Hepatic Toxicity

MSc in Molecular Pathology and Toxicology 2001

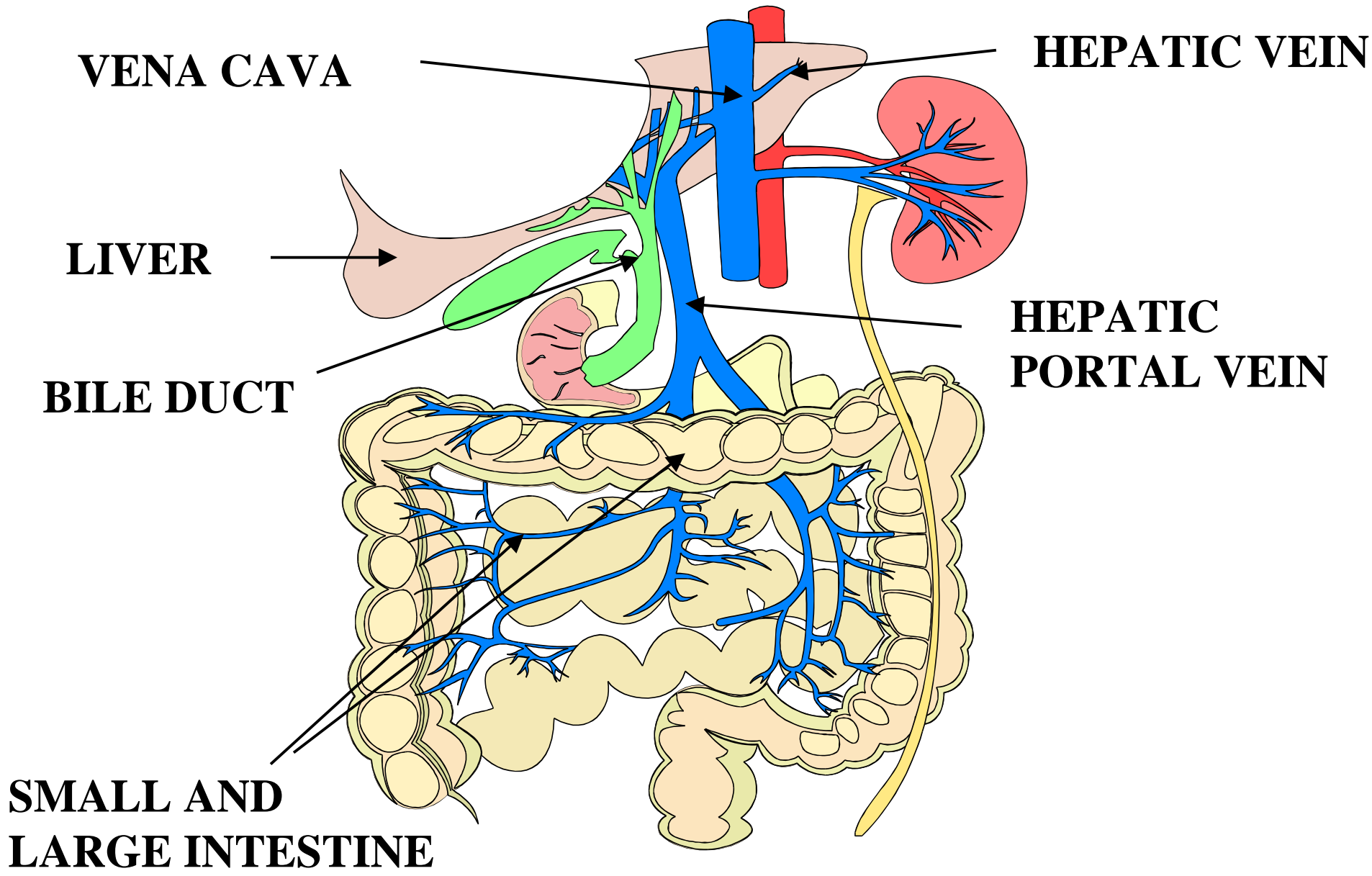**Andy Smith**

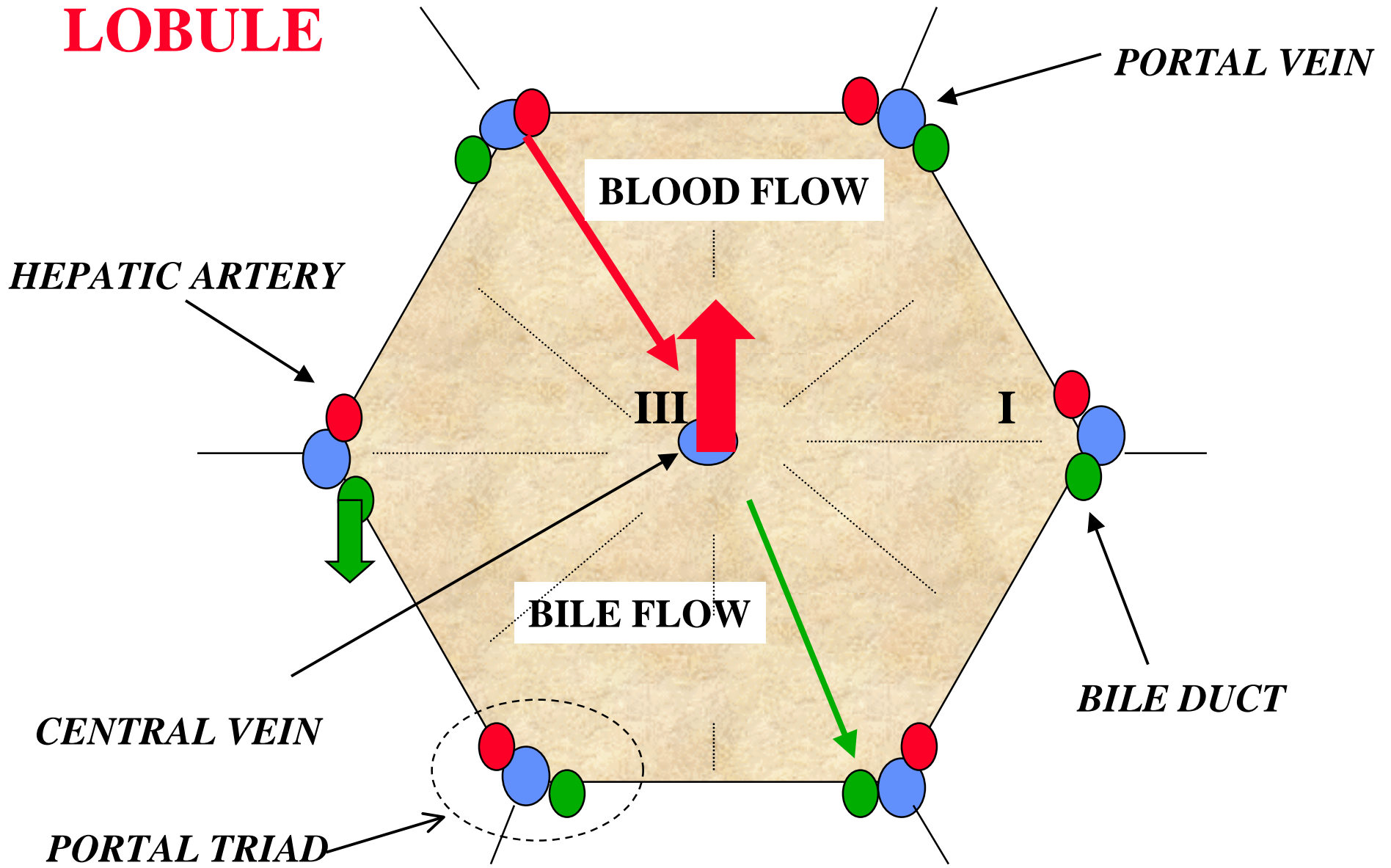**MRC Toxicology Unit**

THE LIVER

- **The liver constitutes about 5% of the body mass of a rodent or human.**

- **It has many functions eg.**
    - Carbohydrate storage and metabolism
    - Synthesis of fibrinogen and albumin etc.
    - Fat metabolism
    - Synthesis of bile acids
    - Metabolism of hormones
    - Formation of urea from amino acids

- **Blood supply is about 20% arterial-80% venous.**

- **May contain 10-15% of blood volume**

INFLUENCE OF TOXIC CHEMICALS ON THE LIVER

- The liver is the most common site of damage in laboratory animals administered drugs and other chemicals. There are many reasons including the fact that the liver is the first major organ to be exposed to ingested chemicals due to its portal blood supply.

- Although chemicals are delivered to the liver to be metabolized and excreted, this can frequently lead to activation and liver injury.

- Study of the liver has been and continues to be important in understanding fundamental molecular mechanisms of toxicity as well as in assessment of risks to humans.

VENA CAVA

HEPATIC VEIN

LIVER

HEPATIC
PORTAL VEIN

BILE DUCT

SMALL AND
LARGE INTESTINE

LOBULE

PORTAL VEIN

BLOOD FLOW

HEPATIC ARTERY

III

I

BILE FLOW

BILE DUCT

CENTRAL VEIN

PORTAL TRIAD

TYPES OF LIVER CELLS

Hepatocytes- Not all the same; depends on lobular site

      Zone I   **Higher in respiratory enzymes (periportal)**

      Zone III   **Higher in cytochrome P450 (centrilobular)**

Endothelial cells

Bile duct cells

Oval cells- Possibly stem cells

Kupffer cells- Phagocytic cells Important role in inflammation

Ito cells- Fat storing or stellate cells

TYPES OF HEPATIC INJURY OR RESPONSES

Each of the different cell types may respond to a toxic insult. If severe, toxicity can result in cell death but hepatocytes have remarkable adaptive responses. These responses depend on the chemical and dose.

- Necrosis
- Lipidosis (fatty liver)
- Infiltration and Pigmentation
- Cholestasis
- Cirrhosis
- Hepatitis
- Vascular injury
- Neoplasia

EXAMPLES OF ACUTE HEPATOTOXIC CHEMICALS

|	Necrosis	Fatty Liver
Carbon tetrachloride	+	+
Bromobenzene	+	-
Dimethylnitrosamine	+	+
Thioacetamide	+	-
Pyrrolizidine alkaloids	+	+
Aflatoxin	+	-
Ethanol	+	-
Berylluim	+	-
Allyl alcohol	+	-
Paracetamol	+	-
Diquat	+	-

NECROSIS

- Cell death of hepatocytes. Damage occurs in different parts of the liver lobule depending on oxygen tension or levels of particular drug metabolizing enzymes.

- Allyl alcohol causes periportal necrosis (III)because the enzyme metbolizing it is particularly located there.

$$CH_2=CHCH_2OH \longrightarrow CH_2=CHCHO$$
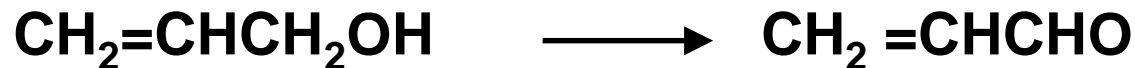
- Carbon tetrachloride causes centrilobular necrosis - endothelial and Kupffer cells adjacent to hepatocytes may be normal - with diethylnitrosamine, endothelial cells are also killed. Due to activation by higher concentrations of cytochrome P450 in zone I.

- Some cells undergo apoptosis which may be increased by agents such as ethanol. Unlike necrosis there is no release of cellular contents and thus no inflammatory cells.

LIPIDOSIS

- Many chemicals cause a fatty liver. Sometimes associated with necrosis but often not.

  Not really understood but essentially is due to an imbalance between uptake of fatty acids and their secretion as VLDL.

- Carbon tetrachloride can cause lipidosis by interfering in apolipoprotein synthesis as well as oxidation of fatty acids.

- Other chemicals can cause lipidosis by interfering with export via the Golgi apparatus.

- Ethanol can induce increased production of fatty acids.

INFILTRATION & PIGMENTATION

•Some chemicals such as CS_2 cause hydropic degeneration(water accumulation in hepatocytes) possibly related to failure of sodium balance.

•Other chemicals, especially the so called peroxisome proliferators, induce lipofuscin (age pigment) accumulation due to poorly metabolized lipid probably accumulating in lysosomes.

•Some drugs eg griseofulvin, cause the deposition of the lipophilic haem precursor protoporphyrin.

CHOLESTASIS

- **Accumulation of bile pigments and other products in the bile canliculi interfering with bile flow. Can occur without damage to hepatocytes.**

  α-**naphthylisocyanate disrupts hepatocyte junctions forming caniculi.**

CIRRHOSIS

- **Hepatic fibrosis and hypoxia leading tonodular regeneration.**
- **Can occur after repeated insult and may lead to hepatic failure. Iron in gerbils is a good example. This mimics human disease of secondary haemochromatosis.**
- **Also carbon tetrachloride.**

Damage to other cell types

- Damage to hepatocytes is not always observed. In some circumstances bile duct necrosis is observed (alpha-naphthylisocyanate). Other chemicals cause bile duct hyperplasia, oval cell proliferation and cholangiofibrosis (2-acetylaminofluorene).

- Other toxins can affect endothelial cells, Ito cells and Kupffer cells. Ricin and beryllium can initially first attack Kupffer cells.

Consequences of impaired hepatic function

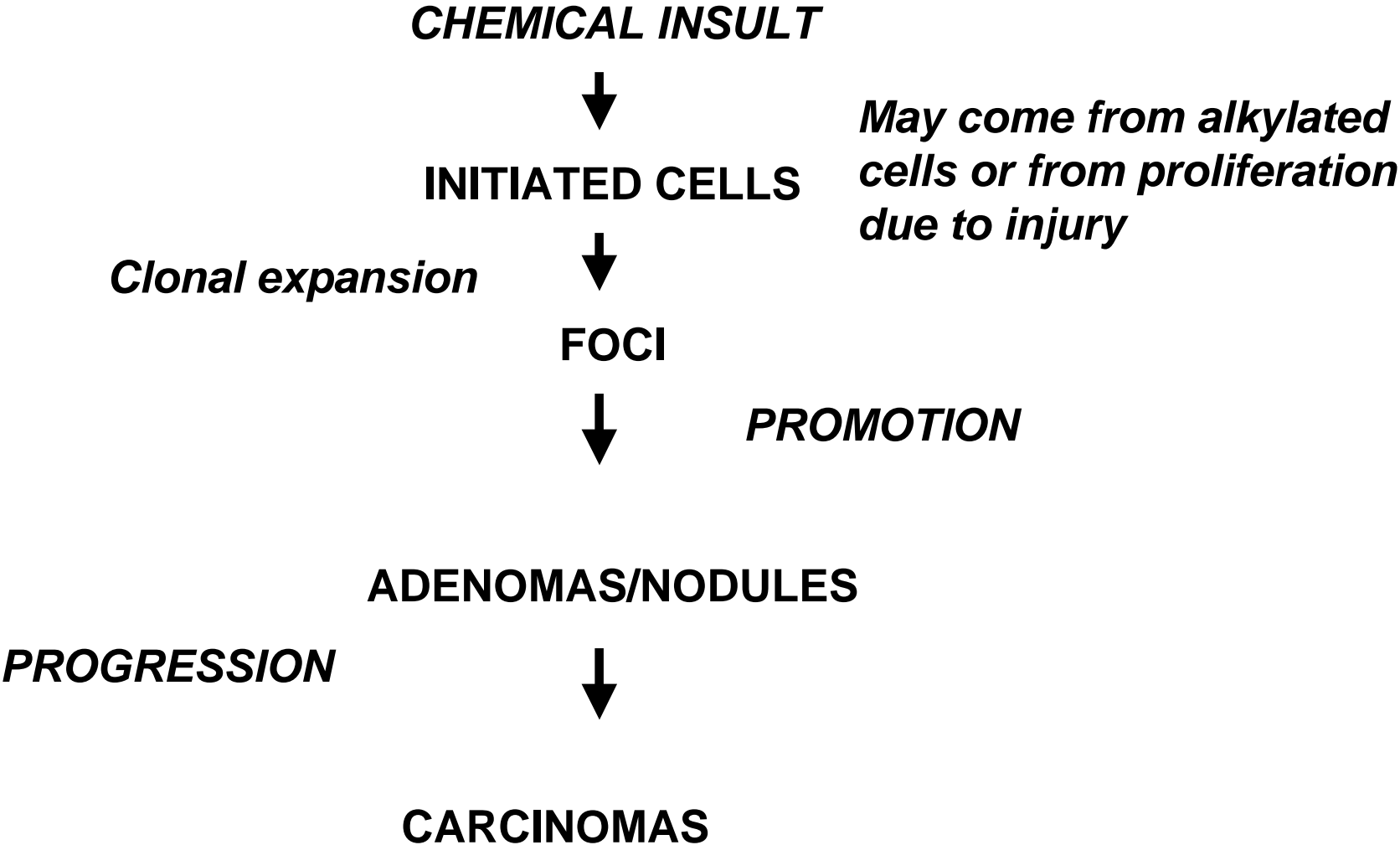| Type of function	Example	Malfunction
Nutrient homeostasis	Glucose storage & synthesis	Hypoglycemia, confusion
	Cholesterol uptake	Hypercholesterolemia
Filtration of particulates	Endotoxin from bacteria	Endotoxemia
Protein synthesis	Clotting factors	Bleeding
	Albumin	Hypoalbuminemia
	Transport proteins	Fatty liver
Biotransformation	Bilirubin	Jaundice
	Xenobiotics	Low drug metabolism
	Steroids	Loss 2nd male characteristics
Bile and biliary excretion	Bilirubin,	Jaundice
	Cholesterol	Hypercholesterolemia
	Xenobiotics	Drug clearance

NEOPLASIA

- **Many chemicals that cause neoplasms in rodents cause hepatocellular neoplasia (often more than 50%) although in humans, in the Western world, this is a relatively rare cancer.**

  On the other hand, it does occur with alcohol abuse in humans and is much more common elsewhere in the world e.g. as caused by aflatoxin.

- **In rodent liver definition of neoplastic lesions has proved immensely difficult. Some strains of mice have high spontaneous incidences (30-50%) whereas in others it is extremely low (<5%).**

- As far as regulatory toxicology is concerned, liver neoplasia is a very important and contraversial topic.

- Most rodent tumours are hepatocyte derived but bile duct neoplasms (cholangiocarcinoma etc.) are observed with some carcinogens such as *N*-nitrosomorpholine.

- Haemangiomas and haemangiosarcomas also occur.

- Often with any one carcinogen e.g. aflatoxin B_1 a range of tumours can be observed.

- Hepatic chemical-induced carcinogenesis is a topic which is studied in great detail as a model for neoplasia in other organs.

NEOPLASTIC PROGRESSION

CHEMICAL INSULT

↓

INITIATED CELLS

May come from alkylated cells or from proliferation due to injury

Clonal expansion ↓

FOCI

↓ *PROMOTION*

ADENOMAS/NODULES

PROGRESSION ↓

CARCINOMAS

FOCI OF HEPATOCELLULAR ALTERATIONS AND NEOPLASIA

- **CYTOLOGIC PHENOTYPES**

    Clear cell

    Acidophilic cell

    Basophic cell

    Tigroid cell

- **HISTOCHEMICAL PHENOTYPES**

    Glycogen storage

    Iron loading resistance

    Enzyme induction

      y-glutamyl transpeptidase

      GSH-transferase

      Glucose 6-phospate dehydorgenase

    Enzyme Deficiency

      ATPase

      Glucose-6 phosphatase

      Serine dehydratase

Mechanisms in hepatocellular injury

Aspects of mechanisms	Components identified
Metabolic activation of chemical	Cytochrome P450s Glutathione transferases Alcohol dehydrogenases
Effect of chemical activation	Covalent binding to protein, lipid, DNA Oxidative stress
Critical targets	Membrane peroxidation Protein thiol depletion Calcium transport

For many chemicals the exact mechanisms are unknown

METABOLIC ACTIVATION OF CHEMICALS

CYTOCHROME P450

• Chemicals and drugs carried to the liver are metabolized initially by the cytochrome P450 haemoprotein system. This is usually to render them more polar i.e. more hydrophilic, and more excretable by conjunction with glutathione and by formation of glucuronides.

• Many cytochrome P450 isoenzymes are constitutive in rat liver which is probably the most active of all tissues in this respect.

• This is a vast subject and cannot be covered here but some essential points can be made

- Although some isoenzymes will only catalyse certain monooxygenase steps - isoenzymes in the hepatic endoplasmic reticulum can catalyse oxidation of many chemicals to varying degrees including drugs, chemicals, pesticides.

- Substrate specificities, amino acid sequences and inducibilities are highly conserved across species. This has allowed a universal classification system to be constructed which often enables direct comparisons to be made between experimental systems and human isozymes.

- For some studies of drug metabolism human cytochrome P450 isozymes have been inserted into cell lines either singly or in combinations to determine the exact role of each isozyme

DIFFERENT DRUGS / CHEMICALS WILL INDUCE SYNTHESIS OF A VARIETY OF P450 ISOENZYMES

'3-Methylcholanthrene' type	CYP1A1 CYP1A2
	CYP1B1
'Phenobarbital' type	CYP2B1 CY2B2
'Ethanol' type	CYP2E1
'Steroid drug' type	CYP3A1 CYP3A2
'Hypolipidaemic drug' type	CYP4A1

SUICIDAL INACTIVATION OF CYTOCHROME P450

Many chemicals when being metabolized by P450 react with these 'detoxifying enzymes.'

CS_2 and other sulphur-containing drugs and chemicals react with the protein.

$CC1_4$ and halothane destroy cytochrome P450 by reacting with the haem moiety at the meso positions.

Many unsaturated chemicals e.g. ethylene, griseofulvin, diethylnitrosamine alkylate pyrrole nitrogens of the haem moiety with the loss of iron. If the substituent is only a methyl or ethyl group, the resulting N-methylproto-porphyrin acts as an inhibitor of the crucial haem biosynthesis enzyme ferrochelatase.

Compounds such as diquat or menadione undergo one-electron reduction to yield the semiquinone radical perhaps catalysed by cytochrome P450 reductase. Auto-oxidation by O_2 gives the parent chemical + $O_2^{-\cdot}$.

Chemicals such as bromobenzene probably react with glutathione very rapidly either with/without prior activation. This can deplete the cell of reductive capacity to critical levels.

- One explanation for the hepatic toxicity of paracetamol is that N-acetyl-p-benzoquinone imine both depletes GSH directly and cycles to produce $0_2^{-\cdot}$.

- At first the imine is reacting with the GSH but eventually activation by P450 overwhelms the GSH and the imine reacts with critical proteins. This then become critical and possibly irreversible.

- KO 1A2/2E1 mice are protected from paracetamol action.

- N-acetylcysteine protects, however the precise mechanism is unknown.

CONJUGATION OF METABOLITES

•**GLUTATHIONE TRANSFERASES**

These conjugate many hydrophobic and electrophilic compounds and their metabolites with glutathione. The conjugates are eventually cleaved to the mercapturates for excretion. Some glutathione conjugates of halogenated olefins are reactive and toxic.

Both cytosolic and microsomal GSTs have been demonstrated with overlapping substrate specificities and can be induced although not to the extent of the cytochrome P450 isoenzymes.

•GLUCURONIDASES

Usually conjugation of hydroxy metabolites of drugs to increase hydrophilicity for excretion. Again there are a number of these with some inducibility and some products which increase toxicity.

•SULPHATASES

Many hydroxylated endogenous chemicals (e.g. steroids) and drug metabolites are excreted as the sulphates.

In some circumstances, as with the carcinogen N-hydroxyacetylamino-fluorene N,O-sulphate is very unstable and probably more carcinogenic than the parent molecule.

CHEMICAL TOXICITY - INFLUDENCE OF BIOACTIVATION & DETOXIFICATION PROCESSES

EXAMPLES

Compound	Pathway	Product
Type 1		*NON-TOXIC*
Sulfur dioxide	Sulfite oxidase	Sulphate
Phenol	UDP-GT	X-glucuronide
Ethylene oxide	Epoxide hydrolase	Ethyleneglycol
Type 2		*TOXIC*
Chloroform	P450	Phosgene
Di-(2-ethylhexyl) phthalate	non-specific esterases	mono-ester
Red 2G	Azo reductase	Aniline

- **AFLATOXIN B$_1$**

   This is a mould metabolite well established as a hepatic toxin and carcinogen in rats and humans.

   The crucial reaction is cytochrome P450 activation of aflatoxin B$_1$ to the 2,3-epoxide. Elevated levels of particular glutathionetransferases will protect by conjugating the eopoxide with glutathione. If no, alkylation of guanine at N^{4}or 0^6 leads to neoplasia.

   Although reactions with DNA appear to be simple it is a\ complex process which determines whether DNA alkylation actually leads eventually to cancer.

CONSEQUENCES OF TOXIC MECHANISMS

- PEROXIDIATION OF MEMBRANE LIPIDS

  Well known as a parameter of cell damage but is it before/after other critical cellular damage? Blebbing of plasma membrane

- PROTEIN THIOL DEPLETION

  Depletion of protein thiols can precede loss of viability.

- ALTERATIONS IN CALCIUM HOMEOSTASIS

  Both alkylation and oxidative toxicities. The plasma membrane is a prime regulator of calcium concentrations. In addition, calcium is sequestered in organelles.

- **MITOCHONDRIAL DAMAGE**

Loss of ATP synthesis, superoxide generation, rupture and loss of cytochrome c leading to apoptosis.

- **ENDOPLASMIC RETICULUM DAMAGE**

Dilation, hypertrophy

- **DNA DAMAGE**

Clearly DNA damage both by alkylation or by oxidation can lead to cell death e.g. strand breaks.

More subtle changes contribute to neoplasia. Depends on repair, site and growth status of the cells.

Chromatin disruption.

Activation of endonucleases.

$CC1_3^{\cdot} + R\text{-}SH \longrightarrow RS^{\cdot}, R\text{-}S\text{-}CC1_3, CHL_3,$

$CC1_3^{\cdot} + \text{protein, lipid} \longrightarrow \text{covalent binding}$

$CC1_3 + \text{polyunsaturated lipid} \longrightarrow \text{lipid peroxidation}$

Lipid peroxidation $\longrightarrow$ membrane damage, enzyme inactivation, toxic aldehyde and peroxide products

Aldehydes and peroxides $\longrightarrow$ protein cross linking, capillary permeability,

decreased enzyme activities

DNA damage

OXIDATIVE STRESS

Toxic agents may oxidatively injure liver cells in two general ways.

1. They may cause the formation of excess $O_2^{-\cdot}$ and H_2O_2.

2. They may deplete cells of protective constituents such as glutathione or vitamin E, so that cells are more susceptible to normal levels of reactive oxygen species.

3. Some chemicals may cause $O_2^{-\cdot}$ formation and react with glutathione.

Whether OH. is the key ultimate toxicant is still open to debate.

- Probably an important consideration is the role of iron.

$$Fe^{3+} + O_2^{-\cdot} \longrightarrow Fe^{2+} + O_2^{-\cdot}$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH\cdot + OH^-$$
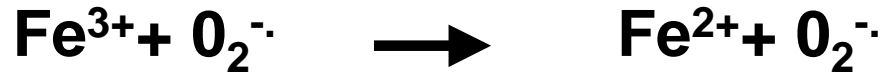
 or related iron oxygen species.

 However, there may be circumstances where iron release from ferritin becomes a critical event. Xenobiotics are starting to be implicated in iron pathobiology.

Haemochromatoses are diseases in which the liver has very high amounts of iron and suffers, necrosis, cirrhosis and liver cancer.

Factors that influence toxicity

- **Dosage**
- **Degree and route of absorption**
- **Nutrition**
- **Disease**
- **Sex**
- **Age**
- **Genetic propensity**

.Autoimmune responses

- Sometimes it seems that in the metabolism of a drug protein-drug adducts are produced which are expressed on the hepatocyte cell surface and act as antigens.

- Hepatocytes can then be destroyed by cell-mediated immune responses. Accompanying inflammation.

- May occur in humans after multi exposures and no dose response.

- Classic cases of halothane hepatitis in humans. Rare but high mortality.

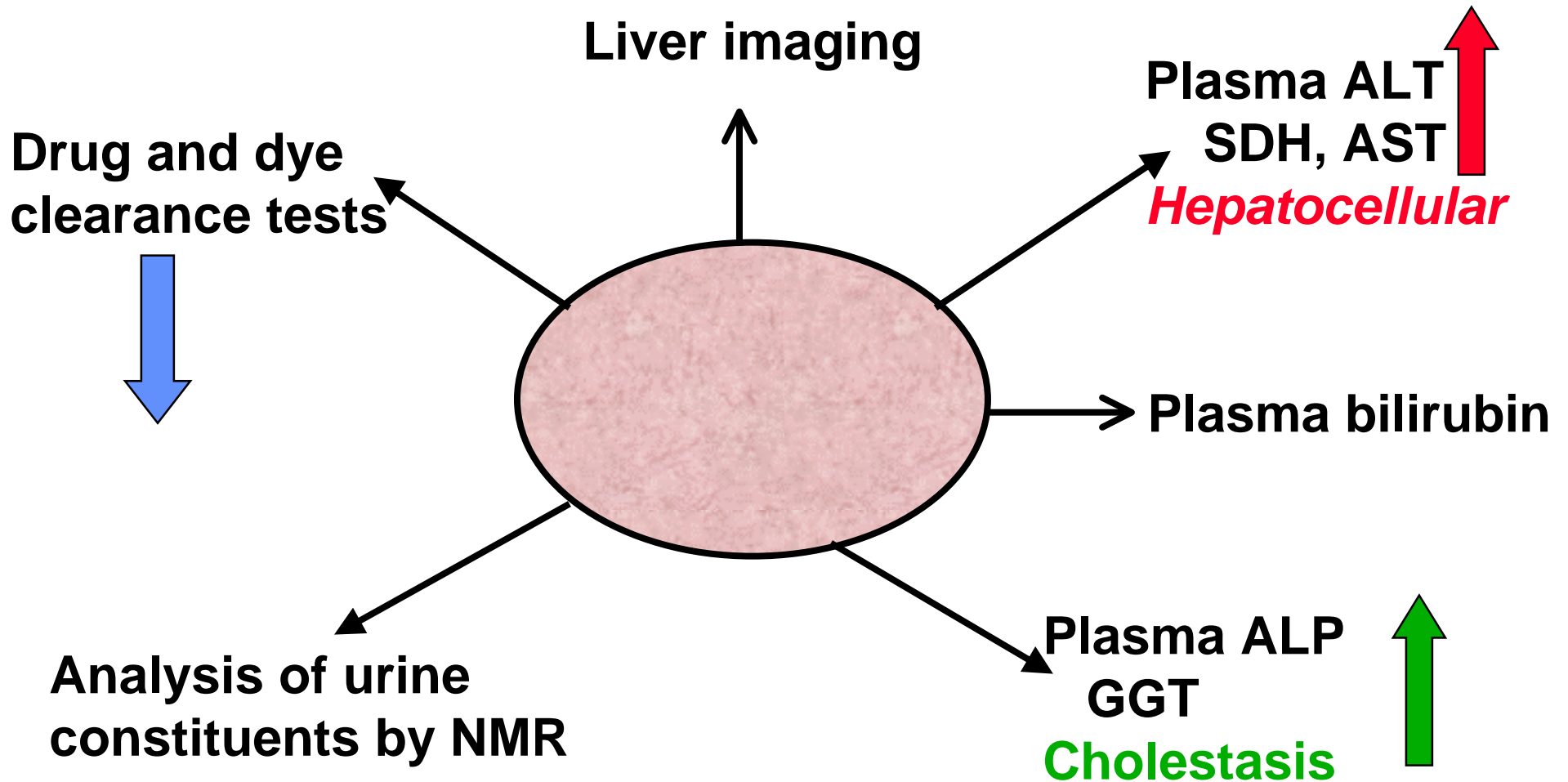# Genetic variation in drug metabolism enzymes

Eskimos	95-100%	INH
Japanese	88%	INH
White Americans	48%	INH
Afro-Caribbean Americans	52%	INH
South Indians	39%	INH
Britons	38%	SMZ
Egyptians	18%	INH

INH - isoniazid   SMZ - sulfamethazine

DETECTING HEPATIC INJURY

Genomic and proteomic approaches

- Soon we will be able to apply global gene expression and protein profiles to understanding mechanisms of liver injury from gene activation through to pathology.

- This will allow us to predict responses to agents much more accurately and focus treatments.

- Multigene responses to hepatotoxic and carcinogenic agents are now under investigations in many labs.

End stage liver disease

In the end liver injury may be very serious leading to

- Jaundice
- Cirrhosis and ruptured portal hypertension
- Anorexia
- Ascites
- Hepatic encephalopathy
- Liver failure
- Renal failure
- Hepatocellular carcinoma