Journal of Surgical Research **155,** 195–200 (2009)
doi:10.1016/j.jss.2008.09.011

# Inhibitory Effect of Curcumin on Early Liver Regeneration Following Partial Hepatectomy in Rats

Daniel Seehofer, M.D.,*[,1] Anja Schirmeier,* Stig Bengmark, M.D.,† Jessica Carter,* Martin Koch, M.D.,‡ Matthias Glanemann, M.D.,* Andreas K. Nüssler, Ph.D.,§ Peter Neuhaus, M.D.,* and Michael D. Menger, M.D.‖

*Department of General, Visceral, and Transplant Surgery, Charité Campus Virchow, Berlin, Germany;
†Department of Hepatology and Surgery, University College of London, Liver Institute, London, United Kingdom;
‡Department of Pathology, Charité Campus Mitte, Berlin, Germany;
§Department of Trauma Surgery, Klinikum Rechts der Isar, Technical University, Munich, Germany; and
‖Institute for Clinical and Experimental Surgery, University of Saarland, Homburg, Germany

Submitted for publication October 16, 2007

***Background.*** **Curcumin (Cur) is a nontoxic, hepatoprotective antioxidant. Recent investigations have demonstrated a protective effect of curcumin pretreatment during cold ischemia of hepatocytes, but its impact on liver regeneration *per se* has not been investigated so far.**

***Material and Methods.*** **Male Sprague-Dawley rats ($n = 6$ per group) underwent sham operation, 70% partial hepatectomy (PH), or PH with curcumin application (100 mg per kg bodyweight per day) starting 48 h before surgery. Rats were sacrificed 24 h after surgery. Liver regeneration was analyzed by measurement of relative liver weight, mitotic-index, bromo-deoxy-uridine (BrdU)-incorporation and Ki-67 expression.**

***Results.*** **The relative liver weight 24 h after surgery was similar in the PH groups with and without curcumin treatment. Also, a comparably high number of Ki-67 positive proliferating hepatocytes was detected in both groups. In contrast, the mitotic index in the untreated PH group (83 ± 20 mitosis/2000 hepatocytes) was significantly higher than in the curcumin treated group (21 ± 6). The BrdU labeling index was slightly higher in the curcumin treated group with PH (24% ± 5%) than in the untreated group (16% ± 2%). The hepatocyte density as marker of cellular hypertrophy was significantly lower in the curcumin group (474 ± 23) than in the untreated group (609 ± 22).**

***Conclusions.*** **Curcumin inhibits cell cycle progression during normal liver regeneration in rats, predominantly at the level of the G2/M transition point. However, the total liver mass and function was not significantly altered. Nevertheless, application of curcumin in conditions of high physiological cell proliferation should be performed with caution.** 

***Key Words:*** **curcumin; liver regeneration; partial hepatectomy.**

## INTRODUCTION

Curcumin is an inexpensive and nontoxic antioxidant that has been shown to be hepatoprotective [1]. It is a polyphenol, which is found in dietary spices like curry or turmeric, and is derived from dried rhizomes of the perennial herb *Curcuma longa*. The antioxidant capacity of curcumin is very high, for example markedly higher than that of vitamin E [2]. Accordingly, curcumin has been shown to efficiently prevent lipid peroxidation in rat hepatocytes during oxidative stress [3]. Other studies have confirmed its ability to scavenge oxygen free radicals [4–6], to increase intracellular glutathione concentrations [6, 7] and to prevent lipid peroxidation [8]. Normal cells are supposed not to be affected by curcumin [9].

A recent investigation demonstrated a protective effect of curcumin pretreatment during ischemia of hepatocytes by induction of hemoxygenase-1 [10] However, the impact of curcumin on liver regeneration after partial hepatectomy (PH) has not been addressed so far. Curcumin is also a known inhibitor of cell cycle

[1] To whom correspondence and reprint requests should be addressed at Department of General, Visceral, and Transplant Surgery, Charité Campus Virchow, Augustenburger Platz 1, D-13353 Berlin, Germany. E-mail: daniel.seehofer@charite.de.



0022-4804/09 $36.00

progression in various tumor cell lines, predominantly at the level of G2/M transition point [11, 12]. So far, it is unclear how these ambiguous effects of curcumin finally affect liver regeneration *in vivo*.

## MATERIAL AND METHODS

### Experimental Groups

Male Sprague Dawley rats (Winkelmann, Bochum, Germany) of 200 to 300 g bodyweight were housed four to six animals per cage and had free access to standard rat chow and water. Animals were kept at a 12-h day and night cycle at a constant room temperature. All experiments were performed in accordance with the German legislation on the protection of animals.

Rats were divided in three experimental groups ($n = 6$ animals per group): sham operation (group I), 70% partial hepatectomy (group II, PH) and 70% partial hepatectomy with curcumin application, [(group III, PH + curcumin (Cur)]. Rats were killed 24 h after surgery, since previous investigations [13] have shown that the most significant alterations in the regeneration process were found at this interval. All rats were operated by one of two surgeons, and were given isoflurane inhalation anesthesia.

Curcumin (Sigma-Aldrich, Steinem, Germany) was given orally by gavage at a dosage of 100 mg pure curcumin per kilogram bodyweight. Due to the poor water solubility of curcumin, it was dissolved immediately before application in 1.5 mL of a commercially available enteral feeding solution. In the curcumin group, all animals received three preoperative doses of curcumin (48 h, 24 h, and 30 min before surgery) and one postoperative dose 6 h after surgery.

### Surgery

Using a midline abdominal incision liver resection was performed using standard techniques as described in more detail previously [13]. Briefly, during sham operation, the liver was separated from its ligaments and the cecum was gently moved. Partial hepatectomy (PH) resulted in removal of approximately 70% of the liver tissue by resection of the middle and left lateral lobe after ligation of the vascular pedicles.

Postoperatively, rats received subcutaneous injections of 5 mL sterile isotonic saline to prevent exsiccation and metamizol plus tramadol for pain relief. These drugs were also added to the drinking water postoperatively. Re-laparotomy was performed after 24 h, the bile duct was cannulated, and bile was collected for 10 min. Afterwards, blood was drawn by aortic puncture and the liver was excised, weighed, and tissue samples were snap-frozen in liquid nitrogen or fixed in formalin.

### Histological Investigation

For histomorphological analysis, a paraffin embedded sample of the right liver lobe was cut in 4 $\mu$m sections and stained with hematoxylin and eosin (HE). The mitotic index was calculated per 2000 hepatocytes after counting the number of mitosis in 20 randomly selected high power fields (HPF).

### BrdU Immunohistology

Rats received an intraperitoneal injection of 100 mg Bromo-deoxy-uridine (BrdU; Roche, Mannheim; Germany) per kg bodyweight 30 min before harvesting. After this, *in vivo* BrdU labeling cells undergoing DNA synthesis in the S-phase can be identified by immunohistology. For this, 5 $\mu$m paraffin embedded sections were fixed, deparaffinized, and antigens were demasked by microwave treatment. BrdU incorporation was detected using a commercial detection kit following the manufacturer's protocol with slight modifications (Bromo-Deoxy-Uridine Labeling and Detection Kit II; Roche). Sections were counterstained with hematoxylin. BrdU positive hepatocytes were observed in 20 randomly selected HPF and quantified as percentage of total hepatocytes. The BrdU positive nonparenchymal cells (NPC) were calculated per 20 HPF.

### Ki 67 Immunohistology

The total number of proliferating hepatocytes was quantified by Ki 67 immunostaining as described in detail before [13]. Paraffin sections were fixed and deparaffinized. Epitopes were demasked by heat treatment in a pressure cooker. After blockage of endogenous peroxidase, sections were incubated for 30 min at room temperature with a mouse anti-rat Ki67 antibody (MIB-5; DAKO, Hamburg, Germany). The slides were washed and incubated with a biotinylated secondary anti-mouse antibody. Streptavidin peroxidase conjugate was added and developed with DAB and chromogen (DAKO). Counterstaining was done with hematoxylin. Positive hepatocytes were calculated as relative number of positive cells in 20 randomly selected HPF. All markers of hepatocyte proliferation (mitotic index, BrdU, and Ki67 index) were evaluated by one observer each, who was blinded for the corresponding study groups.

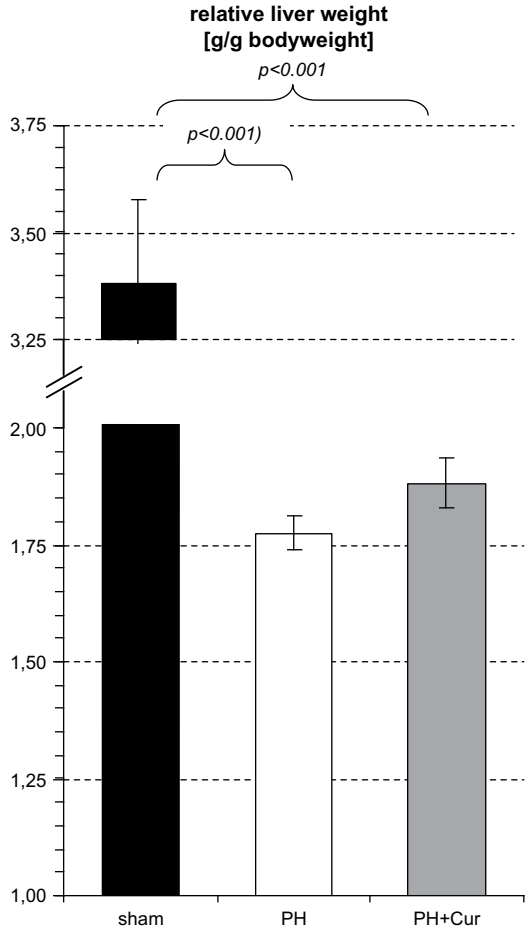


**FIG. 1.** Relative liver weight 24 h after surgery in relation to the bodyweight (*P* values of significant differences are indicated).

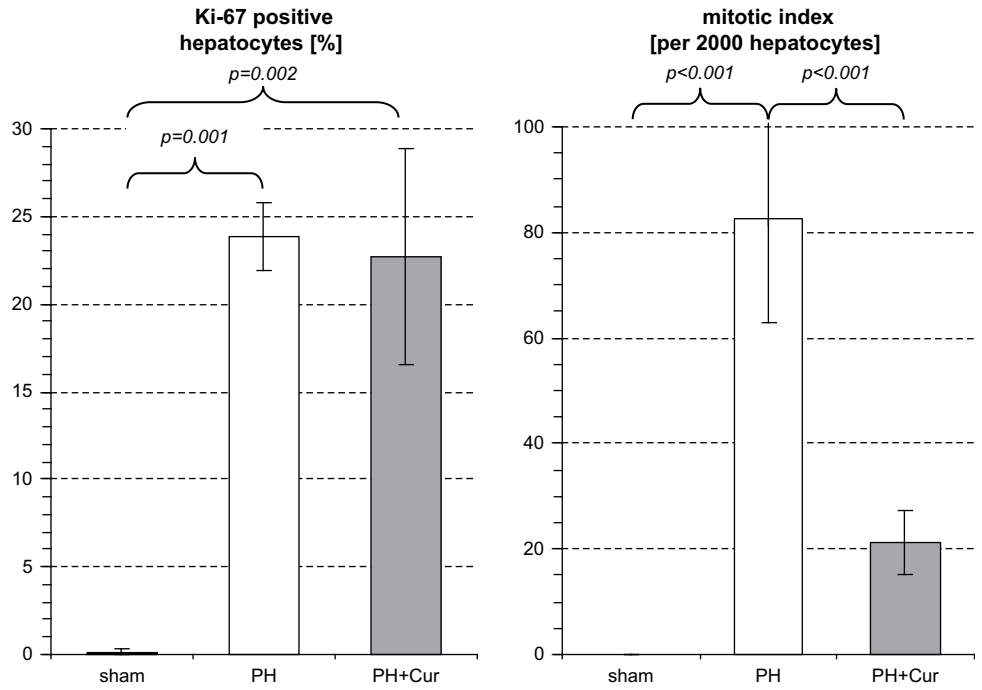


**FIG. 2.** Percentage of proliferating hepatocytes determined by Ki67 immunohistology (left) and mitotic index given as number of mitosis per 2000 hepatocytes (right) in the different experimental groups (*P* values of significant differences are indicated).

### Statistical Analysis

All values are given as mean and standard error of mean (SEM). Data were analyzed for normal distribution and equality of variance, and differences were then calculated by the Student's *t*-test for unpaired variables. Differences between the groups underwent a *post hoc* pairwise comparison using the Tukey test. Differences were considered significant if *P* was less than 0.05. All statistical analyses were performed by using SPSS 13.0 (SPSS Inc., Chicago, IL).

## RESULTS

All animals survived until the second operation. The bodyweight of the animals before primary and before secondary operation did no significantly differ between the partially hepatectomized groups. In the sham operated group, the weight loss between both operations

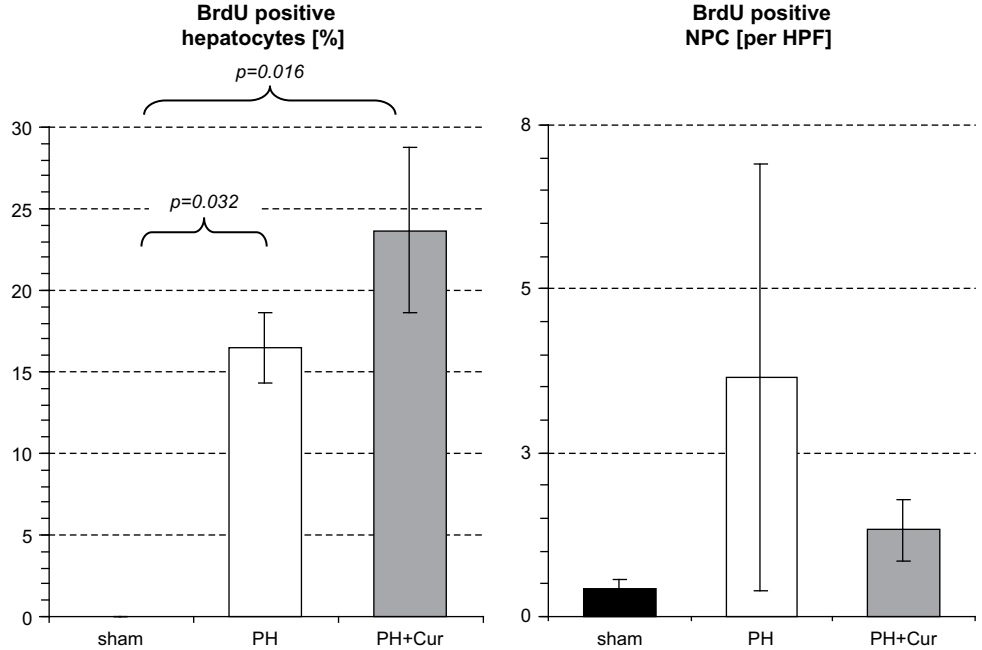


**FIG. 3.** Relative number of BrdU positive hepatocytes (left) and number of BrdU positive NPC per high power field (right) (*P* values of significant differences are indicated).

was significantly lower. The relative liver weight 24 h after liver resection was 1.78% ± 0.04% after partial hepatectomy (PH). Curcumin treatment increased the relative liver weight after 24 h slightly to 1.88% ± 0.05%, but the difference was not statistically significant (Fig. 1).

Immunostaining for Ki-67 as marker of the overall growth fraction showed only singular Ki-67 positive hepatocytes after sham operation (0.2% ± 0.2%). After PH with or without curcumin application, a comparably high number of Ki-67 positive hepatocytes was detected (Fig. 2).

The mitotic index was significantly higher in the untreated PH group (83 ± 20 mitosis/2000 hepatocytes) than in the curcumin treated group (21 ± 6 mitosis/2000 hepatocytes). No mitotic activity was found in the sham operated group (Fig. 2).

In contrast to the mitotic index, the relative number of BrdU positive cells as marker of S-phase activity was slightly higher in the curcumin treated group (24% ± 5%) than in the untreated group (16% ± 2%), but the difference was not statistically significant. In the sham operated group, no BrdU positive hepatocytes were seen (Fig. 3). No significant differences between all groups were observed in the number of BrdU positive nonparenchymal cells (Fig. 3).

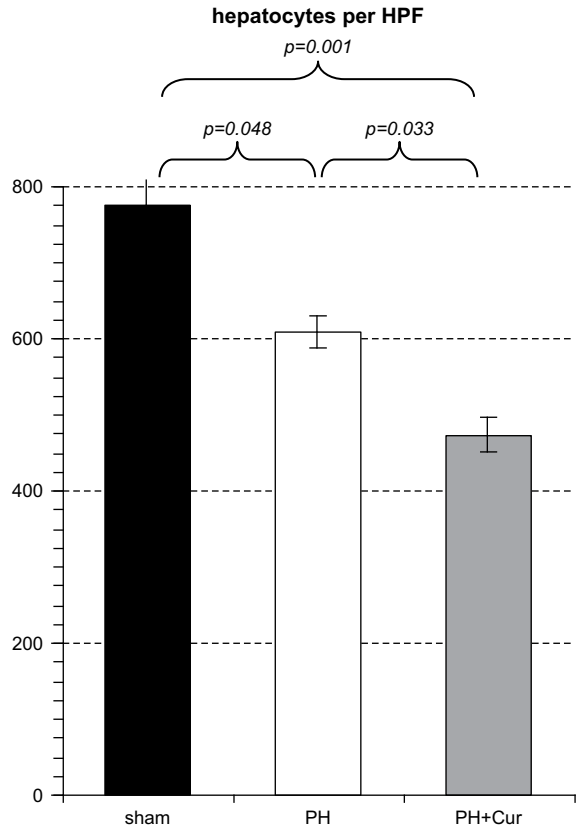


**FIG. 4.** Mean number of hepatocytes per HPF in the different groups. The numbers counted in the BrdU labeled slides are exemplary shown, the same tendency was seen in HE slides and Ki-67 stained slides (data not shown) (*P* values of significant differences are indicated).

The mean number of hepatocytes per HPF as marker of hepatocellular hypertrophy was evaluated in HE-sections, after Ki-67 and BrdU immunostaining. The values in all staining showed comparable tendencies. In the following the detailed data of the BrdU slides are exemplary shown (Fig. 4). The number of hepatocytes per HPF was highest in the sham operated group. Significantly less hepatocytes per HPF were seen after liver resection, pointing out cellular hypertrophy as one mechanism during the regeneration process. In the curcumin treated group, the hepatocyte density per HPF was significantly lower than in the untreated group after liver resection (Fig. 5).

Bile production per gram liver weight was similar 24 h after sham operation (79 ± 10) and PH (80 ± 9), but it was significantly higher in the curcumin treatment group (Fig. 6). However, the serum concentration of bilirubin was not different between groups II (PH) and III (PH + curcumin).

Histomorphological evaluation revealed no relevant inflammatory activity and no necrosis in any of the groups. Marked microvesicular fatty changes were seen after PH, whereas only minimal steatosis was present in the curcumin treated group. Other histomorphological differences were not observed, with the exception of different mitotic indices and hepatocyte density described above (Fig. 5).

## DISCUSSION

In the present study, perioperative curcumin treatment resulted in a significant suppression of liver regeneration and especially of the mitotic activity after liver resection. The present findings point to an inhibition of the cell cycle progression at the level of the G2/M restriction point by curcumin. In contrast, from the present data the earlier G1/S restriction point was not influenced by curcumin application. A comparable inhibitory effect of curcumin on cell cycle progression has been observed in various tumor cell lines [11, 12] in which also the predominantly mitotic phase was affected [14]. This was mediated among others by down-regulation of cyclin A, up-regulation of the cyclin-dependent kinase inhibitors p21 [11], p53, p27, and checkpoint kinase 2 [12]. In the present study, a curcumin mediated inhibition of cell cycle progression could also be proven during physiological regeneration of hepatocytes.

In general, the effect of oxidative stress on cellular proliferation is supposed to depend on its intensity.

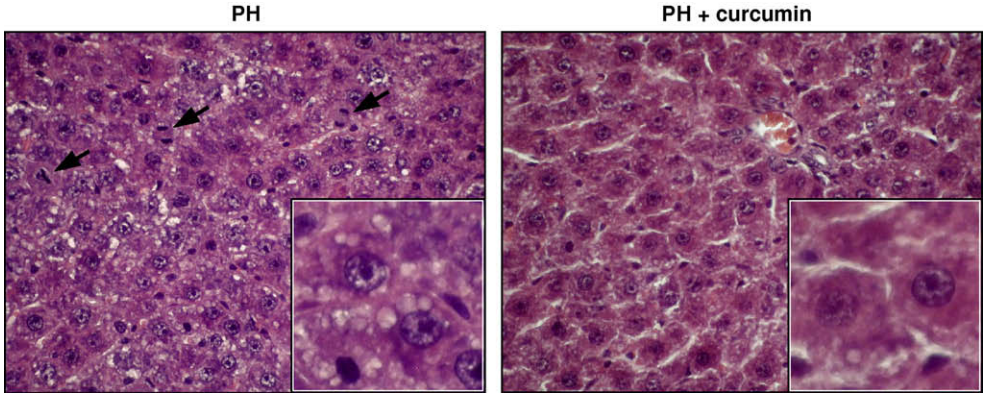


**FIG. 5.** Exemplary histomorphological specimen 24 h after partial hepatectomy (left) showing high mitotic activity (arrow) and microvesicular fatty changes. In the curcumin treated group (right), only minimal fatty changes, a very low number of mitosis and a low hepatocyte density due to hepatocellular hypertrophy is shown. (Color version of figure is available online.)

Whereas low oxidative stress has been shown to increase cell proliferation, high concentrations inhibit proliferation and even induce apoptosis or necrosis [15]. In the regenerating liver an increased antioxidant capacity leads to minimal peroxide values [16], but a low level of oxidative stress occurs during liver regeneration, correlating with the magnitude of hepatic loss and hepatocellular proliferation [17]. Antioxidant treatment with vitamin E has been shown to inhibit liver regeneration after partial hepatectomy [18]. In this study by Trejo-Solis *et al.*, a similar delay of hepatocellular proliferation was observed as in the present study and vitamin E application entailed a striking reduction of liver mass recovery. In contrast, curcumin application in the present model did not reduce the S-phase activity and not inhibit the restoration of liver mass. This points out distinct mechanisms of cell cycle inhibition.

A possible explanation for the restoration of liver weight despite inhibition of mitotic activity is an increase of the cellular mass of individual hepatocytes. This cellular hypertrophy is supposed to be a subsidiary mechanism of the hepatic regeneration process in rats. It is known that after complete blockade of mitosis, a normal increase of liver weight can be achieved in rats after 70% PH without DNA-synthesis or mitosis only by hypertrophy of hepatocytes [19]. Also in the present model hypertrophy of hepatocytes had to be considered, but the DNA-synthesis was maintained. However, in a distinct experimental setup, using a rat model of delayed liver regeneration, it could be shown that curcumin not as monotherapy but in combination with erythropoietin could significantly stimulate hepatocellular proliferation [20].

One limitation of the present model is that only the early phase of liver regeneration was studied. Differences later than 24 h cannot be excluded. However, most similar studies could show that differences in the regeneration process in rats are only observed at early time points [13].

As in the present study, a choleretic effect of curcumin with increase of the bile volume was observed in different models [21]. Since the excretion of bile acids was mostly not stimulated [21], the clinical relevance is unclear. Likewise, no differences in serum bilirubin levels were observed in the curcumin group despite a significantly higher bile production in the present experiments.

In conclusion, the present study shows an inhibitory effect of curcumin on cell cycle progression during normal liver regeneration in rats. Although this is the

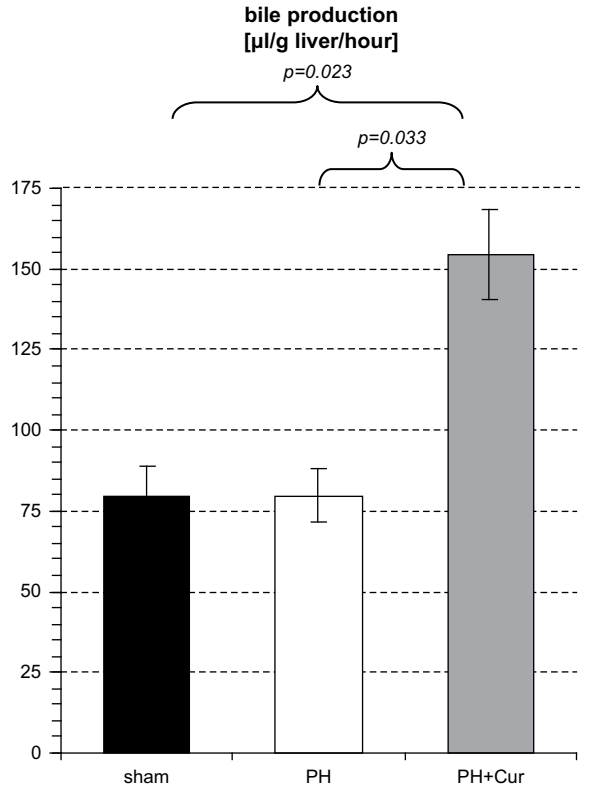


**FIG. 6.** Relative bile production per gram liver weight and h (*P* values of significant differences are indicated).

most striking effect at first glance, no severe negative effects were seen in the PH group. Nevertheless, due to the inhibition of the proliferation process, application of curcumin in conditions of high physiological cell proliferation has to be performed cautiously.

## ACKNOWLEDGMENTS

This study was supported by grants from the Deutsche Forschungsgemeinschaft (Se1694/2-1).

## REFERENCES

1. Bengmark S. Curcumin, an atoxic antioxidant and natural NFkappaB, cyclooxygenase-2, lipooxygenase, and inducible nitric oxide synthase inhibitor: A shield against acute and chronic diseases. JPEN J Parenter Enteral Nutr 2006;30:45.
2. Khopde SM, Priyadarsini KI, Venkatesan P, et al. Free radical scavenging ability and antioxidant efficiency of curcumin and its substituted analogue. Biophys Chem 1999;80:85.
3. Wei QY, Chen WF, Zhou B, et al. Inhibition of lipid peroxidation and protein oxidation in rat liver mitochondria by curcumin and its analogues. Biochim Biophys Acta 2006;1760:70.
4. Reddy AC, Lokesh BR. Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. Mol Cell Biochem 1994;137:1.
5. Ghoneim AI, Abdel-Naim AB, Khalifa AE, et al. Protective effects of curcumin against ischemia/reperfusion insult in rat forebrain. Pharmacol Res 2002;46:273.
6. Strasser EM, Wessner B, Manhart N, et al. The relationship between the anti-inflammatory effects of curcumin and cellular glutathione content in myelomonocytic cells. Biochem Pharmacol 2005;70:552.
7. Dickinson DA, Iles KE, Zhang H, et al. Curcumin alters EpRE and AP-1 binding complexes and elevates glutamate-cysteine ligase gene expression. FASB J 2003;17:473.
8. Reddy AC, Lokesh BR. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Mol Cell Biochem 1992;111:117.
9. Khar A, Ali AM, Pardhasaradhi BVV, et al. Induction of stress response renders human tumor cell lines resistant to curcumin-mediated apoptosis: Role of reactive oxygen intermediates. Cell Stress Chaperones 2001;6:368.
10. McNally SJ, Harrison EM, Ross JA, et al. Curcumin induces heme oxygenase-1 in hepatocytes and is protective in simulated cold preservation and warm reperfusion injury. Transplantation 2006;81:623.
11. Park C, Kim GY, Kim GD, et al. Induction of G2/M arrest and inhibition of cyclooxygenase-2 activity by curcumin in human bladder cancer T24 cells. Oncol Rep 2006;15:1225.
12. Zheng M, Ekmekcioglu S, Walch ET, et al. Inhibition of nuclear factor-kappaB and nitric oxide by curcumin induces G2/M cell cycle arrest and apoptosis in human melanoma cells. Melanoma Res 2004;14:165.
13. Seehofer D, Stockmann M, Schirmeier A, et al. Intraabdominal bacterial infections significantly alter regeneration and function of the liver in a rat model of major hepatectomy. Langenbeck's Arch Surg 2007;392:273.
14. Holy JM. Curcumin disrupts mitotic spindle structure and induces micronucleation in MCF-7 breast cancer cells. Mutat Res 2002;518:71.
15. Kang J, Zheng R. Dose dependent regulation of superoxide anion on the proliferation, differentiation, apoptosis and necrosis of human hepatoma cells: The role of intracellular Ca2+. Redox Rep 2004;9:37.
16. Wolfson N, Wilbur KM, Bernheim F. Lipid peroxide formation in regenerating liver. Exp Cell Res 1956;10:556.
17. Aguilar-Delfin I, Lopez-Barrera F, Hernandez-Munoz R. Selective enhancement of lipid peroxidation in plasma membrane in two experimental models of liver regeneration: Partial hepatectomy and acute $CCl_4$ administration. Hepatology 1996;24:657.
18. Trejo-Solis C, Chagoya de Sanchez V, Aranda-Fraustro A, et al. Inhibitory effect of vitamin E administration on the progression of liver regeneration induced by partial hepatectomy in rats. Lab Invest 2003;83:1669.
19. Nagy P, Teramoto T, Factor VM, et al. Reconstitution of liver mass via cellular hypertrophy in the rat. Hepatology 2001; 33:339.
20. Seehofer D, Neumann UP, Schirmeier A, et al. Synergistic effect of erythropoietin but not G-CSF in combination with curcumin on impaired liver regeneration in rats. Langenbecks Arch Surg 2008;393:325.
21. Deters M, Siegers C, Muhl P, et al. Choleretic effects of curcuminoids on an acute cyclosporin-induced cholestasis in the rat. Planta Med 1999;65:610.