Survival Surgery: Partial Hepatectomy

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Mouse Research Models/Mouse 101 Course
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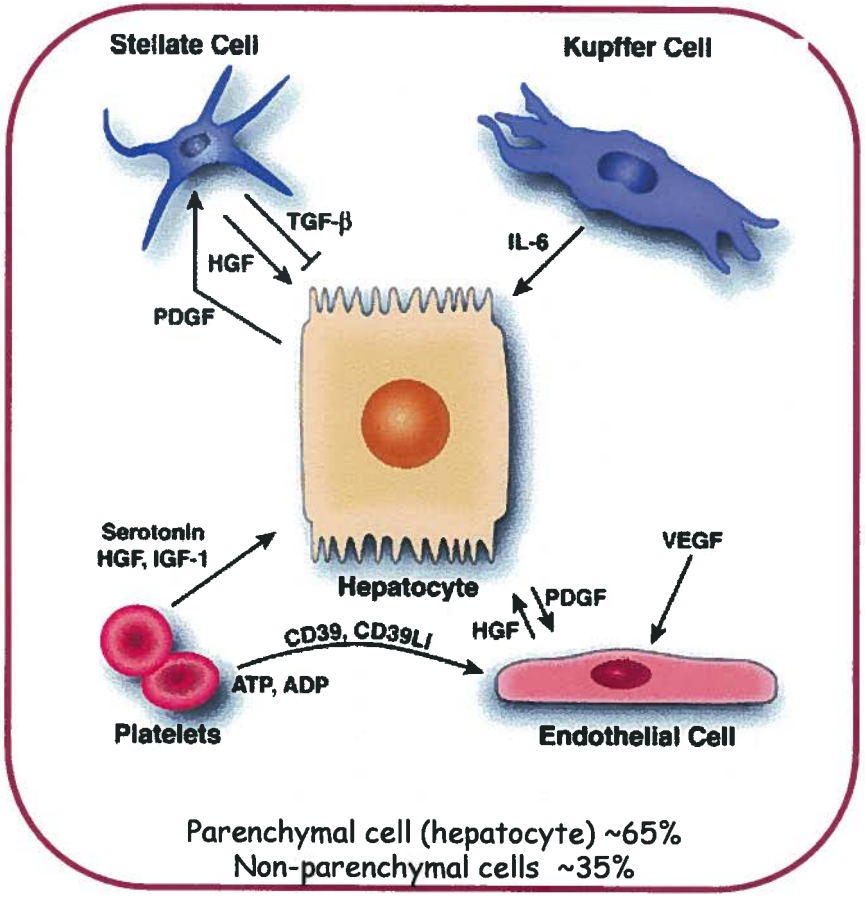
Prometheus - Early Champion of Liver Regeneration

- Human and rodent liver has an amazing regenerative capacity
- It can fully restore its mass within a short time after a significant tissue loss either from partial resection or toxic and viral injury
- The remarkable regenerating capacity of the liver was first described in Greek mythology
- Prometheus was a Titan who stole fire from Zeus and gave it to mortals. Zeus then punished him for this crime by tying him to a rock and making a great eagle to eat his liver every day. Liver grew back and was eaten again the next day.

Painting by Jacob Jordaens, 1640

Major Functions and Cell Types

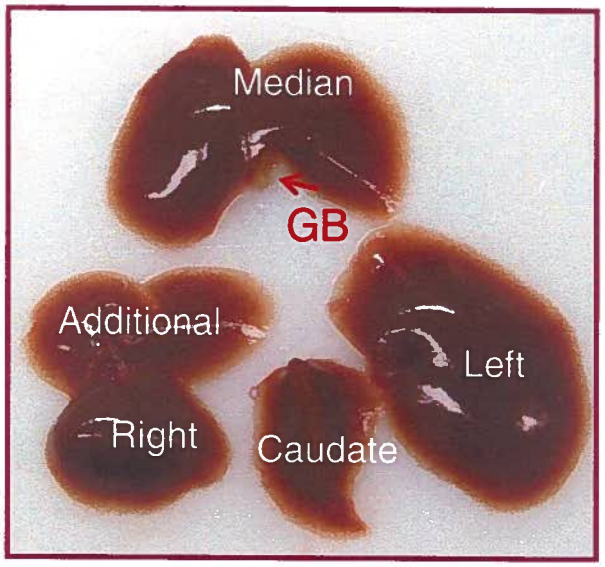
- The liver is the largest gland in the body and performs an enormously large number of tasks
- Metabolic homeostasis
  - Synthesis, storage and redistribution of nutrients, carbohydrates, lipids, hormones and vitamins
- Protein synthesis
  - Clotting factors
  - Albumin
  - Acute phase proteins
  - Transport proteins (e.g. low density lipoproteins)
- Biotransformation and detoxification
  - Bilirubin and ammonia
  - Steroid hormones
  - Xenobiotics
- Formation of bile and biliary excretion
  - Bile acid-dependent uptake of dietary lipids and vitamins
  - Bilirubin and cholesterol, metals
  - Xenobiotics
- The main cell type of liver that carries out most of these functions is the parenchymal cell, or hepatocyte

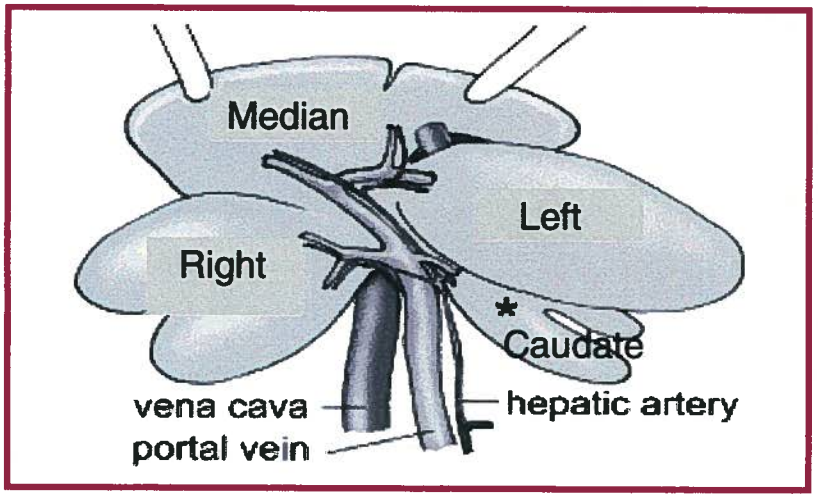


From S. Karp, Am J Transpl., 2009, 9:1973

Mouse Liver Anatomy

- The mouse liver is anatomically similar to the rat and human liver
- It is made up of several lobes that are anatomically distinct, each with its own blood supply and biliary drainage
- Each lobe can be securely tied off the base leaving as small stump
- Four major liver lobes are identified:
  - Median
  - Left lateral
  - Right lateral
  - Caudate

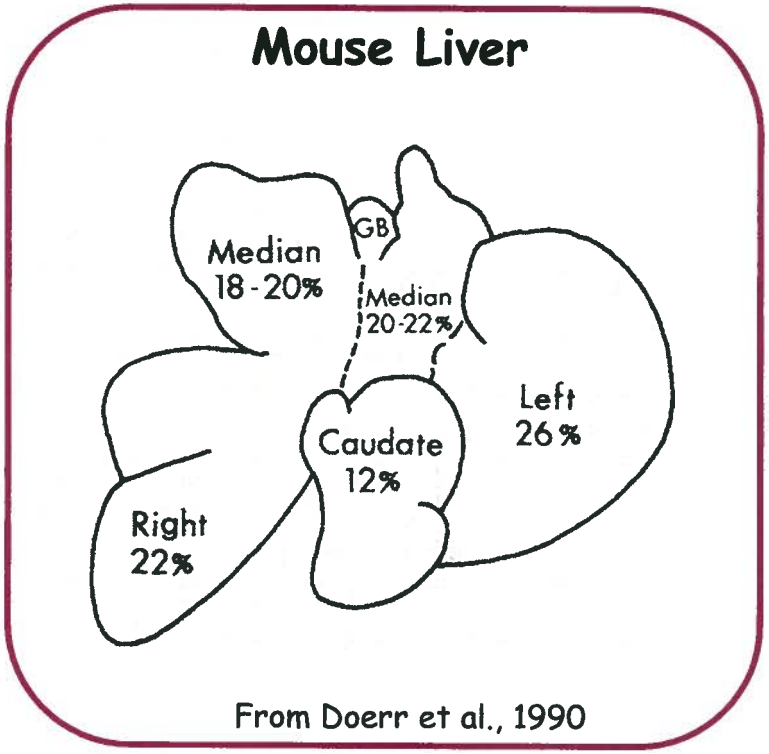




- Median lobe is divided into right and left segments separated by the gall bladder
- Right lobe has two additional segments
- The number of liver lobes may be referenced as 5 or even 7 lobes if all segments are counted
- Resection of the median and left lateral lobes results in removal of about 64-68% of the total liver mass
- A technique known as 2/3 partial hepatectomy (PH)

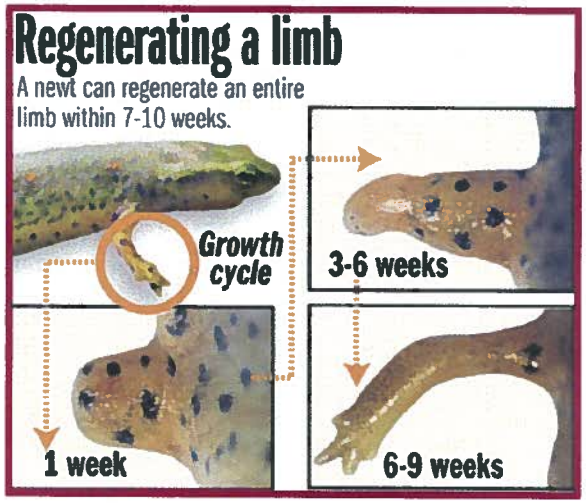
2/3 Partial Hepatectomy

- The technique of 2/3 PH for the study of liver regeneration was first described in rats by Higgins and Anderson in 1931 (Arch. Pathol. 1931: 12, 186-202)
- In most strains of mice the relative weights of the respective lobes are constant
- Mammals can survive surgical removal of up to 75% of the total liver mass
- If more than 75% of liver is removed, the remaining liver mass may be insufficient to maintain critical levels of blood glucose
- After resecting 2/3ds of the liver, the remaining hepatocytes undergo one or two rounds of replication without complications of hypoglycemia
- The removal of less than 1/3 of liver does not elicit a generalized proliferative response
- 2/3ds partial hepatectomy is the method of choice to study the mechanism of liver regeneration and cell cycle dynamics

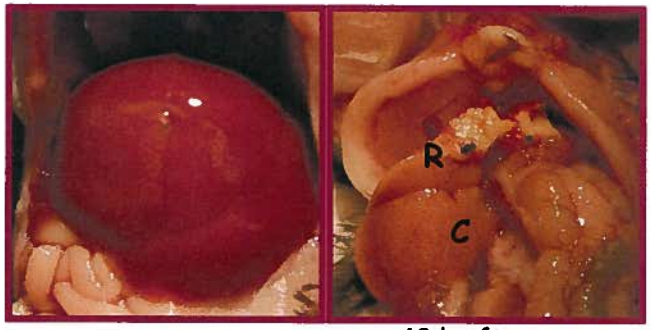


Compensatory Liver Growth

- Unlike the complete re-growth of the limbs in amphibian models, liver regeneration after PH is a very different process
- The liver is not restored to its original form because the excised parts do not grow back
- The remaining liver expands in mass to compensate for lost tissue
- Liver regeneration is a process of compensatory growth which stops when the original liver mass is restored



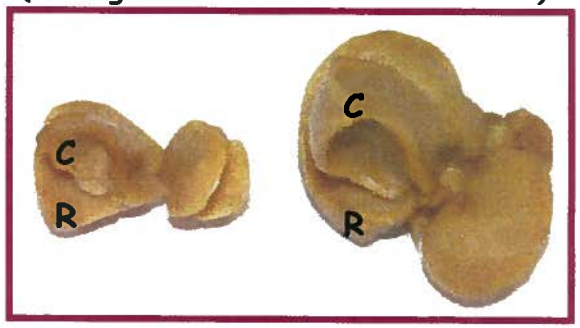
Mouse Liver Regeneration



48 h after surgery

Compensatory Liver Hyperplasia

(Enlargement of the residual lobes)



Adapted from Boyce et al., 2008

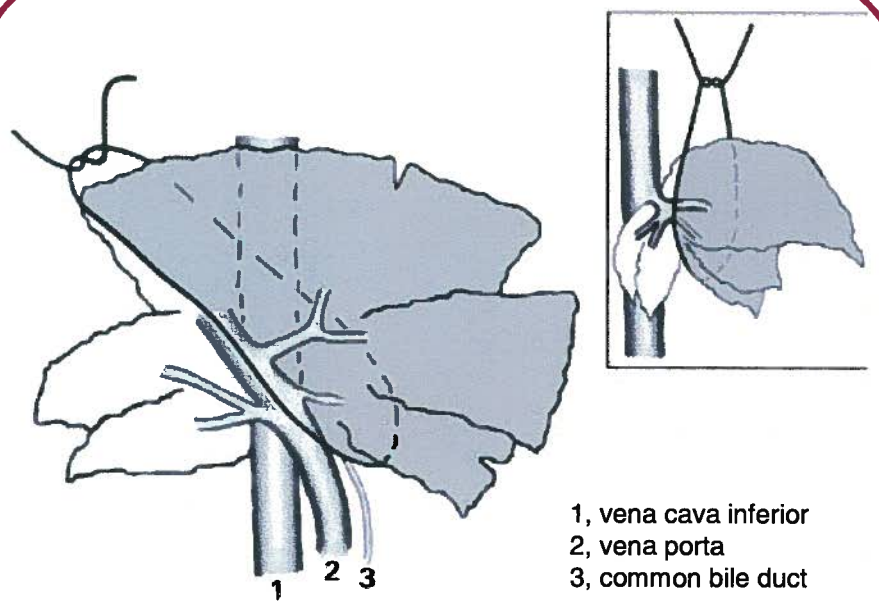
Hepatic Surgery

- Hepatic surgery in mice is challenging because of the delicate nature of the liver and risk of obstructing blood and bile flow resulting in hemorrhage and necrosis
- The procedure is not difficult and only requires basic surgical skills
- It is not associated with mortality and can give highly reproducible results
- The outcome can greatly vary if the operation is not performed properly
- The extent of the proliferative stimulus is directly related to the amount of liver resected
- Consistency in the surgical approach is critical

Before Beginning Any Procedures with Animals (including pilot studies), Ensure that ALL Procedures Have Been Described in an Animal Study Protocol (ASP) and Approved by the Institutional Animal Care and Use Committee

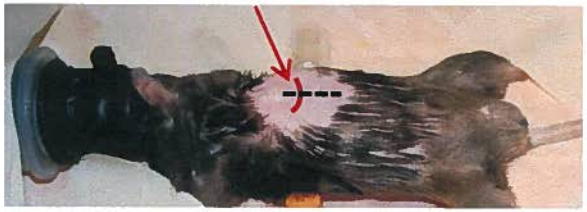
Schematic Illustration of PH

First Method - One Knot



Adapted from A.C.Aoun Tannuri et al., 2007

Xyphoid process, easily seen and palpable



- A midline abdominal skin and muscle incision (about 2 cm) to expose the xyphoid process and allow for the extrusion and extra-abdominal ligation of the lobes

An advantage:

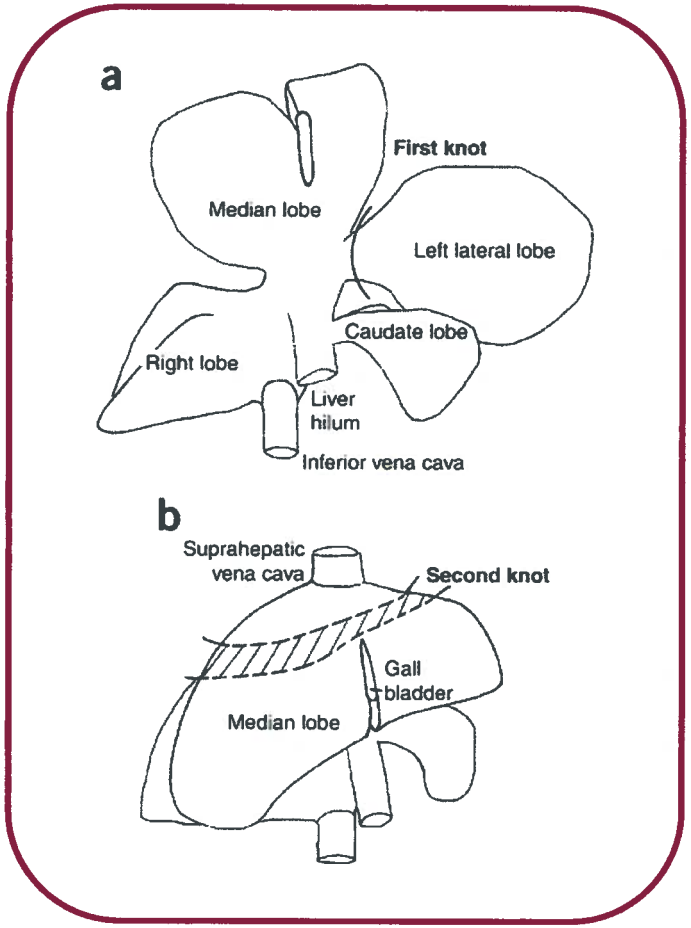
- Requires only a small incision
- Can be performed fast (less than 10 min)

The disadvantages:

- Limited visibility impairs the precision
- Lack of precision can cause complications such as vena cava stenosis, bile duct obstruction and liver congestion

Schematic Illustration of PH

Second Method - Two Knots



- To achieve more reproducible results, the second method of PH is based on a larger abdominal incision (about 3 cm) and two separate ligatures
- The thread for the first knot is positioned between the caudate and the left lateral lobes at the base of the left lobe
- The second knot should be tied within the dashed area, above the gall bladder which will be removed but not close to the suprahepatic vena cava to avoid stenosis and necrosis of the remaining liver lobes

Timing

The surgery takes about 10 -15 min

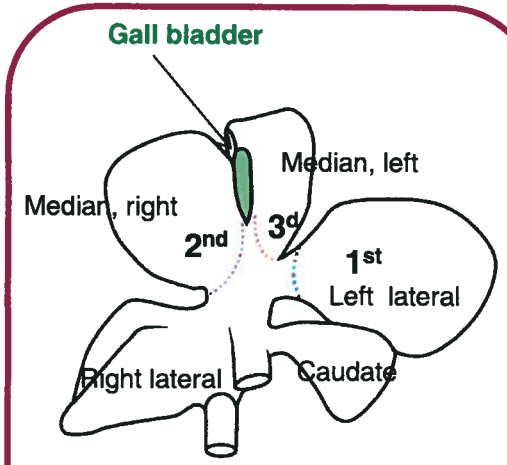
This method is published in Nature Protocols, 2008, 3:1167-1170 by Mitchell and Willenbring and illustrated by corresponding videos.

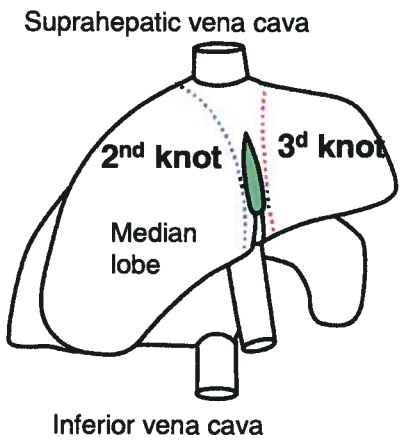
All technical details can be found in this publication.

Mitchell and Willenbring, Nature Protocols, 2008, 3:1167-1170

Schematic Illustration of PH

Third Method - Three knots





- The presence of gall bladder in the mouse presents a problem
- Currently, the most accepted protocol of PH is based on three separate ligatures
- This method keeps the gall bladder intact to prevent the obstruction of the extra-hepatic biliary tree
- The first knot is tied at the base of the left lobe
- The second knot is tied at the base of the right portion of the median lobe avoiding the gall bladder
- The third knot is positioned at the base of the left portion of the median lobe

A premade loop of silk suture to assist in positioning of knots around liver lobe to be resected

References

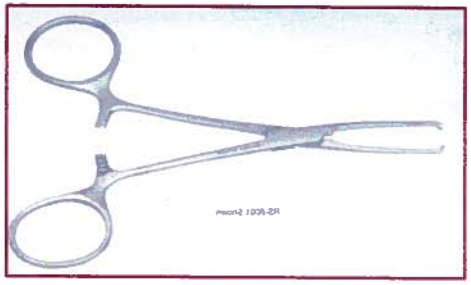
1.Boyce & Harrison, Lab Animal, 2008, 37:529

2.Greene & Puder, J Invest Surgery, 2003, 16:99

Bolster (sterile roll, 1 ml syringe)

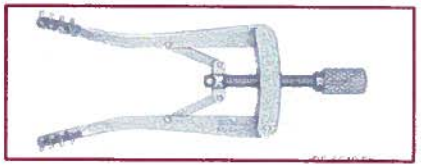
To increase operative exposure to the liver

Allis clamp



To lift the xyphoid process backward

Alam retractor



To increase the superior exposure of the liver and falciform ligament

Recovery from Surgery

Mice should recover within minutes after the end of surgery and isoflurane anesthesia

No mortality is seen in wild-type mice

Perioperative mortality is due to technical errors:

- Accidental penetration into the chest cavity during the cutting of the falciform ligament or by the tips of the forceps when placing silk suture underneath the liver lobe

- A bowel injury during the opening of the peritoneum

Minimal morbidity within the first 12 h

- Shivering
- Decreased mobility
- Hunched posture

Fluid replacement (Optional)

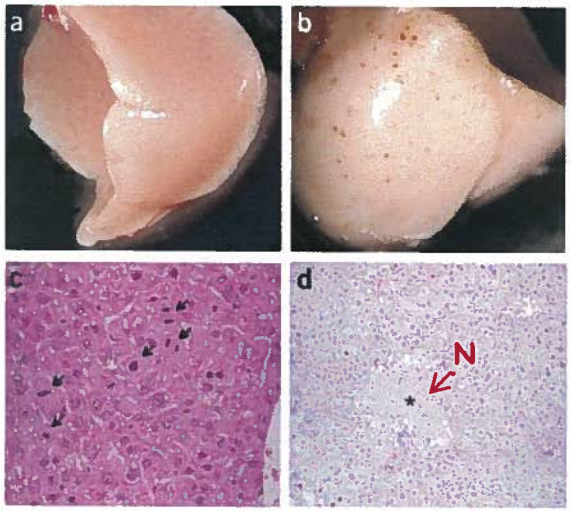
- From liver removal and prolong operative exposure of abdominal cavity
- s.c. Bolus of warm dextrose saline (0.75-1 ml, NaCl 0.19%, dextrose 4%)
- A warming blanket to maintain the mouse's core temperature

Mechanical obstruction

- Placing ligatures too close to the suprahepatic vena cava or inferior vena cava and common bile duct can cause stenosis, jaundice and necrosis in the remaining liver lobes
- The liver will become pale and spotted with mild bleeding

These livers do not regenerate properly and should not be included in the study

Pale liver with necrotic areas (N)



Adapted from Mitchel and Willenbring, Nature Protocols, 2008

Experimental Design

Anesthesia

- The second most important factor in 2/3 PH
- Has a profound impact on postoperative morbidity and mortality
- Some agents are hepatotoxic, and their toxicity can be amplified in mice that carry mutations in genes relevant to hepatocyte survival

Injectable anesthetic agents

Avertin
- Highly hepatotoxic
- High postoperative morbidity and mortality

Ketamine/xylazine
- Is not associated with mortality
- Has more side effects (longer awakening times, higher hepatoxicity) than isoflurane

Inhaled anesthetic agents

Isoflurane
- Anesthetic of choice for 2/3 PH
- Least toxic
- Removed immediately after the procedure
- Allows rapid recovery of the animal after surgery

If a vaporizer is not available, use ketamine/xylazine

Analgesia

- Required for rodents after surgery
- Should be carefully considered

Buprenorphine

- The commonly used analgesic buprenorphine has been shown to have hepatotoxic effects (Skoulis et al., Toxicol. Appl. Pharmacol., 1989)
- Narcotic analgetic, should not been administered to an animal that is already under anesthesia with an injectable agent which cannot been reversed
- If buprenorphine is used for anesthetic induction, the dose of anesthesia must be reduced by 10-25%

Marcaine (bupivacaine)

- Injected subcutaneously at the incision site
- Provides 8-10 h of post surgical local anesthesia
- Used as 1/10 dilution of 0.25% Marcaine in sterile water, saline or PBS, 0.1 ml/25 g mouse

Experimental Design

Time of Surgery

- Circadian rhythms affect the timing of DNA synthesis and mitosis in the liver
- Most researchers perform 2/3 PH experiments during morning hours

Fasting

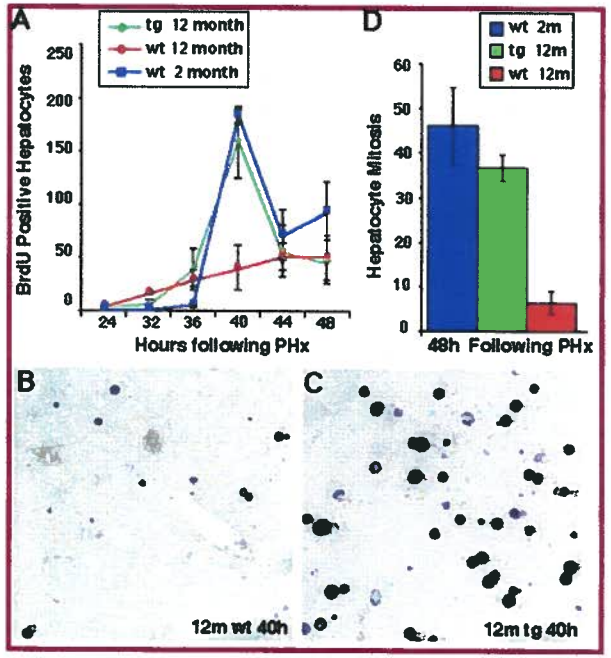
- To synchronize the metabolic state of hepatocytes before 2/3 PH, some investigators use fasting before surgery
- Fasting induces hepatic steatosis (accumulation of lipids)
- This can cause impairment of liver regeneration as an excessive fat accumulation in hepatocytes impairs proliferation

Gender

- The gender has no obvious effect on the regenerative process in wild-type animals
- There is a possibility that certain genetic modifications could induce sex-dependent effects after PH
- Animals of the same sex are used for PH studies

Age

- Affects hepatic regeneration
- Young animals (4-6 wk) restore their liver mass more rapidly than adults
- Mice older than 10 months of age, present a lag in liver regeneration
- It is recommended to use 8 to 14 wk old mice

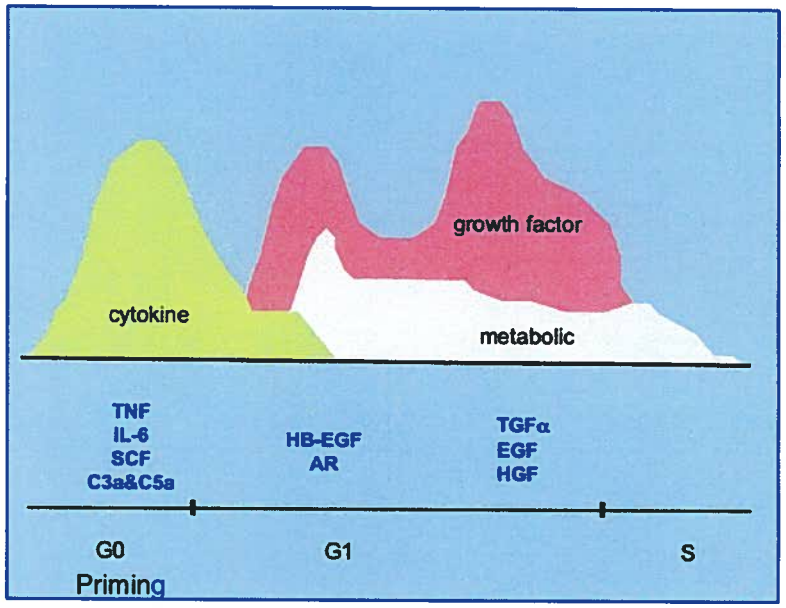


Wang et al. , PNAS, 2001;98:11468-11473

Molecular Basis of Regeneration

- Rapid and synchronized response to injury
- Involves all cell types and depends on a multitude of interrelating signaling networks:
  - Cytokine
  - Growth factor
  - Metabolic
- Redundancy within and between each network
- No single mouse model shows a complete blockage of DNA replication and cell division
- Distinct stages that are regulated by different mechanisms:
  - Initiation of liver regeneration or "priming" phase is regulated by activation of a cytokine network
  - Cell cycle progression driven by growth factors, including EGF, TGFa and HGF

Major Regulatory Networks



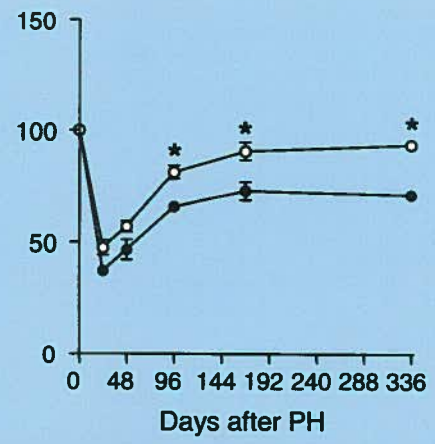
From N. Fausto, Hepatology, 2006

Evaluation of Regeneration

Liver Weight

- Restoration of liver mass is the simplest but the least reliable index of regeneration
  - Liver weight may change with deposition of lipids, glycogen, etc., not directly related to the proliferative response
  - Precise weighing is difficult because of the variable amount of blood
- The liver to body weight ratios can be used for estimating the rate of regeneration
- When harvesting the regenerating livers, only the right and caudate lobes are used for analysis
- The stumps and the part of the median lobe that might have remained should be excluded, as they may contain necrotic tissue
- Seven days after 2/3 PH, 80% to 90% of the original liver mass is recovered (i.e., liver weight corresponds to about 3.5-4% of total body)

Kinetics of Liver Regeneration



A = Weight of liver at autopsy/ Weight of mouse

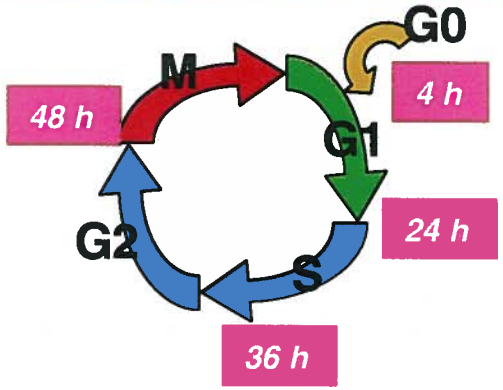
B = Weight of liver before surgery/ Weight of mouse

% Regeneration = A/B x 100

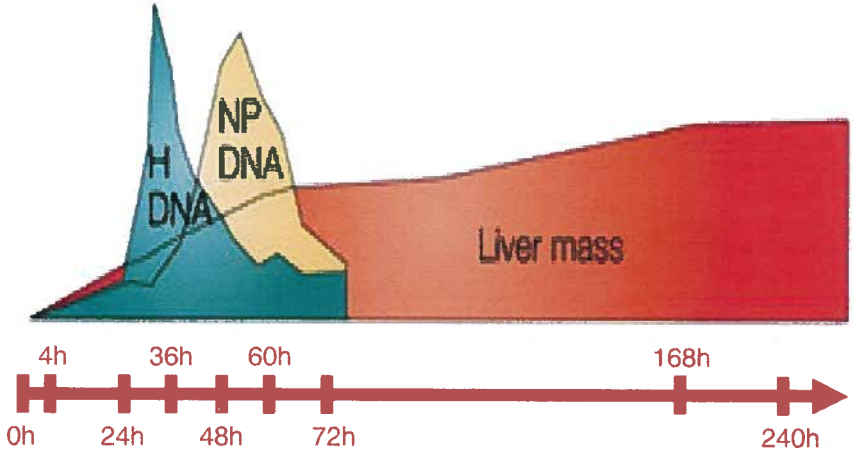
Evaluation of Regeneration

Histological Criteria

- Morphology
- Quantification of DNA synthesis
  - BrdU incorporation followed by IHC
  - Analysis of Ki67 or PCNA expression
- Count of mitoses



Kinetics of DNA Synthesis



From R. Traub, Nature Review, 2004

- Hepatocytes are normally quiescent cells
- Enter into a pre-replicative or priming phase
- The rate of hepatocyte proliferation begins to increase after 24 h and peaks around 36 h
- A smaller percent of cells enter into a second round of DNA synthesis to re-establish the original number of hepatocytes
- In the non-parenchymal (NP) cells, the induction of DNA synthesis occurs later
- A small wave of apoptosis is seen at the end of DNA synthesis
- In young adult mice, the process of liver regeneration is complete within 7-14 days