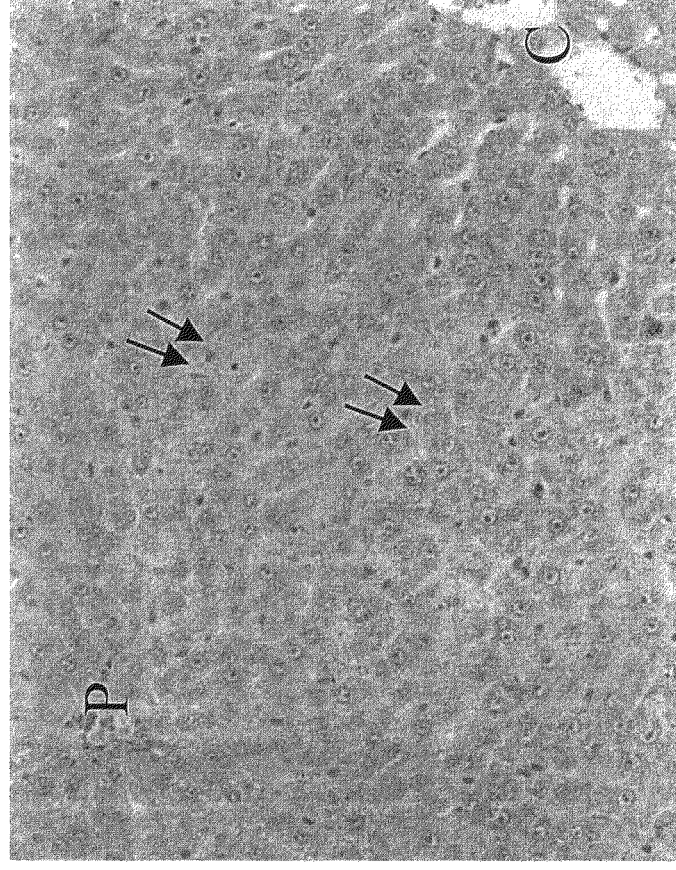
PHS

Fig. 3

B 18h



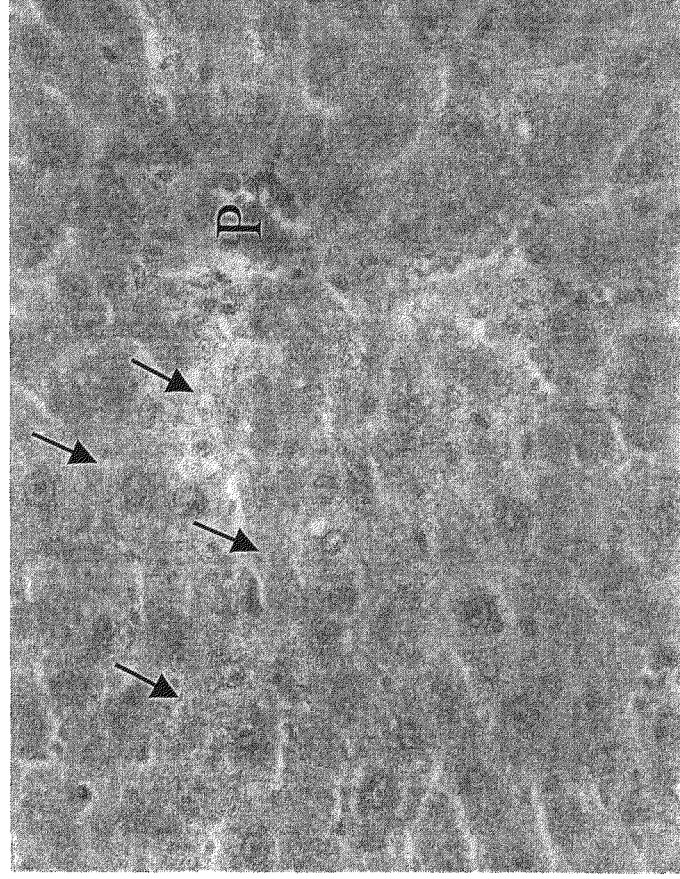
Control



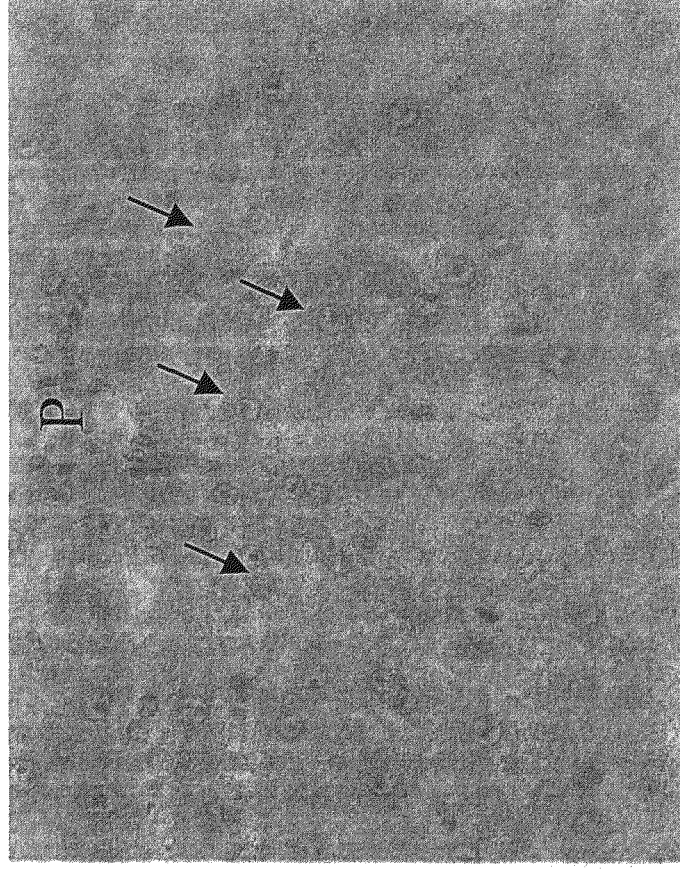
PHS

Fig. 3

C 18h



Control



PHS

Fig. 3

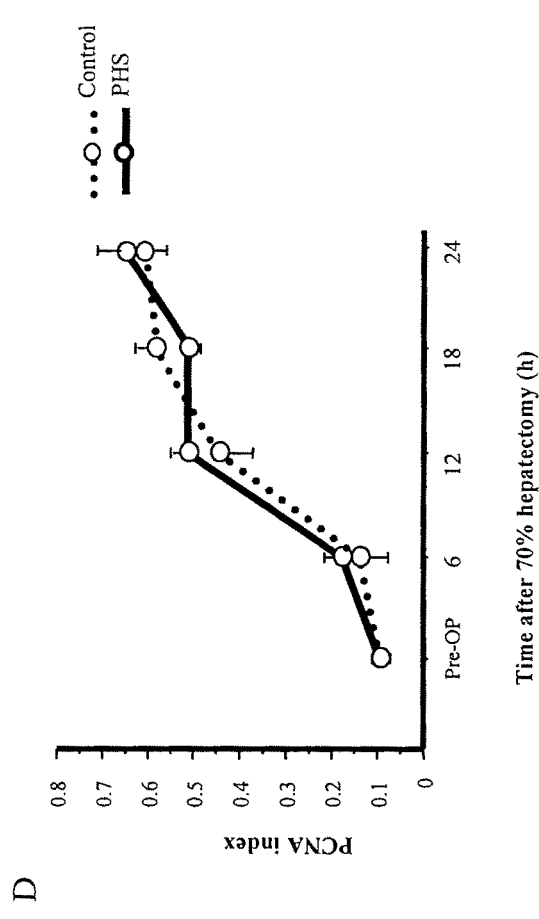
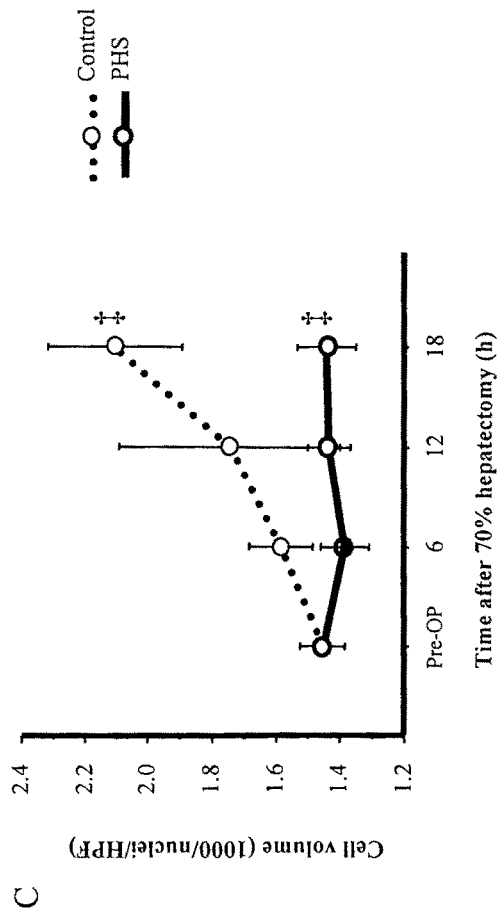
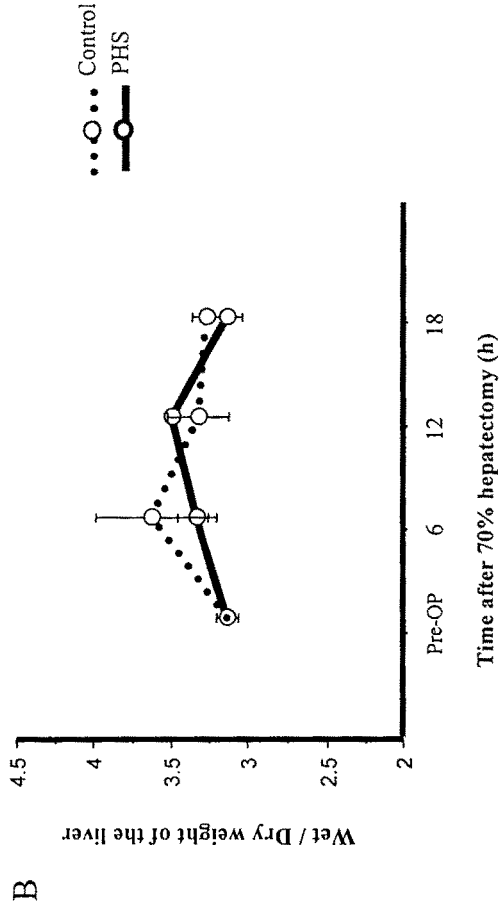
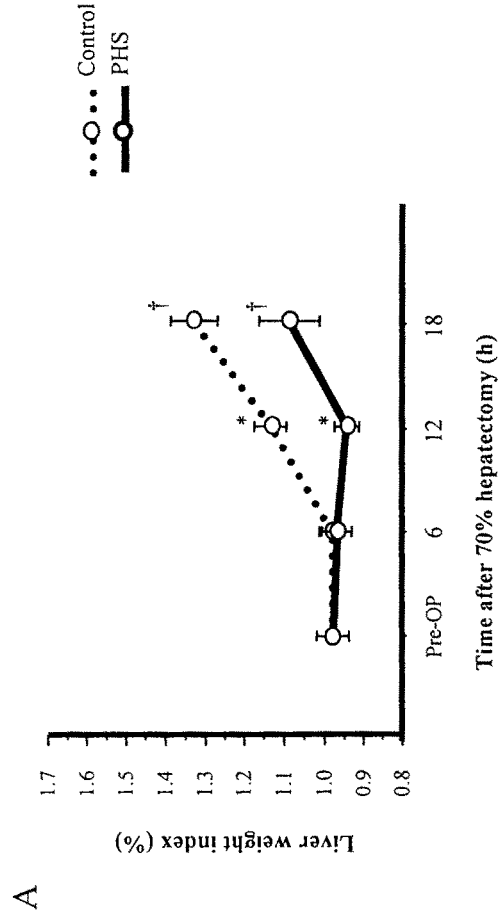


Fig. 4

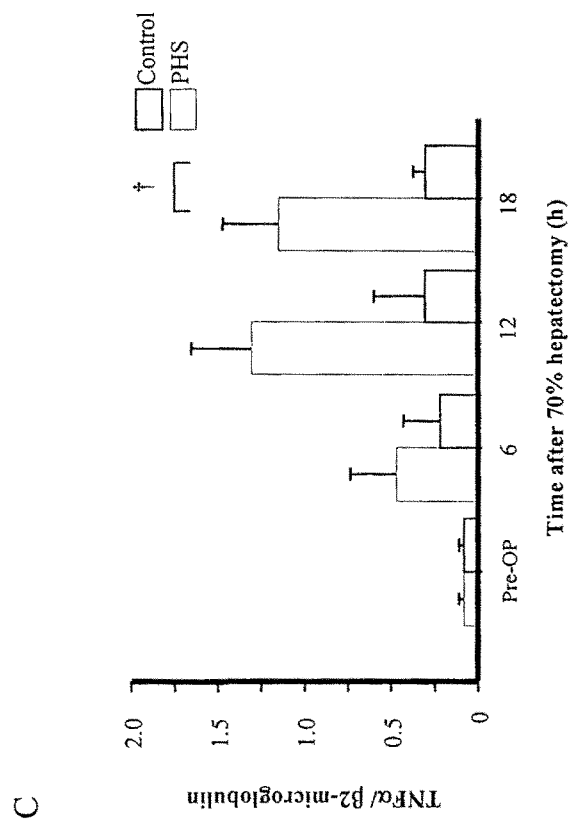
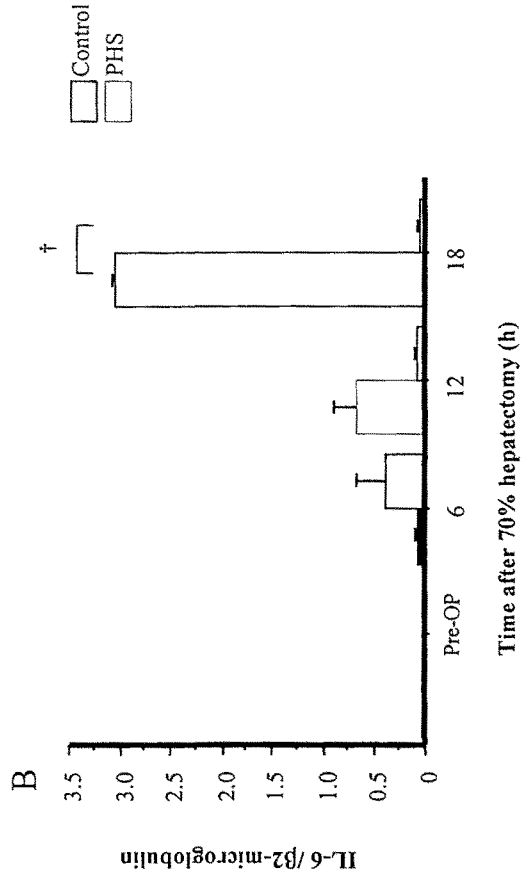
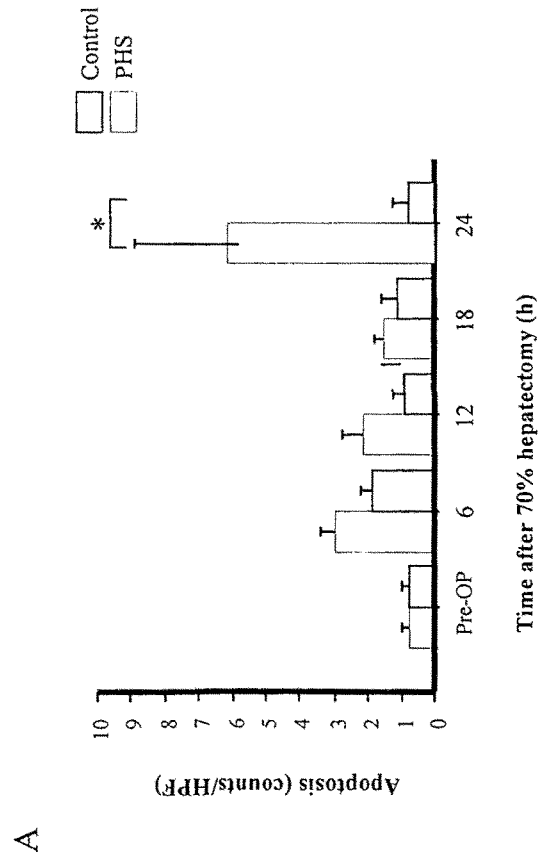
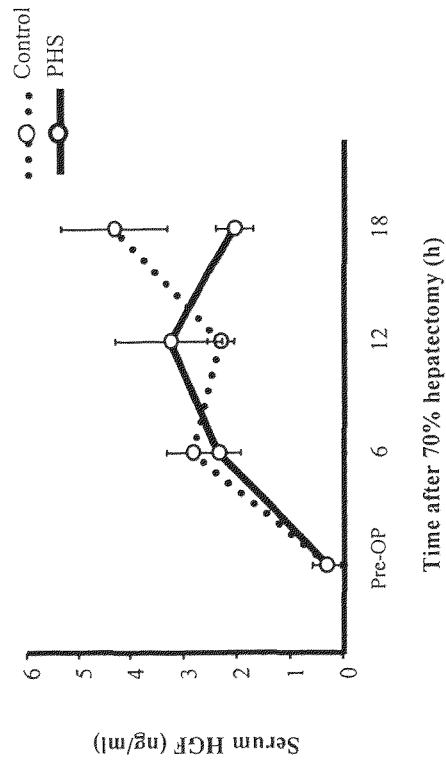
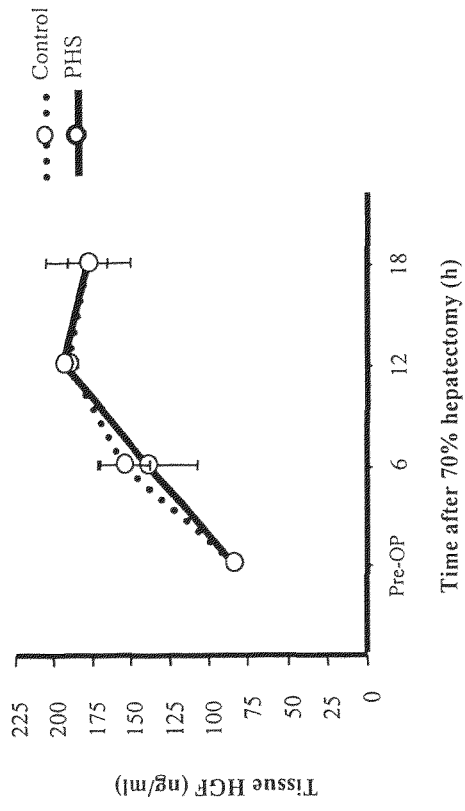


Fig. 5

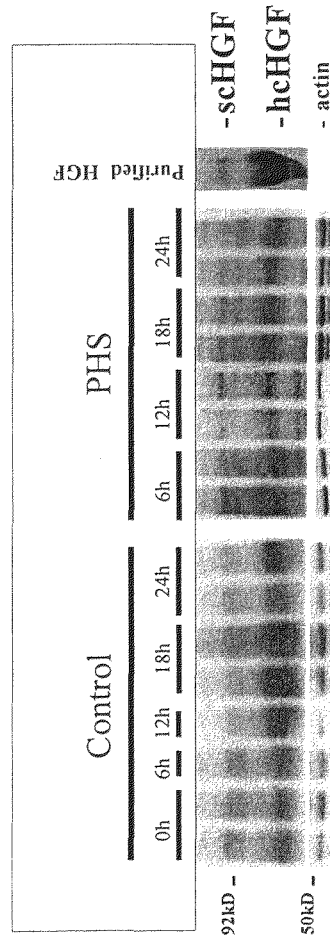
A



B



C



D

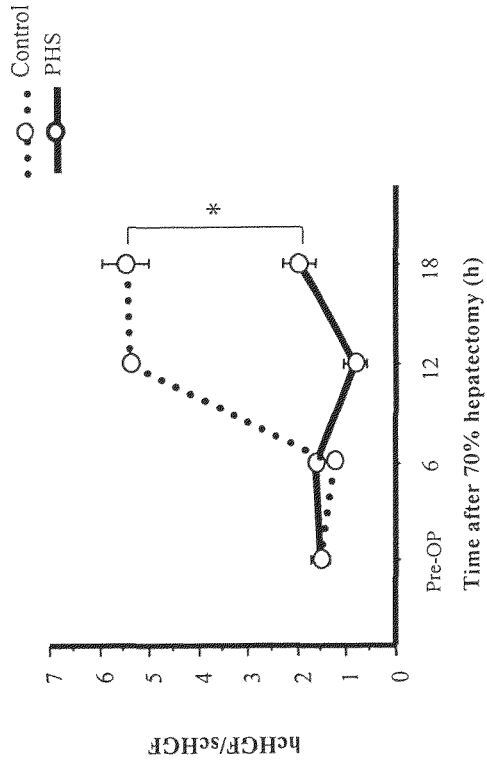


Fig. 6

SURGERY

Editors in Chief

Andrew L. Warshaw, MD
Surgeon-in-Chief
Massachusetts General Hospital
55 Fruit Street, White 506
Boston, MA 02114

April 5, 2004

Michael G. Sarr, MD
Department of Surgery
GI Unit, SMH
Mayo Clinic (Alfred 2-435)
200 First Street S.W.
Rochester, MN 55905

Managing Editor

Jenea R. Lomas
731 Cypress Road
Warminster, PA 18974
Tel: 215-442-0241
FAX: 215-328-9043

Masato Sakon, M.D.
Department of Surgery & Clinical Oncology
Osaka University Medical School
2-2 Yamadaoka, Suita City
Osaka 565-0871
Japan

Editorial Board

Adrian Barbul
Barbara L. Bass
R. Daniel Beauchamp
Kevin E. Behrns
Jacques Belghiti
Richard H. Bell, Jr.
John D. Birkmeyer
Kirby I. Bland
Murray F. Brennan
Colleen M. Brophy
Markus W. Buchler
Richard P. Cambria
William G. Cance
William G. Cioffi
G. Patrick Clagett
Pierre-Alain Clavien
A. Benedict Cosimi
Ara Darzi
Timothy J. Eberlein
Douglas B. Evans
B. Mark Evers
Victor W. Fazio
Lewis M. Flint, Jr.
M. Wayne Flye
Yuman Fong
Henri R. Ford
Julie A. Freischlag
Alden H. Harken
Richard Hodin
John G. Hunter
Jakob R. Izbicke
Danny O. Jacobs
Bernard M. Jaffe
V. Suzanne Klimberg
Stuart J. Knechtle
Steven D. Leach
Chung Mau Lo
Mark A. Malangoni
Jeffrey B. Matthews
Robin S. McLeod
Anthony A. Meyer
Ernest E. Moore
David M. Nagorney
Bernard Nordlinger
Carlos A. Pellegrini
Henry A. Pitt
Richard A. Prinz
Steven E. Raper
David W. Rattner
Robert V. Rege
Robert S. Rhodes
David A. Rothenberger
Ori D. Rotstein
Ajit K. Sachdeva
Diane M. Simeone
Geoffrey B. Thompson
Jon A. van Heerden
Michael A. West
Charles J. Yeo

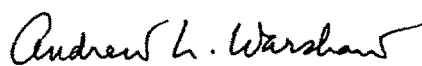
RE: OR 20030505

Dear Dr. Sakon:

Your revised manuscript entitled "EFFECT OF PORTAL HEMODYNAMICS ON LIVER REGENERATION STUDIED IN A NOVEL PORTOHEPATIC SHUNT RAT MODEL" has been reviewed by members of the Editorial Board of SURGERY and has been accepted for publication in SURGERY. Within the next few months, you shall be receiving the galley proofs for your corrections.

On behalf of the members of our Editorial Board, we would like to thank you for your submission of this excellent manuscript and for your continued interest in SURGERY.

Sincerely,



Andrew L. Warshaw, M.D.



Michael G. Sarr, M.D.

/jrl