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Experimental Studies on Liver Regeneration after Partial Hepatectomy in Rats with Obstructive Jaundice after Biliary Drainage: with Special Reference to Lipid Metabolism

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The present experimental study was carried out to determine how lipid metabolism was involved in the clinically important liver regeneration after hepatectomy for obstructive jaundice followed by biliary drainage. Experimental obstructive jaundice was prepared in rats, 1 week after which biliary drainage was performed. After 1 additional week, an approximate 70%-partial hepatectomy was performed in some rats. Similarly, the control rats received a 70%-partial hepatectomy. A profile of the time-sequential changes in lipid metabolism and liver regeneration in the residual liver was investigated and compared between both animal groups that underwent hepatectomy. Liver regeneration after hepatectomy in obstructive jaundice after biliary drainage was delayed compared with that of a resected normal liver. In the injured liver, however, lipid metabolism greatly contributed to rapid synthesis of cholesterol necessary for liver regeneration. The synthesized cholesterol was not utilized for the formation of cell membrane in the liver, which suggests potential damage in these processes. Future studies would warrant clarification of why the cholesterol synthesized in the liver was not used for cell membrane synthesis.

Key words: liver regeneration, hepatectomy, obstructive jaundice, biliary drainage, lipid metabolism

Introduction

Hepatobiliary disease with obstructive jaundice is often complicated by liver dysfunction and other organic disorders. The general concept for treatment for obstructive jaundice is that the obstructive jaundice is treated first followed by a radical hepatectomy after recovery of the liver function[1)~3)], not merely by a one-step surgical operation. Nevertheless, in actual clinical practice, we often experience liver failure developing after hepatectomy performed after appropriate biliary drainage. As the cause, it has been reported that it took many days to recover mitochondrial function[4)], microsomal function[5)], and glucose tolerance[6)] in the status with obstructive jaundice after biliary drainage. Delayed liver regeneration has also been suggested[7)8)].

In general, the synthesis of cholesterol and phospholipids required for the formation of cellular membranes are stimulated in liver regeneration[9)10)]. However, there are few reports that studied lipid metabolism in liver regeneration after hepatectomy followed by biliary drainage. The authors paid attention to this lipid metabolism as a cause that would delay liver regeneration in obstructive jaundice after biliary drainage.

In this study, we prepared partial hepatectomy models in rats with obstructive jaundice after biliary drainage, and the relation of lipid metabolism and liver regeneration in the residual liver was experimentally investigated.

Materials and Methods

1. Animals

Male Wistar rats aged 10 weeks (250 to 300 g, Charles River Japan Inc) were used in the experiment. All animal experiments were conducted in accordance with guideline of Animal Experiment Ethical Review Committee of Tokyo Women's Medical University.

2. Experimental models

1) Preparation of obstructive jaundice and the procedure for biliary drainage

The rat's abdomen was opened under anesthesia with intraperitoneal Nembutal (40 mg/kg). Common bile duct was removed, and a 3 Fr polyethylene catheter (Imamura Co Ltd, Tokyo) connected to a SP8 polyethylene catheter (NATSUME Co Ltd, Tokyo) was inserted and fixed by ligation. The common bile duct at the duodenal side was ligated. A 3-Fr polyethylene catheter (Imamura Co Ltd) was inserted and fixed to the duodenum. The distal end of both tubes was led outside the abdominal cavity as a blind ending tube to produce obstructive jaundice (Fig. 1A).

In the biliary drainage procedure, 2 blind ending tubes were connected to each other using a connector tube after 1 week of jaundice, and the bile juice was drained into the duodenum for biliary drainage (Fig. 1B).

2) Assessment of biliary drainage

A preliminary experiment was performed to assess if the biliary drainage was effective: experimental obstructive jaundice was prepared in rats, and biliary drainage was performed 1 week later (n = 20). Rats were sacrificed immediately before and 1, 3, 5 and 7 days after biliary drainage (n = 4, each). Untreated rats were used as a control (n = 4). Blood was collected from the inferior vena cava, and serum total bilirubin (T-bil) was measured.

3) Hepatectomy

Experimental obstructive jaundice was prepared in rats, and biliary drainage was performed 1 week later (n = 30, the injured group). An approximate 70%-partial hepatectomy was performed at 1 week after biliary drainage (n = 24) according to the procedures described by Higgins and Anderson[11]. In the normal control rats (n = 30, the normal group), 24 rats similarly received a 70%-partial hepatectomy.

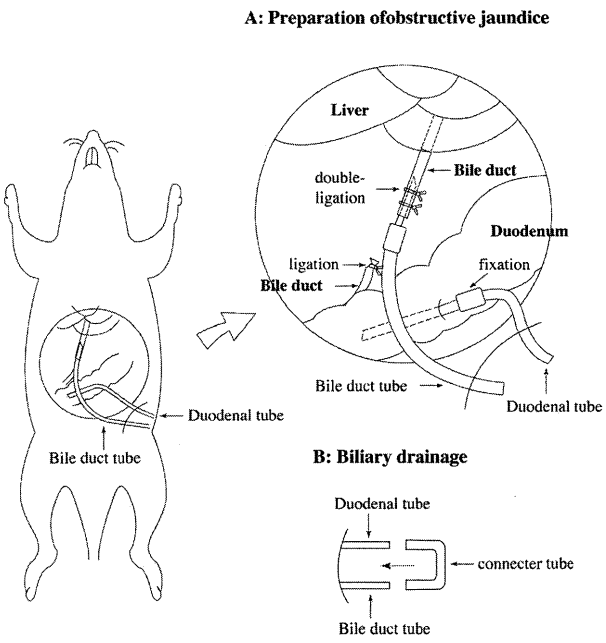

Fig. 1 Experimental rat model
Jaundice was prepared by canulating each blind-ending tube for 1 week, followed by biliary drainage for 1 week. A: Preparation of obstructive jaundice, B: Biliary drainage.

3. Biochemical analysis

Rats of both groups were sacrificed before hepatectomy and 1, 3, 5, and 7 days after hepatectomy (n = 6, each). Blood was collected from the inferior vena cava. The residual liver was isolated, weighed, and a part of resected liver tissue was frozen and stored. The remaining liver tissue was fixed in formalin, and paraffin embedded specimens were prepared. These blood and residual liver specimens were collected between 10: 00 a.m. and 11: 30 a.m. in consideration of the circadian rhythm[12], and the following parameters were measured.

Serum was separated from venous blood, and alanine aminotransferase (ALT), aspartate aminotransferase (AST) and T-bil were measured as a parameter of liver function. Total cholesterol (T-chol) and low-density lipoprotein (LDL) of blood lipid were measured as a marker related to lipid metabolism. Using the frozen liver specimens, LDL receptor (LR) m-RNA expression level ($\times 10^4$/copy/

ml) that expresses capacity of cholesterol uptake[13] and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity (pmol/mg/min at 37℃) that reflects capacity of cholesterol synthesis[14] were measured. LR m-RNA expression level was measured by PCR assay according to the procedures described by Lee[15] and Reinholz et al[16]. The Taq Man primer probe specific to the LR was designed. Total RNA was extracted from the frozen liver specimens (total RNA RNeasy kits, QIAGEN KK, Tokyo). This primer was hybridized to perform PCR, and the LR m-RNA expression level was measured using SDS7700 (Applied Biosystems Japan Ltd, Tokyo). HMG-CoA reductase activity was measured using the modification method (twofold scaled up) of the procedure of Kuroda and Endo[17]. The microsomes of the frozen liver specimens were extracted, and were incubated with reaction mixture containing [^{14}C] HMG-CoA. [^{14}C] mevalonolactone was separated from [^{14}C] HMG-CoA in accordance with the procedures by Nagata et al[18], and HMG-CoA reductase activity was assayed using a liquid scintilation counter LS 6000 SC type (Beckman Coulter Inc, California).

Liver regeneration capacity was estimated from the ratio (%) that divided the isolated residual liver weight by the estimated residual liver weight at hepatectomy and the mitotic index. The mitotic index was estimated as follows. The paraffin embedded specimen was sliced, stained with hematoxylin and eosin, and examined microscopically under a visual field of 400× amplification. The numbers of hepatic cells and mitotic cells in the M-phase in the 10 visual field were counted, and a ratio of the number of mitotic cells to 1,000 hepatic cells was calculated[19].

4. Statistical analysis

The results were presented as mean ± SD. Repeated-measures ANOVA (ANOVA) and the Mann-Whitney U test (MWU test) were used for statistical analysis. A difference of $p < 0.05$ was considered statistically significant.

Results

1. Assessment of biliary drainage in the experimental model

Serum T-bil was 0.1 ± 0.05 mg/dl prior to the preparation of obstructive jaundice (normal rat, control) and 9.8 ± 2.1 mg/dl immediately before biliary drainage. Serum T-bil steadily lowered after biliary drainage, at 1 week after biliary drainage, it was 0.3 ± 0.2 mg/dl, returned to the normal range, and the jaundice was eliminated (Fig. 2). Therefore, the biliary drainage in this experimental model was considered successful, and the injured group underwent a 70%-partial hepatectomy after 1 week of post-biliary drainage, the jaundice was completely eliminated.

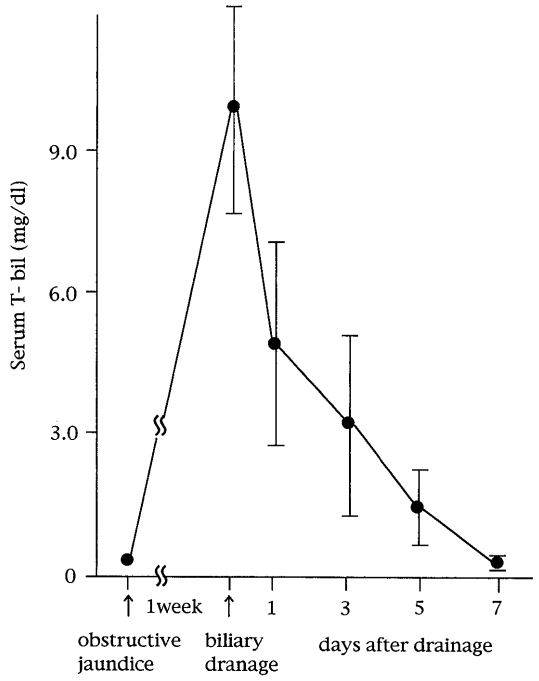

Fig. 2 A profile of changes in serum T-bil induced by obstructive jaundice after biliary drainage

2. Profile of liver function after hepatectomy

1) Serum AST and ALT

Serum AST and ALT in both the injured and normal groups were the highest on day 1, and recovered to their normal ranges on day 7 with no statistical difference between these 2 groups (Figs. 3 and 4).

2) Serum T-bil

Serum T-bil on day 1 in the injured group was 1.6 ± 0.7 mg/dl and increased significantly ($p = 0.004$, MWU test) with jaundice. Serum T-bil in the injured group was higher throughout the experimental period compared with the normal group ($p = 0.003$, ANOVA), and this jaundice showed a tend-

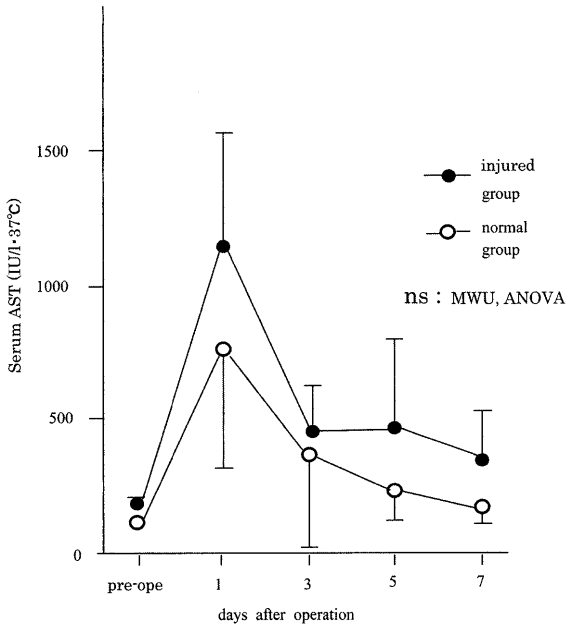

Fig. 3 Changes in serum AST after hepatectomy in the injured group and the normal group

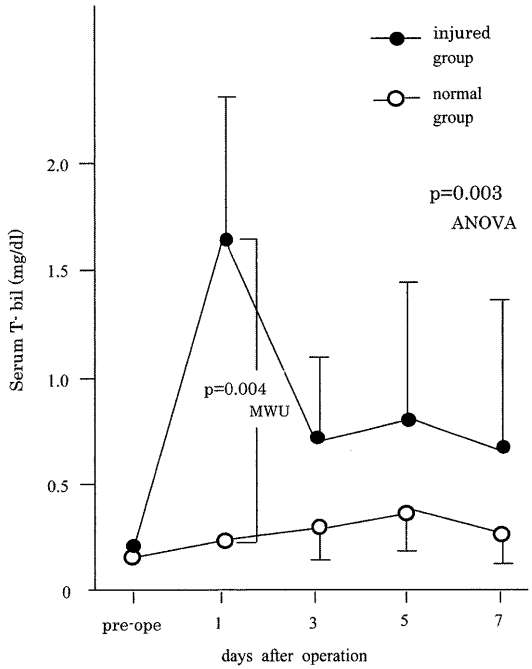

Fig. 5 Changes in serum T-bil after hepatectomy in the injured group and the normal group

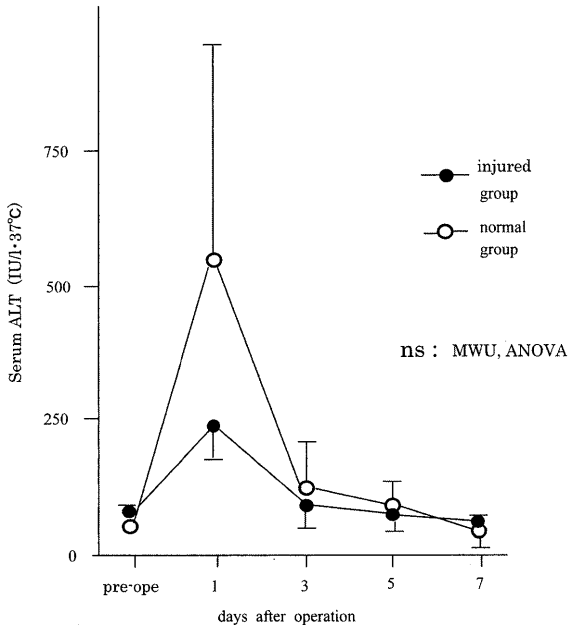

Fig. 4 Changes in serum ALT after hepatectomy in the injured group and the normal group

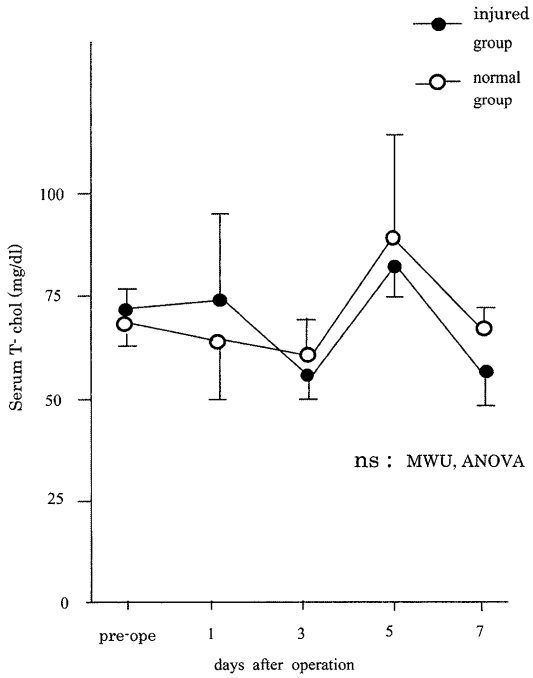

Fig. 6 Changes in serum T-chol after hepatectomy in the injured group and the normal group

ency to persist. No jaundice was observed in the normal group (Fig. 5).

3. Profile of lipid metabolism after hepatectomy

1) Serum T-chol

No marked changes in serum T-chol were observed in the injured and normal groups throughout the experimental period, and with no significant difference between these 2 groups (Fig. 6).

2) Serum LDL

Serum LDL at hepatectomy in the injured group

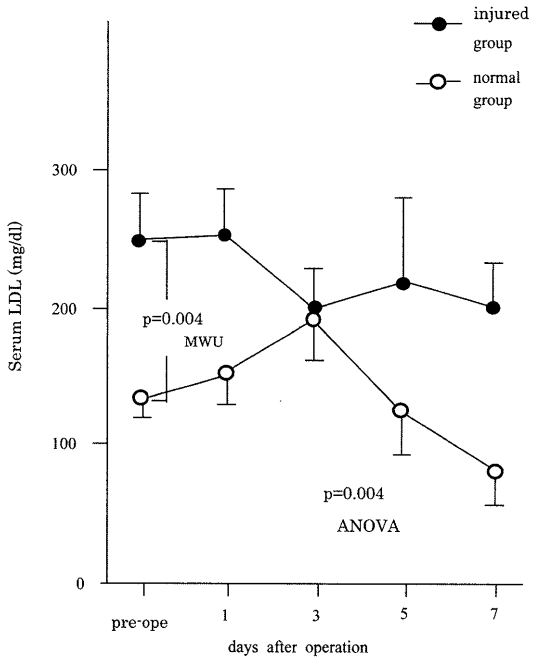

Fig. 7 Changes in serum LDL after hepatectomy in the injured group and the normal group

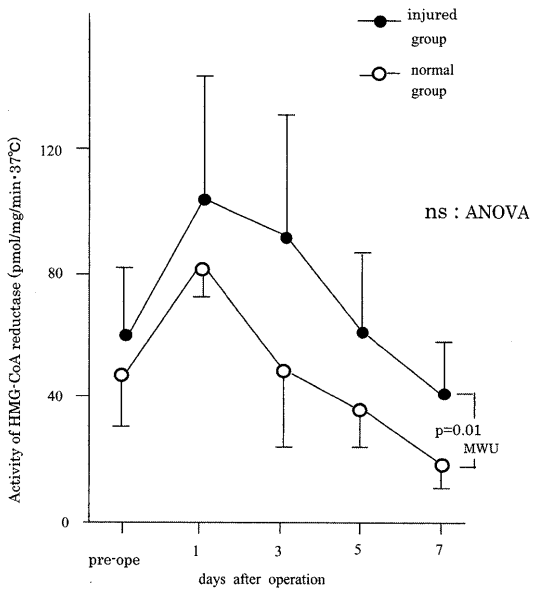

Fig. 9 Changes in hepatic HMG-CoA reductase activity after hepatectomy in the injured group and the normal group

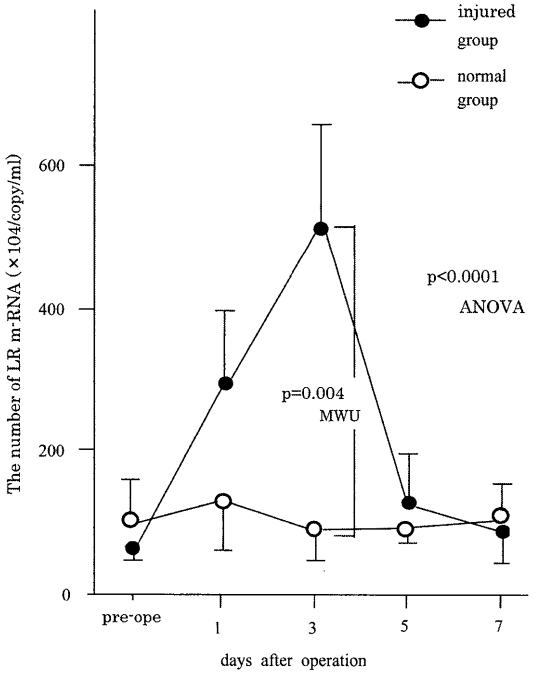

Fig. 8 Changes in yield of hepatic LR m-RNA expression level after hepatectomy in the injured group and the normal group

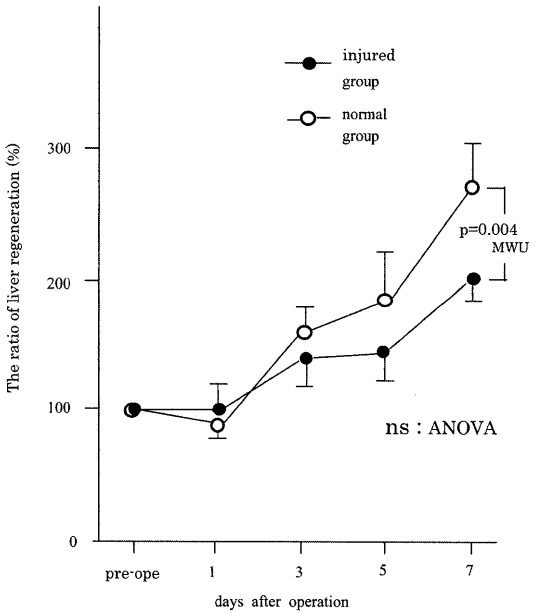

Fig. 10 Changes in the ratio of liver regeneration after hepatectomy in the injured group and the normal group

was 250.0 ± 41.1 mg/dl, and was significantly higher compared with the normal group ($p = 0.004$, MWU test). Serum LDL in the normal group showed a tendency to increase on day 1 and day 3, thereafter decreasing. Serum LDL in the injured group was significantly higher compared with the normal group throughout the experimental period ($p = 0.004$, ANOVA) (Fig. 7).

3) LR m-RNA expression level of liver tissue

LR m-RNA expression level of liver tissue ele-

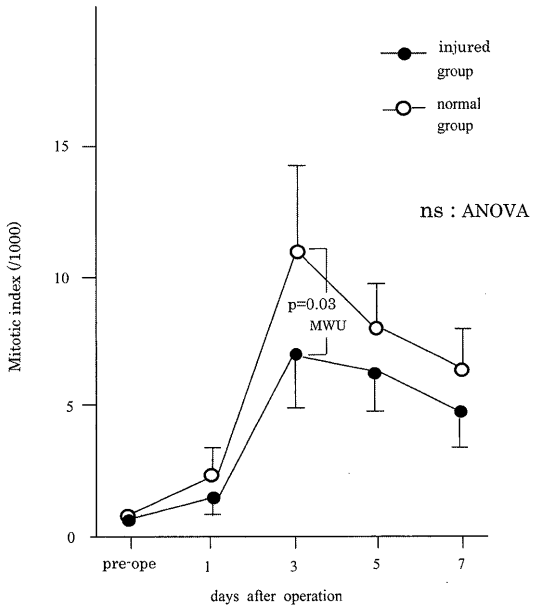

Fig. 11 Changes in the mitotic index after hepatectomy in the injured group and the normal group

vated from day 1, and on day 3 reached the maximum level of $506.10 \pm 142.7 \times 10^4$/copy/ml, which was significantly different from the normal group ($p = 0.004$, MWU test). The normal group did not show any marked changes. The LR m-RNA expression level of liver tissue in the injured group was significantly higher compared with the normal group throughout the experimental period ($p < 0.0001$, ANOVA) (Fig. 8).

4) HMG-CoA reductase activity of liver tissue

HMG-CoA reductase activity of liver tissue in both groups reached the maximum level on day 1, and thereafter lowered. HMG-CoA reductase activity of liver tissue in the injured group was higher compared with the normal group throughout the experimental period, and showed a significant difference on day 7 ($p = 0.01$, MWU test) (Fig. 9).

4. Profile of liver regeneration capacity after hepatectomy

1) Ratio of liver regeneration

Ratio of liver regeneration gradually increased in both injured and normal groups. Ratio of liver regeneration in the injured group maintained a lower level compared with the normal group throughout the experimental period, and showed a significant difference on day 7 ($p = 0.004$, MWU test) (Fig. 10).

2) Mitotic index

Mitotic index in both the groups reached the maximum level on day 3, and thereafter decreased. Mitotic index in the injured group maintained a lower level compared with the normal group throughout the experimental period, and showed a significant difference on day 3 showing the maximum level ($p = 0.03$, MWU test) (Fig. 11).

Discussion

One of the factors involved in liver regeneration is lipid metabolism, particularly cholesterol in the liver, needed for the synthesis and maintenance of cell membranes[9)10)]. The authors paid attention to this lipid metabolism as a cause to delay liver regeneration in obstructive jaundice after biliary drainage. In lipid metabolism, it has been reported that 7α-hydroxylase, a synthase of bile acid, and HMG-CoA reductase activity, a rate-limiting enzyme of the *de novo* synthesis of cholesterol, were stimulated in obstructive jaundice[20)], and both the LR m-RNA expression level and HMG-CoA reductase activity were stimulated in the regeneration of normal liver[9)21)]. The authors had previously investigated the changes in lipid metabolism induced by obstructive jaundice after biliary drainage[22)]. Based on these findings, we examined, in the experimental model, how lipid metabolism was involved in the clinically important liver regeneration in obstructive jaundice after biliary drainage.

In the experimental model of a rat whose bile duct was ligated, it was reported that blood flow in the hepatic tissue lowered from after 1 week. Moreover, there was a marked decrease of hepatic blood flow even after biliary drainage when the jaundice continued for 2 weeks or longer, and energy metabolism did not improve[23)]. In the present experiment, the period of jaundice was selected for 1 week that might improve hepatic tissue blood flow and hepatic energy metabolism, and a 70%-partial hepatectomy was performed after confirming that serum T-bil was normalized after 1 week post-biliary drainage.

In a profile of the changes in liver function after hepatectomy, serum AST and ALT did not show statistical differences between the injured and nor-

mal groups, while serum T-bil in the injured group increased significantly, and jaundice persisted longer compared with the normal group. These results suggested that recovery of the capacity of biliary bilirubin excretion delayed in the liver with obstructive jaundice even if the liver function was normalized after biliary drainage.

In lipid metabolism, in regard to serum T-chol, there was no statistical difference between the groups. However, LDL in the injured group was already higher prior to hepatectomy compared with the normal group, and it remained constant at a higher level even after AST and ALT were normalized. This LDL binds with LR as described below and is taken into hepatic cells where cholesterol is synthesized. In the injured group, a metabolic disorder associated with cholesterol was apparent even after liver function was normalized.

Cholesterol metabolism involves a variety of factors including food intake, fecal excretion, the amount of biosynthesized cholesterol in the liver, excretion from the liver and bile, or into the blood, and peripheral utilization[13)24)].

In the present study, we focused on lipid metabolism in liver regeneration in obstructive jaundice after biliary drainage, particularly hepatic cholesterol synthesis, and measured the LR m-RNA expression level and HMG-CoA reductase activity in the liver tissue. In the injured group, the LR m-RNA expression level and HMG-CoA reductase activity reached their maximum levels after 3 days and 1 day of post-hepatectomy, respectively, and maintained at higher levels throughout the experimental period compared with the normal group. On the other hand, LR m-RNA expression level in the normal group did not show marked changes, however, HMG-CoA reductase activity showed the maximum level 1 day after the hepatectomy. Considering LDL was at a high level in the injured group, uptake and synthesis of cholesterol in the liver appears to have been stimulated from the early stage of the post-hepatectomy.

In general, a feedback mechanism exists between the LR expression and HMG-CoA reductase activity, and cholesterol synthesis in the liver is controlled by both of them[10)]. The levels in the injured group were higher compared with those in the normal group, which suggest a high demand of cholesterol in the liver.

In regeneration of normal liver, content of cholesterol in liver tissue increased[10)], and both the LR m-RNA expression level and HMG-CoA reductase activity were stimulated[9)21)]. Most of these increased cholesterol were utilized for the formation of regenerating hepatic cell membranes[25)]. Hepatic cell proliferation has been reported starting within 12 hours of a partial hepatectomy in rats[26)27)]. Considering these findings, liver regeneration might be closely related to the demand of cholesterol. In particular, cholesterol synthesis in liver regeneration in the injured group was not damaged compared with the normal group, and if anything, it was much more stimulated in the injured group, so that the status of liver regeneration was better compared with the normal group.

However, ratios of liver regeneration and mitotic index were at lower levels in the injured group, and the liver regeneration corresponding to cholesterol synthesis was not observed. That is, recovery of liver regeneration capacity was insufficient in the injured group even if serum AST, ALT and parameters of liver function were normalized after biliary drainage.

In conclusion, liver regeneration after hepatectomy in obstructive jaundice after biliary drainage was delayed compared with that of a resected normal liver. In the injured liver, however, lipid metabolism greatly contributed to the rapid synthesis of cholesterol necessary for the formation of hepatic cell membranes in liver regeneration. However, the synthesized cholesterol was not utilized for the synthesis of hepatic cell membranes, consequently liver regeneration was delayed in the injured liver, which suggests potential damage in these processes. Future studies would warrant the clarification of why the cholesterol synthesized in the liver was not used for cell membrane synthesis, as well as the molecular mechanism of lipid metabolism and liver regeneration, particularly the relation to the hepatocyte growth factor.

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閉塞性黄疸解除後における肝切除後の肝再生
—脂質代謝からみた実験的研究—

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閉塞性黄疸を有する肝・胆道疾患に対して肝切除を行う場合，適切な黄疸解除処置を行った上で肝切除を施行しても術後に肝不全となる症例を経験する．この原因の一つに，黄疸解除後も肝再生が遷延することが挙げられる．一般に，肝再生時には細胞膜の合成に必要なコレステロール，リン脂質の合成が亢進する．しかし，黄疸解除後の肝臓における肝再生について，こうした脂質代謝の面から検討した報告はない．そこで本研究では，とくに臨床上問題となる閉塞性黄疸解除後の肝再生に脂質代謝がどのように関わるか実験的に検討した．Wistar 系雄性ラット（10 週齢）に閉塞性黄疸を作製し，黄疸解除後 1 週目に 70% 肝切除を行った．対照として，正常ラットに同じ方法で 70% 肝切除を加えた．肝切除前および切除後 1，3，5，7 日目にラットを犠牲死させ，下大静脈より採血し，肝機能や脂質代謝の推移を検索した．また残肝を全摘出し，肝再生能と肝再生に重要な肝組織におけるコレステロール取り込み能および合成能を測定した．結果として①閉塞性黄疸では，黄疸解除によりみかけの肝機能は回復しても脂質代謝の障害は遷延する，②閉塞性黄疸解除後の肝切除では，正常肝の肝切除に比べて肝再生が遷延する，③肝再生の遷延を脂質代謝の面からみると，こうした肝臓では，肝再生のために必要なコレステロールは急速に生合成されているが，このコレステロールが細胞膜合成に利用されていない，などが明らかとなった．利用されない原因は不明であるが，このことを中心とした肝臓における脂質代謝の障害が肝再生を遷延させる一因と考えられる．今後は，この利用されない原因の究明が重要と考えている．