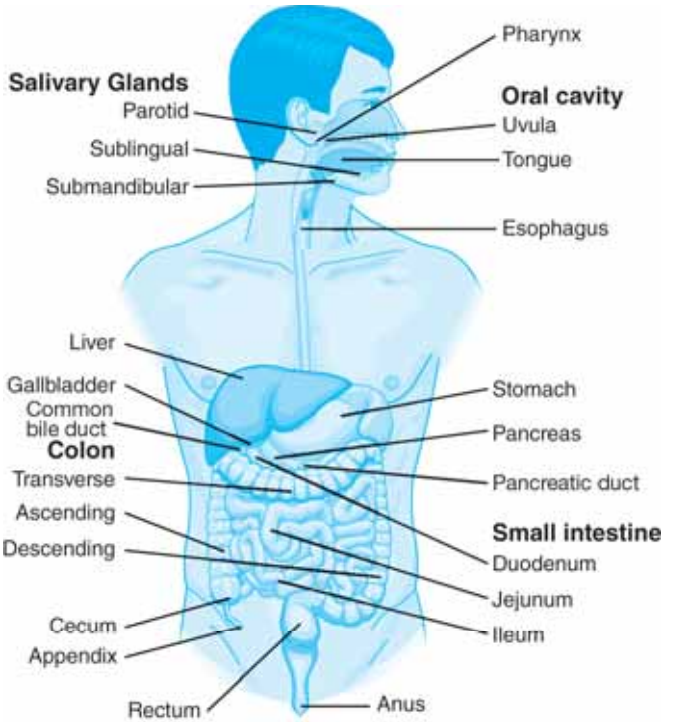
CHAPTER 16

Gastrointestinal System Toxicity and Oral Exposure (GI Tract, Pancreas, Liver)

I. Gastrointestinal (GI) system



A. Most likely accidental poisoning of animals, infant humans, and route of contaminated food or suicidal ingestions

B. Damage to GI structures (proximal → distal)—see Table 16-1

1. Mouth

a. Teeth—stain with food products (coffee, tea), tetracycline, chlorhexidine (in prescription mouthwash), fluoride; TCDD arrests molar development (AhR stimulation → apoptosis) and formation of enamel; cleft palate caused by cell cycle progression and proliferation disruption (TCDD, mycotoxin, glucocorticoids, secalonic acid, retinoic acid)

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2. Bone—osteonecrosis of jaw by bisphosphonates via ↑ infection, ischemia, low bone turnover, direct bone toxicity, soft tissue toxicity
3. Tongue—discoloration by V_2O_5, edema by 30% H_2O_2; paralysis of tongue = D-tubocurarine (reversible) and *Clostridium botulinum* toxin (months to reversal due to destroyed mechanisms of ACh release or fatal)
   a. Mucositis—anticancer medications
   b. Strong H_2O_2 solutions (> 30%) → irritation and burns and tissue emphysema (blood or tissue protein contact) → hypoxanthine-guanine phosphoribosyltransferase mutations in human T lymphocytes (genotoxicity)
   c. Radiation → dry mouth, sore throat, altered taste, dental decay, voice quality alterations, chewing and swallowing difficulties (salivary glands affected) → cancer; ethanol (via ADH) → acetaldehyde → cancer; tobacco (via CYP) → active metabolites → cancer
   d. Anticholinergic medications also lead to dry mouth
   e. Bitter yields reflex rejection due to bitter taste receptors (quinine) or high concentration of salt/acid (affect K^+ channels)
   f. Mercury leads to gingivitis (one of triad of symptoms of Hg poisoning) → damage to salivary gland DNA
4. Esophagus
   a. See 1 g for effects of radiation on swallowing
   b. Bullae and vesicles in mouth caused by anticancer medication methotrexate leads to difficulties in swallowing
   c. Anticholinergic atropine → inhibits cough reflex in dogs
   d. Strong acids and alkali (pH > 11.5) → strictures which inhibit swallowing
   e. NSAIDs exacerbates gastric reflux or KCl + quinidine → strictures → cytokine-mediated esophagitis → leads to esophageal cancer
   f. Antibiotic tetracycline → transient, self-limiting esophagitis
   g. Bisphosphonates → severe injury when taken by person without water who then lies down
   h. Oxidative stress → lipid peroxidation (malondialdehyde) and ↓ SOD → esophageal mucosa injury
   i. Bile acids → ROS and RNS induction → DNA damage with short-term apoptosis and long term apoptosis resistance → cancers of esophagus, stomach, small intestine, liver, biliary tract, pancreas, and colon/rectum
   j. Hyperglycemia due to diabetes mellitus induced by natural chemical streptozotocin → RNS → nitrosative stress (peroxynitrite) + glycoconjugates of epithelial barrier → esophageal injury
   k. Mycophenolic acid (immunosuppressant) → apoptosis and GI disturbances
   l. Ethanol via ADH → acetaldehyde (and worse with CYP activation of tobacco products) → G:C > T:A transversions → TP53 mutations (exons 4–10) → oral and esophageal cancers
5. Stomach
   a. NSAIDs (via COX inhibition and ↓ formation of PGE2 and PGI_2 resulting in less mucus and increased acid and pepsin secretion) or *Helicobacter pylori* infection (H_2S generation usually anti-inflammatory and cytoprotective but actually proinflammatory in the presence of *H. pylori* + NO generation through macrophage L-arginine/nitric oxide pathway → nitrosating compounds) → ulcers (type I–IV) + duodenal ulcers
   b. Formation of nitrosamines from nitrates → nitrites and cancer (prevented by antioxidants); isothiocyanates in cruciferous vegetables protect gastric mucosal cells from genotoxicity related to *H. pylori* infection
   c. Chronic ethanol damage to stomach prevented by lupeol and NAC (restore non-protein sulfhydryl depletion), however this gastroprotection reversed by COX inhibition (indomethacin) or NO-synthase inhibition (L-NAME), Ca^{2+} also plays a role in ethanol toxicity as indicated by action of Ca^{2+}-channel blockers

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d. Three models of stomach ulceration: HCl/ethanol ± omeprazole (proton pump inhibitor to prevent acid secretion); indomethacin (strong NSAID) + bethanechol (muscarinic agonist); mice undergoing cold stress

e. Cancer from thiophenes in coal-tar fumes; road workers exposed to bitumen fumes → overexpression of TNF-related apoptosis-inducing ligand, DR5 death receptor, and caspase-3 and TUNEL

6. Duodenal toxicity

a. Acute ethanol administration ↑ lipid absorption, while chronic ethanol use ↓ lipid absorption and interferes with Na^+ and H_2O absorption due to ↓ ATP and effects on ATPase and secretion of antidiuretic hormone; EtOH also ↑ Mg^{2+} and ↓ Ca^{2+} absorption and causes necrosis of villus epithelium + lymphocyte and plasma cell infiltration

b. Cancer chemotherapy agents → DNA strand breaks, clonogenic death of basal epithelial cells, ↑ ROS → lipid peroxidation → NF- κB, Wnt, p53 signaling pathways → ceramide pathway → cell death and activated macrophage metalloproteinases; TNF can amplify NF-κB signaling → MAPK signaling → signal overload and ulceration → bacterial invasion → cytokine inflammation or sepsis; anticancer drug methotrexate also damages via nitrosative stress

c. NSAIDs still damage the duodenum via COX-1 inhibition

d. Immunosuppressive medication mycophenolate mofetil → ulcerative esophagitis, reactive gastropathy, duodenal and ileal graft-versus host disease

e. Heavy metals compete for absorption in this part of the small bowel → transport across luminal membrane → transport across basolateral membrane (this portion determines residence time in mucosal epithelial cytoplasm → mucosal sloughing); Ca^{2+} mainly absorbed here; Hg^{2+} absorbed in proximal jejunum; Zn^{2+} mainly absorbed in jejunum and ilium; selenomethionine absorbed in entire intestinal tract

(1) Pb → tissue desiccation and mucosal damage (damage from neurotoxicity)

(2) Hg → sloughing of intestinal mucosa + edema in capillary walls (also affects hematopoiesis) → shock and peripheral vascular collapse (high Hg levels; death usually neurotoxicity)

(3) Cr(VI) → oral cancer in rats, small intestine cancer in mice

(4) Carcinogenic sodium arsenite → stimulates pregnane X receptor and forms heterodimer with retinoid X receptor α → induces CYP3A4

(5) Iron transport mediated by intramembrane divalent metal transporter 1 and then by ferroportin (concentrates in liver)

f. Accidental ingestion of plastic hardener methyl ethyl ketone peroxide → oxidative damage → ulceration of proximal GI tract (death from liver necrosis)

g. Bacterial and fungal toxins—*Clostridium perfringens* β-toxin → hemorrhagic luminal fluid in small intestine (mainly jejunum and ilium); *Staphylococcus aureus* toxins A–E + enterotoxins G and I → toxic shock; *Fusarium* mycotoxins → ↓ villus height in turkey duodenum

h. Immune reactivity—LPS → ↓ duodenal and ileal proline transport in Meishan pigs (not Yorkshire), ↑ glycyl sarcosine transport in both pig breeds

7. Jejunum

a. In this region, nutrient mineral deficiencies (i.e., Fe) increase carcinogenic Ni absorption due to less Ni export from jejunal mucosa

b. D-glucose addition to mucosal side of voltage clamped intestinal sections yielded highest current increases in jejunum—interference with glucose transport by deoxynivalenol or vomitoxin most important here and duodenum; Cd inhibits D-galactose transport in the presence of Ca; also transports toxic glycosides such as prunasin from amygdalin

c. Ouabain or vanadium inhibition of $3Na^+$,$2K^+$-ATPase leads to ↓ amino acid and H_2O transport

d. Jejunum produces more ROS and RNS than many other organs (duodenum, kidney, ileum, blood, cerebellum, brain, heart, liver), so GSH important

CONCEPTUALIZING TOXICOLOGY 16-1 (*continued*)

8. Ileum
   a. Bacterial toxins—this region and jejunum succumbs to *C. perfringens* type A toxin in chickens due to contaminated wheat- and barley-based diets; *S. aureus* α toxin and *C. perfringens* NetB → jejunal and ileal necrosis; *C. difficile* toxins A (antibiotic-induced diarrhea and pseudomembranous colitis—prevented by A2A adenosine receptor agonist) and B → pathogenesis in this area; bacterial flora also can activate a cancer chemotherapy prodrug irinotecan → toxic SN-38 via β-glucuronidase activity → diffuse small and large intestinal damage (higher flora in large bowel in normal humans); cholera toxin → secretory diarrhea (controlled by 2-thioxo-4-thiozolidinone cystic fibrosis transmembrane conductance regulator)
   b. Endotoxin or LPS decrease jejunal radical formation and activate phospholipase A_2 → increased permeability of ileal wall to bacterial invasion and sepsis; high concentrations of the herbicide glyphosate also increases permeability and disrupt actin cytoskeleton; Cd^{2+} also disrupts the paracellular barrier increasing its own absorption and toxicity
   c. Okadaic acid → tumor-promoting specific cell-permeating inhibitor of protein phosphatases → diarrhetic shellfish poisoning and genotoxicity in ileum, liver and kidney
   d. Immune system—IP injection of zymosan → neutrophil migration → increased myeloperoxidase activity in ileum and lung → IL-1 and TNF-α (controlled by TNF-α- soluble inhibitor etanercept) → ileal and pancreatic injury; depleted U ingestion → ↑ COX-2 expression, IL-1β and IL-10 → inflammation via neutrophil response (short-term hypersensitivity), but ↓ macrophage function (CCL-2 mRNA) = chronic poisoning of immune response
   e. Chronic cocaine → ischemia/infarction and hemorrhage in distal ileum
   f. Bacterial bile acids include lithocholic acid, which is prevented by vitamin D-induced metabolism by CYP3A1 and CYPA2; however, liver-derived chenodeoxycholic bile acids prevent the induction of CYP3A in rat liver and increase the toxicity of lithocholic acid
   g. Isoliquiritigenin, a licorice flavonoid → muscarinic receptor agonist-related spasmogenic action on rat stomach fundus, but an opposite Ca^{2+} channel antagonism-related spasmolytic effect on rabbit jejunum, guinea pig ileum, and atropine-treated rat stomach fundus (change in motility)
   h. Mycotoxin aflatoxin B_1 → release of ACh → ileal spasms
9. Colon
   a. Excess Fe → free radicals and lipid peroxidation → cecum and proximal colon cancer (prevented by difluoromethylornithine which inhibits ornithine decarboxylase = less polyamine synthesis for inflammation and NSAIDs as anti-inflammatory agents via COX inhibition)
   b. Dimethylhydrazine metabolized → azoxymethane → DNA methylation and cell signaling changes (upregulation of Wnt-β catenin, phospholipase A_2, MAPK) associated with colon cancer
   c. Crohn's colitis from bacterial infections, ischemia, autoimmunity → chronic inflammation and cancer
   d. Mesenteric vessel effects of alosetron, amphetamines, cocaine, ergotamine, estrogen, pseudoephedrine, sodium polystyrene, and vasopressin → colonic ischemia
   e. Colon pseudo-obstruction caused by atropine, narcotics, nifedipine, phenothiazines, tricyclic antidepressants, vincristine (↓ motility)
   f. Antibiotics → pseudomembranous colitis; ampicillin → hemorrhagic colitis
   g. Cancer chemotherapy agents → neutropenic colitis
   h. Au, α-methyldopa, and NSAIDs → cytotoxic colitis (allergic reaction, antimetabolite action or mucosal cytotoxicity)
   i. Lymphocytic colitis produced by altered immune response due to cyclo 3 fort, flutamide, lansoprazole, NSAIDs, or ticlopidine

C. Pancreatic damage—see Table 16-2
   1. Soybean trypsin inhibitor (heat labile) → pancreatic damage
   2. Ethanol → acetaldehyde → acinar atrophy and fibrosis; ↑ permeability of intestinal wall → endotoxemia and necroinflammation → stellate cell activation → progressive deterioration of pancreas

CONCEPTUALIZING TOXICOLOGY 16-1 (*continued*)

3. Choline deficiency + ethionine → ↑ IL-6 → serotonin 5-HT_{2A} receptor activation (blocked by risperidone) → necrotic pancreatitis
4. Mercaptopurine immunosuppressants → inflammatory bowel disease (IBD)
5. Estrogens → ↑ VLDL and ↓ triglyceride lipase in liver → hypertriglyceridemia → pancreatitis
6. Diuretics furosemide and hydrochlorothiazide + ACE inhibitor lisinopril → necrotizing pancreatitis
7. Medications classed by cases: class I (> 20), class II (> 10), class III (any pancreatitis); oral toxic medications may cause pancreatitis in sensitive individuals
8. Oleic acid → mitochondrial membrane dysfunction (prevented by topiramate, an antiseizure compound ↑ expression of nutrient sensor PPARα and mitochondrial fatty acid carrier CPT-1- which leads to β-oxidation of the lipid) → exocrine pancreas ↑ secretion of H_2O and HCO_3^- and endocrine ↓ insulin response to glucose
9. Alloxan reduction → dialuric acid → redox cycling → $O_2^{-\cdot}$ + ↑ cytosolic Ca^{2+} → β-cell damage → ↓ insulin
10. Streptozotocin → GLUT2 glucose transporter → gains entrance to pancreatic β-cells → alkylates DNA → activation of poly ADP-ribosylation → depletes cellular NAD^+ and ATP → ATP dephosphorylation → substrate for xanthine oxidase → $O_2^{-\cdot}$ (and Fenton to H_2O_2 + $HO^{\cdot}$); also ↑ NO-inhibiting aconitase activity → DNA damage
11. Pancreatic cancers from DNA damage in exocrine pancreas from compounds such a DMBA and other PAHs activated by cells of pancreatic duct: preneoplastic acidophilic atypical acinar cell foci and nodules → growth mediated by cholecystokinin-A receptors
12. 4-nitroquinoline-1-oxide → 4-hydroxyaminoquinoline-1-oxide metabolite → DNA adducts → ↑ apoptosis and induction of p53 proliferative gene(s)
13. Rat pancreas → acinar adenomas and adenocarcinomas while human develops ductal adenocarcinomas as is seen in hamster exposed to *N,N*-dipropylnitrosamine → β-oxidation → *N*-nitrosamines

D. Liver damage—see Table 16-3

1. A study of biochemical anatomy (zones 1–3; see Figure 16-1)
   a. Zone 1 = periportal = high direct and oxidative toxicity (high O_2) but high GSH
      (1) Metals highest here and bile outflow in this area
      (2) Fe oxidation products → lipid peroxidation
      (3) Cu → generates GSSG + $O_2^{-\cdot}$ → mitochondrial ↓ Mn SOD and thiol/disulphide ratio → ↓ ATP → collapse of mitochondrial membrane potential → induction of mitochondrial permeability transition → hepatocellular apoptosis
      (4) Doxorubicin reduced by CYP reductase → generates ROS → interferes with macromolecule synthesis → covalently binds to and X-links DNA, inhibits topoisomerase II, arrests cells in G^2 phase → induces apoptosis → attracts inflammatory cells → periportal fibrosis (fibrosis is precursor to cirrhosis and then liver cancer if survive the severely compromised liver function)
      (5) Cisplatin → CYP activation → cross-links DNA → G^2 phase arrest → apoptosis → periportal fibrosis
      (6) Methapyrilene → S-oxidation of thiophene group → depletes periportal GSH but ↑ GSH centrilobular region → ↑ HO-1 and glutamate cysteine ligase catalytic subunit → apoptosis → necrosis → hepatic cancer if animal survives liver damage
      (7) Allyl alcohol → model oxidation activation in periportal region
      (8) Ethanol metabolized extensive periportal and inhibits gluconeogenesis decreases O_2 uptake
      (9) Aflatoxin → CYP activation → diffuse and severe hydropic (intracellular edema) degeneration, bile duct hyperplasia, periportal fibrosis
      (10) Dexamethasone → fat accumulation in periportal region and causes liver enlargement by increased excretion of p-glycoprotein expressed in this region → microvesicular steatosis (fatty)

CONCEPTUALIZING TOXICOLOGY 16-1 (*continued*)

(11) α-naphthyl isothiocyanate attracts neutrophils via β2-integrin CD18 → periportal inflammation, wide-spread hepatic necrosis, acute cholestatic hepatitis

b. Zone 3 = reductive metabolic radical formation (low O_2), more CYP, less GSH

(1) Fe damage from iron-supplemented vitamins → hemosiderin → centrilobular fibrosis

(2) Acetaminophen → CYP activation → N-acetyl-p-benzoquinone imine + GSH → GSH depletion, covalent adduct formation, initiates mitochondrial damage → ↓ ATP → massive centrilobular necrosis + extensive inflammatory cell infiltration; NAC protective (bolstering GSH) early but later delays recovery by impairing glucose metabolism (periportal hepatocyte vacuolation)

(3) Ethanol in this region → ↑ endotoxin, plasma aminotransferase (leakage), CYP2E1, lipid peroxidation, NF-κB, TNF-α, iNOS, COX-2, procollagen-I + hypoxia generated by EtOH → acetaldehyde → Na^+ influx and decreased hepatocyte pH

(4) CCl_4 model of centrilobular necrosis, increased halogens draw electrons away from electropositive carbon → CYP2E1 → reductive dehalogenation → electron withdrawal stabilizes radical formation (carbon-centered) → necrosis + steatosis (via protein synthesis inhibition at the ribosome which is protected by cycloheximide), however if protein synthesis is inhibited then steatosis increases and necrosis decreases

(5) Tienilic acid → CYP2C9 → electrophilic reactive intermediates bind covalently to macromolecules → ↓ GSH and ↑ lipid peroxidation → upregulation of GSH synthase and glutamate-cysteine ligase (attempts to ↑ GSH synthesis), HO-1 and NAD(P)H dehydrogenase quinone 1 (oxidative stress), GSH-transferase and UDP glycosyltransferase 1A6 (phase II drug metabolism); inhibition of glutamine-cysteine ligase by buthionine-(S,R)-sulfoximine → extensive centrilobular necrosis from tienilic acid

c. Kupffer cells

(1) Produce cytokines mediating hepatic acute phase response and cholestasis to LPS in circulation → bile acid transport sensitivity resides with regulation of Ntcp by retinoid X receptor-retinoid acid receptor nuclear heterodimer and liver-enriched transcription factor hepatocyte nuclear factor 1α inhibited by cytokine release

2. Sex-linked damage

a. Female human and animals have higher rates of certain liver toxicities related to:

(1) Continuous release of growth hormone from the pituitary (vs. male stochastic release) → activate STATs (signal transducers and activators of transcription) genes

(2) Males involve DNA methylation in gene expression and ↑ CYP7B1 (oxysterol 7α-hydroxylase) expression in males represses androgen biosynthesis (↓ bioavailability of DHEA precursor to testosterone)

(3) Women who take estrogen-containing birth control → activate estrogen receptor → regulates *CYP7b1* expression → inflammation and hepatotoxicity including intrahepatic cholestasis (most frequent liver toxicity of pregnancy)

(4) PCBs, PBBs, hexachlorobenzene → induce CYP1A1 (ethoxyresorufin O-deethylase) which is already high in centrilobular region of female fats → development of uroporphyria

b. Males sensitive to liver production of vitellogenin (egg yolk protein) by environmental estrogens → hypertrophy of the fish liver; formamide → hemangiosarcoma of the liver in male mice

3. Apoptosis/necrosis

a. CYP metabolites from pharmaceuticals (especially acetaminophen and statins) → apoptosis → severe damage = necrosis—worse with ethanol

b. Diversity of damage leads to death of cells including cholestasis, viral hepatitis, ischemia or reperfusion injury, liver preservation for transplantation, and direct toxicity of medications or industrial chemicals

c. Mitochondrial permeability changes and dysfunction lead to apoptosis (requires sufficient ATP synthesis to initiate a death program through Fas → caspase cascade) or necrosis

CONCEPTUALIZING TOXICOLOGY 16-1 (*continued*)

d. Invasion of neutrophils → centrilobular necrosis (macrophages and natural killer cells also promote injury via TNF-α, IL-1β, and NO), while monocyte infiltration (especially M2s) → phagocytosis of apoptotic cells, resolves inflammation and promotes tissue repair (via Il-10, IL-6, and IL-18)

4. Steatosis (fatty liver)
   a. Obesity, insulin resistance, ethanol → triglyceride accumulation
   b. CCl_4 especially with protein synthesis inhibitor puromycin → steatosis (↓ triglyceride secretion via VLDL and other triglyceride transfer proteins)
5. Fibrosis/cirrhosis
   a. Ethanol → macrocyte involvement (M1) and stellate liver cells, which are activate by CD8+ T cells
   b. WBCs responsible for fibrosis vary based on the development of hepatic damage
6. Neoplasms
   a. Develop from severe DNA modification or multiple insults (mutations in tumor suppressor genes [p53 mutations present in 30–60% of liver cancer patients], proto-oncogenes, and DNA mismatch genes); aflatoxin B_1 forms an epoxide via CYP → mutates p53 at codon 249 (promoter of cancer), but aflatoxin is also an initiator (does not require cirrhotic damage) and works synergistically with hepatitis B virus (incorporates in host DNA and activates promoters of several oncogenes, inhibits apoptosis, viral protein ↑ expression of epidermal growth factor receptor and potentiates TGF-α, fibrosis stimulated by upregulating expression of TGF-β, inflammation)
   b. Inflammation → fibrosis or cirrhosis or cancer (hepatitis C model)
   c. Vinyl chloride monomer → CYP oxidation to chloroethylene oxide → exocyclic etheno adducts form with DNA (and generate lipid peroxidation and oxidative stress) → promutagenic and affect proto-oncogenes and tumor suppressor genes
   d. Stimulating the AhR (dioxins and PCBs), constitutive androstane receptor (phenobarbital), or peroxisome proliferators (plasticizers = phthalates) → cancer without gene mutation (mitogens)
   e. Obesity → cancer may be due to fatty liver, gallstone development, or related to food ingredients, excessive calories, loss of protective factors due to ↓ exercise, or signaling factors from adipose tissue
7. Cytoskeleton toxicity
   a. Ethanol affects α-tubulin (hyperacetylation) and microtubule stability
   b. Macrolactones of marine toxins target actin cytoskeleton → severe hepatotoxicity
   c. Microcystins from cyanobacteria (agricultural lakes) → inhibit phosphatases 1 and 2A—disrupts cytoskeleton → hepatic hemorrhage
   d. As disrupts cytoskeleton (soundness, shape, and movement)
   e. Anticancer medication paclitaxel (diterpenoid) → binds to β-tubulin and prevents disassembly of microtubules → mitotic arrest; opposite mechanism of vinca alkaloids that prevent polymerization of microtubules by binding to β-tubulin and not allowing α-tubulin interaction → mitotic arrest (same endpoint)
8. Sinusoidal injury
   a. Binge ethanol intake + acetaminophen → cytoskeletal injury → sinusoidal epithelial cells swell and lose ability to endocytose FITC-FSA → fenestrae form → RBC penetration into Space of Disse → sinusoidal collapse and ↓ blood flow (similar to veno-occlusive disease caused by pyrrolizidine alkaloids of the toxic *Crotalaria* plant species)
9. Immune and inflammatory damage
   a. Metabolic liver injury from medications formation of active metabolites damaging organelles → initiate immune response

CONCEPTUALIZING TOXICOLOGY 16-1 (*continued*)

b. Immune liver injury from anti-medication antibodies or autoimmune antibodies are generated by anesthetic halothane or diuretic tienilic acid (rapid onset on subsequent challenge) or slow-developing autoimmunity in brown Norway rats exposed to anti-arthritis drug penicillamine or cats exposed to the medication for hyperthyroidism propylthiouracil

c. Medications such as antibiotics minocycline and nitrofurantoin, and discontinued antihypertensive medication α-methyldopa develop a condition resembling idiopathic (unknown cause) autoimmune hepatitis—may also involve autoimmune reactions resembling lupus, autoimmune-hemolytic anemia, or vasculitis as well

d. Liver prone to infiltration by immune cells (lymphocytes and neutrophils) due to two vascular supplies, lack of certain adhesion molecule (P-selectin) to prevent infiltration and presence of chemoattractant molecules/receptors (VAP1 and osteoponin among others)

e. Neutrophils generate hypochlorous acid → ROS → cell mortality

10. Cholestasis and bile duct toxicity

a. Retention of toxic bile salts due to damage to drug transporting proteins on hepatocyte canalicular membrane

b. Inhibition of ATP-dependent bile salt transport proteins ↑ damage by medications/toxicants—e.g., anti-diabetic, anti-inflammatory troglitazone + ACE inhibitor (hypertension medication) lisinopril

c. Direct damage to cholangiocytes (epithelial cells of bile duct) sustain direct damage by medications such as the antibiotic flucloxacillin

d. Usually liver cells sense toxic products of metabolism and ↑ elimination (xenobiotic receptors CAR and PXR = members of NR1I nuclear receptor family) induced by phenobarbital (as are some CYPs)

E. GI tests—see Table 16-4

1. DNA analysis of cheek swabs; micronucleus assay of buccal cells
2. ^{13}C-sucrose breath test for small intestinal sucrase activity; $^{13}CO_2$ breath evolution test for mucositis (cancer chemotherapy and jejunal sucrase activity)
3. NSAIDs and cancer chemotherapy → GI permeability probes, oxidative phosphorylation (direct measures + mitochondrial DNA analysis), ROS formation (direct measures + COX-2 mRNA expression)
4. Cell and tissue preparations analyzed for organelle damage + biochemical measures (e.g., keratin in epithelial cells, enzyme markers for brush border, lysosomes)
5. Intact animals assessed by nutrient and toxicant absorption—compared with ulcer model of MPTP in rat, EtOH-induced colitis, 1,2-dimethylhydrazine-induced colon cancer, enterohepatic recirculation in monkey (has gall bladder)
6. Zebrafish GI motility alternative to mammalian testing

F. Pancreatic tests—see Table 16-5

1. Leakage of digestive (exocrine) enzymes amylase and lipase into plasma
2. Endocrine cell damage monitored by insulin and glucagon (β-cells and α-cells, respectively), direct examination of Islets of Langerhans cells (β-cell mass), anti-insulin antibody immunohistochemical staining, or effects of lack of insulin (↑ blood or urine glucose or glucose tolerance test if minor damage)
3. Inflammatory reactions assessed via IL-6, TNF-α + histological grading of vacuolization, inflammation, lobular disarray, and edema
4. Pancreatic cancer from agents such as TCDD need examination of apoptotic bodies, immunohistochemistry of CYP1A1, CCK, AhR, CCKAR, amylase, proliferation of cell nuclear antigen, and development/incidence of lesions

G. Liver tests—see Table 16-6

1. Leakage of enzymes into plasma (ALT = hepatocyte necrosis and AST less indicative as may be ↑ with muscle and heart damage, body mass changes, blood diseases, and pancreatic

CONCEPTUALIZING TOXICOLOGY 16-1 (*continued*)

injury)—transaminases should be 3 × normal activity and coupled to ↑ serum bilirubin (jaundice indicative of lack of liver processing of this hemoglobin degradation product)

2. Plasma GST-α indicative of centrilobular damage as occurs with acetaminophen
3. γ-Glutamyl transpeptidase indicates hepatobiliary damage
4. Paraoxygenase-1 (metabolizes organophosphates) reduction in serum is consistent with liver injury as it is transported there by HDL (cholesterol mobilizing lipoprotein)
5. Leakage of purine nucleotide phosphorylase into hepatic sinusoids = marker for necrosis prior to ALT leakage
6. Liver mitochondrial malate dehydrogenase in plasma = necrosis and cirrhosis
7. Leakage of sorbitol dehydrogenase = necrotic liver
8. Leakage of alkaline phosphatase = cholestatic hepatobiliary injury
9. Function aspects of liver damage = ↑ ammonia (urea cycle not functional), jaundice (bilirubin not conjugated to glucuronic acid and sent on in the bile to be converted to other products by gut flora), lack of plasma proteins albumin, and clotting factors prothrombin and fibrinogen
10. Liver organ weight may be useful in assessing damage but more specific information derived from fatty tissue, glycogen, and bile acid content as does examination for periportal, midzonal, and centrilobular necrosis or random damage
11. Centrilobular hypertrophy may accompany CYP induction
12. Steatosis (fatty liver) can be microvesicular or macrovesicular
13. Liver cancer or precancerous lesions may be divided into hepatocellular alterations, hepatic hyperplasias/adenoma, or primary hepatocyte carcinoma

CONCEPTUALIZING TOXICOLOGY 16-1 (*continued*)

Oral exposure from ingesting contaminated food or water is the next most likely route of exposure after skin and eye exposure. Consider babies' instinctive behavior of putting objects in their mouths for examination. There are a number of structures that deserve closer examination here. The toxicity to absorption structures is the major focus in this chapter. It is appropriate that this chapter be reviewed after the skin chapter, because the skin and the gastrointestinal (GI) tract both have high cell turnover that can be exacerbated by conditions that cause skin (e.g., psoriasis) or GI cell sloughing (e.g. *Helicobacter pylori* infection [Abdalla et al., 1998], stress, or Crohn's disease [Boudry et al., 2007]). Two of the areas that suffer the highest toxicity on exposure to external ionizing radiation are the skin and the GI tract. The radiological treatment of prostate cancer, for example, is indeed limited by concerns of GI damage (Sefrová et al., 2009). This chapter could then warrant the same considerations as the coverage of the skin regarding mechanisms of damage and toxicity as the GI tract is considered "external" to the body. However, the important structures in each area of the tract that involve taste and part of speech, mastication, saliva secretion, swallowing, peristalsis, acid and enzyme production and secretion, specialized absorption, and finally the portal vein processing by the first pass through the liver are essential to consider for activation of toxicants, detoxication, nutrient processing, and conversion of waste products for excretion by the kidneys. The liver is the last structure covered in this chapter, because it represents the final processing of intestinal contents prior to becoming part of the bloodstream.

Forensic Analysis of GI Damage

Table 16-1 is a forensic table for GI toxicity and liver damage resulting from portal vein transport. Note that it is separated into regions for examination of specific problems resulting from toxicity to a given area.

TABLE 16-1 Forensic Chart of GI Toxicity

GI Toxicity	Toxic Agents
Mouth	
Bone	Bisphosphonates (Reid, 2009), tetracycline, TCDD (Alaluusua and Lukinmaa, 2006), mycotoxins, secalonic acid D, cortisol, retinoic acid (Dhulipala et al., 2006)
Burns	Concentrated acids, alkali, peroxides (Naik et al., 2006)
Cancer	Radiation, tobacco, ethanol (McCullough and Farah, 2008)
Edema/irritation	Oxalates (Zhong and Wu, 2006)
Gingivitis	Inorganic mercury (Schmid et al., 2007)
Mucositis	Anticancer (Keefe and Gibson, 2007)
Muscle paralysis	Botulinum toxin (Braun, 2006)
Paresthesias (numbness)	Topical Na^+-channel blockade (local anesthetics), systemic Na^+ channel poisoning (Watkins et al., 2008)
Salivary glands	Radiation (Bhide et al., 2009), anticholinergic medications (psychotropic; Smith et al., 2008)
Esophagus	
Mucosal injury	Caustic agents, aspirin/NSAIDs (Sugimoto et al., 2010), cytokines (Souza et al., 2009), oxidative stress (Liu et al., 2009), tetracycline, KCl^+ quinidine SO_4, bisphosphonates taken without water at high doses (Zografos et al., 2009), diabetes mellitus and generation of RNS/glyco-oxidation (Zayachkivska et al., 2008)
Cancer	Acid, bile refluxes (Bernstein et al., 2009), ethanol, tobacco compounds (Szymańska, 2010)
Swallowing	Atropine (Tsubouchi et al., 2008), caustic agents and strictures (Doo et al., 2009), chemoradiotherapy (Lazarus, 2009)
Stomach	
Cancer	Nitrates forming nitroso compounds with *H. pylori* (Izzotti et al., 2009), bitumen fume condensates (Binet et al., 2002)
Hemorrhage	NSAIDs (Lim et al., 2009), *Panicum maximum* cultivars Mombasa, Tanzania, and Maasai (Cerqueira et al., 2009)
Mucosal damage	Salt (Izzotti et al., 2009), ethanol (Lira, 2009)
Ulcers	NSAIDs (Lim, 2009), *H. pylori* with mutagens (Izzotti et al., 2009)
Duodenum	
Bleeding ulcers	Aspirin (Yeomans et al., 2009)
Cancer	Cr(VI) (Stout et al., 2009)
CYP expression	As and metabolites (Medina-Díaz et al., 2009)
Graft-versus-host disease	Mycophenolate mofetil (Parfitt et al., 2008)
Hemorrhage	*Clostridium perfringens* beta-toxin (Vidal et al., 2008)
Malabsorption of nutrients	Ethanol (Krawitt, 1977), avidin (White et al., 1992)
Mucositis	Anticancer, radiation (Sonis, 2009b)

(*continues*)

TABLE 16-1 Forensic Chart of G.I. Toxicity (*continued*)

GI Toxicity	Toxic Agents
Nitrosative stress	Methotrexate (Kolli et al., 2008)
Sloughing	Acute mercury poisoning (Iino et al., 2009)
Ulcers	Methyl ethyl ketone peroxide (van Enckevort et al., 2008)
Villus height reduction	*Fusarium* mycotoxins (Girish and Smith, 2008)
Jejunum	
Glutathione depletion	Buthionine sulfoximine (Mårtensson et al., 1990)
Increased toxicant absorption	Fe deficiency increasing Ni absorption (Müller-Fassbender et al., 2003)
Inhibited transport of simple sugars	Deoxynivalenol (Awad et al., 2007), Cd (Mesonero et al., 1996)
Nerve-mediated Na and fluid loss	Ethanol (Hallbäck et al., 1990)
ROS and RNS Sodium-potassium ATPase inhibition	Intrinsically high and decreased by endotoxin challenge (Kozlov et al., 2003)
Transport of toxic glycosides	Ouabain, vanadium (vanadate; Hajjar et al., 1989)
	Prunasin (amygdalin metabolite; Strugala et al., 1995)
Ileum	
Acetylcholine release	Aflatoxin B_1 (Luzi et al., 2002)
Antagonism at $Ca2^+$ channels	Isoliquiritigenin (Chen, Zhu, et al., 2009)
Bacterial prodrug activation	Irinotecan (Brandi et al., 2006)
CYP3A expression	Chenodeoxycholic acid enhance toxicity of lithocholic acid (Khan et al., 2010)
Enteritis	*Clostridium perfringens* type A toxins (Cooper and Songer, 2009), *Clostridium difficile* toxin A (Cavalcante et al., 2006)
Genotoxicity	Okadaic acid (Le Hégarat et al., 2006)
Inflammatory alterations	Depleted uranium (Dublineau et al., 2007)
Ischemia, infarction, and hemorrhage	Cocaine (Lingamfelter and Knight, 2010)
Multiple-organ dysfunction syndrome	Zymosan induction of TNF-α (Malleo et al., 2008)
Permeability changes/sepsis	Luminal phospholipase A2 activation by LPS (Zayat et al., 2008), glyphosate (Vasiluk et al., 2005), Cd^{2+} (Duizer et al., 1999)
Colon	
Cancer/clastogenesis	*N*-nitroso compounds (Pearson et al., 2009)
Cancer/lipid peroxidation	Fe (Lund et al., 2001)
Cancer/polyamine synthesis and inflammation	Single nucleotide polymorphism in ornithine decarboxylase promoter (Rial et al., 2009)
Chloride secretion	Trimethyltin chloride (Yu et al., 2009)
Colitis models	Dextran sodium sulfate (Whittem et al., 2010) 2,4,6-trinitrobenzene sulfonic acid (Fichtner-Feigl et al., 2008)
Cytotoxicity	Cobalt-doped tungsten carbide nanoparticle suspensions (Bastian et al., 2009)
DNA methylation/cell signaling	Dimethylhydrazine/azoxymethane (Davidson et al., 2009; Likhachev et al., 1978;

Mouth Toxicity

Dentists can provide information about what might be toxic to structures in the mouth. The dental profession is concerned with the combined effects of high sugar on mouth bacteria and the degradation of teeth by low-pH beverages (carbonic acid) such as soda pop. Certain compounds also discolor teeth. Coffee, tea, and red wine can discolor teeth, as does the use of tobacco products. The antibiotic tetracycline binds strongly to calcium and can produce a blue-gray stain. Fluoride used by dentists to guard against tooth decay also can stain from chalky white to brown. Minocycline similarly stains teeth a green/grey or blue/gray. This discoloration appears to be permanent. Another antibiotic of the quinolones, ciprofloxacin, has caused a greenish discoloration of teeth if given to infants prior to teething. Chlorhexidine used in prescription antiseptic mouthwashes cause yellow-brown stains. Iron can also stain the teeth and cause colon cancer or liver damage depending on the dose (Addy and Moran, 1985). The tongue may be discolored from systemic poisoning; for example, green tongue is observed in workers heavily exposed to vanadium pentoxide (Kawai et al., 1989). More disturbing than coloration changes alone, certain compounds cause cancer and disturb tooth and enamel development. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) through the aryl hydrocarbon receptor (AhR) and epidermal growth factor receptor increases apoptosis of cells. In the developing rat (or Finnish children), mineralization of teeth is decreased by TCDD exposure resulting in arrests in molar development. Polychlorinated dibenzodioxins/polychlorinated dibenzofurans (PCDDs/PCDFs) appear to affect the formation of the protective enamel layer of teeth (Alaluusua and Lukinmaa, 2006). Some chemicals also disturb proper developmental bone fusion in the mouth. Cleft palate results from chemicals that disrupt cell-cycle progression and proliferation such as TCDD, mycotoxin, secalonic acid D, glucocorticoids, and retinoic acid (Dhulipala et al., 2006).

It is appropriate to look at the mouth for the first signs of toxicity or DNA damage, because it represents the route for ingestion and inhalation. Because 90% of all cancers originate from epithelial cells, it is worth examining the oral epithelium. It has four layers: the lamina propria composed of connective tissue, stratum basale or the basal cell layer, the stratum spinosum or prickle cell layer, and the keratinized layer at the surface. Rete pegs project from the lamina propria into the epidermal layer. The basal cell layer has a high mitosis rate, producing new cells that migrate to the surface to replace those shed from continual use. It has CYPs and is capable of activating/metabolizing toxic compounds. This can transform the stem cells in the basal layer, which can be examined for chromosome breakage and loss (Holland et al., 2008). Mucosal injury or mucositis occurs dramatically as mouth ulcers or diarrhea in lower parts of the GI tract with targeted anticancer therapies (Keefe and Gibson, 2007). Because teeth-whitening products contain either hydrogen peroxide or carbamide peroxide, toxicity is of concern for repeated use of high concentrations in the mouth. Animal studies have shown that 30% H_2O_2 causes severe irritation or burns. If the peroxide comes in contact with blood or tissue proteins, the effervescence releases oxygen and causes tissue emphysema. Thirty percent H_2O_2 results in edema of the tongue and subsequent intraepithelial and subepithelial vesiculation. Hydrogen peroxide rinses can result in mouth irritation, dryness, loss of taste, elongation of filiform papillae, and diffuse mucosal whitening. Cellular studies indicate that 0.34–1.35 μM H_2O_2 induces a dose-dependent increase in hypoxanthine-guanine phosphoribosyltransferase mutations in human T lymphocytes and other measures of increased genotoxicity (cytokinesis block micronucleus assay and sister chromatid exchanges [Naik et al., 2006]).

Some plants have a high concentration of nonsoluble calcium oxalate crystals including *Anthurium* species, *Arisaema* species, *Caladium bicolor, Zantedeschia* species, *Aglaonema* species, *Dieffenbachia* species, *Monstera deliciosa*, *Syngonium podophyllum*, *Philodendron* species, *Epipremnum aureum*, and *Symplocarpus foetidus*. The stalk of the *Dieffenbachia* plant is the most potent it this regard. Oxalate needlelike crystals produce pain and edema when an animal tries to ingest

the toxic plant (lips, tongue, oral mucosa) or if they contact their face (conjunctiva or skin). The oxalates cause direct trauma from physical action of the crystals resulting in edema and secondarily from bradykinins and enzymes released from the plant cells (Zhong and Wu, 2006).

Radiation for head and neck cancer in humans may induce dry mouth or xerostomia, sore throat, altered taste, dental decay, changes in voice quality, and impaired chewing and swallowing leading to decreased nutritional intake and weight loss. This is due to damage to the parotid (purely serous and produces most of saliva in stimulated state) and submandibular glands (predominantly serous with 10% mucus-secreting acini and produces most of saliva in unstimulated state) responsible for most of salivary flow. The salivary glands contain secretory units consisting of acinar cells (secrete serous/protein and mucous/mucin portions of saliva), myoepithelial cells, intercalated duct, striated duct, and excretory duct. Parasympathetic stimulation via acetylcholine release to postsynaptic M3 muscarinic receptors activates inositol-1,4,5-trisphosphate, which mobilizes intracellular calcium stores, triggering a watery saliva flow low in amylase for digestion of polysaccharides from acinar cells. Sympathetic activation via β_2 adrenergic receptors activates protein kinase A throughout the cAMP pathway and causes exocytosis of secretory granules (scant, viscous saliva) high in amylase. Salivary flow reductions (50–70% after 10–16 Gy radiation) or loss (after 40–42 Gy radiation) is a phased process, which has been studied in the rat. Days 0–10 or phase I cause a 40% reduction in water without altering amylase secretion or producing cell loss. In 10–120 days, plasma membrane destruction leads to acinar cell death and lack of amylase secretion. Progenitor cell and stem cell death occur during phase III (120–240 days). Radiation changes the saliva to thick, tenacious, and acidic. Additional chemotherapy (not cetuximab, which is an epidermal growth factor receptor inhibitor) results in increased acute toxicity, especially for mucositis (Bhide et al., 2009). Another reason for dry mouth can be the effect of anticholinergic compounds like atropine (used for dental surgery for that reason) or the adverse effects of psychotropic medications (Smith et al., 2008).

Radiation is also a predisposing factor to oral cancer as any dental patient is made aware during their cancer screening for chronic cumulative effects of dental X-rays. Also, it makes sense that because CYPs are present in the cells of the mouth, tobacco chewing or smoking also lead to activated metabolites that result in oral carcinogenesis. Many people do not know that their antiseptic mouthwashes or the consumption of ethanol and tobacco increases the risk of oral cancers. Those who smoke and drink alcohol increase the penetration of the oral mucosa by the carcinogens in smoke in the presence of ethanol. Ethanol can eliminate the lipid component of the barrier present in the oral cavity and increase the permeability of the human ventral tongue mucosa. Chronic ethanol exposure results in epithelial atrophy and decreased basal cell size in rat esophageal mucosa and hyper-regeneration, making the tissues more sensitive to chemical carcinogens. Short-term exposure of rabbit oral mucosa to ethanol caused dysplastic changes with keratoses (premalignant lesions), increased density of basal cell layer, and increased mitotic figures. Alcohol dehydrogenase present both in the epithelial cells and in people with aerobic oral flora including *Streptococcus salivarius*, *S. intermedius*, and *S. mitis* produce high amounts of the primary metabolite of ethanol, acetaldehyde. Acetaldehyde is mutagenic and appears to be covalently bound to protein and DNA in patients with oral cancer and dysplasia, with lipid peroxidation products as well (McCullough and Farah, 2008).

Bone toxicity may result from some toxicants and medications. Bisphosphonates (zoledronate and/or pamidronate) used in cancer management cause osteonecrosis of the jaw. They probably do so via increased infection, ischemia (decreased proliferation of endothelial cells), low bone turnover, direct toxicity to the bone (inhibition of farnesyl pyrophosphate synthase in the mevalonate and ultimately cholesterol result in apoptosis in osteoclasts), and toxicity to soft tissue. Non-osteoclasts may suffer toxicity through inhibition of the mevalonate pathway in the absence of a bone surface.

The presence of a bone surface adjacent to the soft tissue may cause toxicity due to binding and accumulation of the bisphosphonate at the bone surface (Reid, 2009).

Muscle paralysis may result from a variety of toxicants including D-tubocurarine. However, ingestion of the toxin from *Clostridium botulinum* is first indicated by paralysis of the tongue and the muscles of mastication, although death comes from respiratory paralysis (Braun, 2006).

Taste is a discriminator used by a variety of species for initial indication of toxicity. A bitter taste results in reflex rejection. What is bitter taste though? A physiology book might indicate that bitter taste results from blocking potassium channels and downstream activation of a G protein known as transducin. It appears that bitter ligands activate "B-best" neurons in the nucleus of the solitary tract and parabrachial nucleus (PBN). However, the PBN B-best units are activated by intense salt and acid. This indicates a strong bitter rejection that can result from a compound such as quinine due to its bitter nature or a high concentration of salt/acid (Travers and Geran, 2009).

Some systemic toxicities are also diagnosed by signs from damage to mouth cells. A likely example is the "mad hatter's" disease experienced by occupational exposure of workers in the felt hat industry to mercury nitrate. The triad for determining exposure is gingivitis or gum disease, tremor, and erethism or an abnormal form of excitability. Although this represents mainly a neurotoxic action of inorganic mercury, silver-colored amalgam fillings for cavities contain mercury in an inorganic state that can evolve by volatilization or physical grinding and corrosion. Chronic exposure to mercury vapor and/or inorganic mercury leads to oral cavity lesions, tremor, decreased coordination, decreased sensation, and psychiatric symptoms including anxiety, excessive timidity, and pathological fear of ridicule (note triad of symptoms is part of this overall picture). Although methyl mercury has been viewed as more toxic, inorganic mercury can damage salivary gland DNA (Schmid et al., 2007).

Some toxicants cannot be deciphered by taste. Sodium-channel blockade by local anesthetics (as used in dental work) can cause loss of feeling on the lips, mouth, and other oral structures. Brevetoxins (10 lipid-soluble cyclic polyethers) from the dinoflagellate *Karenia brevis* cause neurotoxic shellfish poisoning by binding to receptor site 5 on the voltage-gates sodium channel and induce a channel-mediated sodium influx. Nausea and vomiting occur with paresthesias (burning or tingling sensations) or numbness of mouth, lips, and tongue (and distal paresthesias, ataxia, slurred speech, and dizziness [Watkins et al., 2008]). Mercury poisoning can also result in a metallic taste.

Esophageal Toxicity

Swallowing is a key function mediated by a variety of structures from the top of the esophagus to the sphincter at the entrance to the stomach. Chemoradiotherapy for head and neck cancers not only influences the mouth, but also impairs swallowing and voice (Lazarus, 2009). Even a relatively benign antimetabolite such as methotrexate if taken at toxic concentrations causes development of bullae and vesicles in the mouth and makes swallowing difficult (Bookstaver et al., 2008). Atropine used in oral surgery to dry the area of the mouth also induces a swallowing disorder that inhibits the cough reflex in dogs. This may lead to aspiration pneumonia (Tsubouchi et al., 2008). Caustic agents apparently are readily available in Asia and many children have taken in strong alkali and acids. Swallowing is made difficult by the esophageal strictures that form following injury, but can be repaired surgically with balloon dilatation. However, the caustic injury or the surgical repair attempt can result in esophageal rupture (Doo et al., 2009). Aspirin augments the effects of gastric reflux for those with a lower intragastric pH (Sugimoto et al., 2010). Drug-induced esophageal injury occurs mainly where the esophagus narrows (middle third behind left atrium). Tetracycline-induced injury is transient and self-limiting and represents one class of agents that produces esophagitis. Persistent esophagitis with stricture occurs in patients taking nonsteroidal anti-inflammatory drugs (NSAIDs) enhanced by gastroesophageal reflux or those supplementing KCl while

taking quinidine sulfate. Severe injury has been observed with osteoporosis patients on biphosphonates who have taken high doses without water and then lie down (Zografos et al., 2009). On the other end of the pH range, for example, 2.25% ethyl ammonium chloride was intentionally ingested by a 60-year-old woman. She developed a persistent cough, copious oral secretions, worsening hoarseness, and had poor esophageal motility in the mid to lower third of the esophagus in addition to systemic mild hypotension, nonanion gap metabolic acidosis, and oliguria (Hammond et al., 2009). For NaOH, the toxic pH for esophageal injury is > 11.5 and is time- and pH-dependent (Atug et al., 2009).

Gastroesophageal reflux has been related to development of esophageal cancer. A current alternative to the caustic damage theory for development of esophagitis is a cytokine-mediated mechanism as studied in rats (Souza et al., 2009). Oxidative stress as indicated by an increase in malondialdehyde and decrease in superoxide dismutase content also appears to play a role in esophageal mucosa injury (Liu et al., 2009). What is not generally well known is that bile acids may be involved in cancers of the esophagus, stomach, small intestine, liver, biliary tract, pancreas, and colon/rectum. The untoward effects of bile acids are reactive oxygen species (ROS) and (RNS) induction, DNA damage, increased mutation, short-term induction of apoptosis, and selection for long-term apoptosis resistance (Bernstein et al., 2009). Diabetes mellitus is a condition that also causes damage to the esophagus. In an experimental animal model of uncontrolled hyperglycemia induced by streptozotocin, there is an excess production of RNS initiating nitrosative stress. This stress depends upon the balance of pro- and antioxidative activity, paracrine regulation (NO/NOS and PG/COS signaling pathways), and pre-epithelial and epithelial cellular homeostasis. Identification alterations of glycoconjugates of the epithelial barrier and generation of peroxynitrite are important in the pathophysiology of diabetes mellitus–induced esophageal injury (Zayachkivska et al., 2008). Certain medications other than aspirin have untoward effects on the GI tract. The immunosuppressive medication mycophenolic acid causes increased apoptotic counts in the esophagus in 57% of patients taking the medication between 1 month and 10 years posttransplant. Similar findings were found in the duodenum (82%), but less in gastric biopsies (28%). The symptoms associated with these apoptosis increases were diarrhea (55%), nausea (45%), abdominal pain (35%), vomiting (25%), GI bleed (15%), dysphagia (difficulty swallowing for 10%), dyspepsia, anemia, and hematemesis (5% for each; Nguyen et al., 2009).

Esophageal cancers resulting from TP53 mutations (exons 4–10) are common in South America and appear to involve the same risk factors as oral cancer—ethanol and tobacco product use. Mutations inactivate this gene, which normally makes the tumor protein 53. This protein binds directly to DNA and determines whether damaged regions will be repaired or to signal the process of apoptosis. The mutation rate of this gene increased from 38% in nonsmokers to 66% in current smokers, with G:C > T:A transversions in 15% of smokers alone. Alcohol drinkers were observed to have more G:C > A:T transitions. G:C > A:T transitions at CpG sire occurred in nonexposed individuals (Szymańska et al., 2010).

It is of interest that some toxic substances may also be of therapeutic use in the esophagus. Spasms of the lower esophageal sphincter between the esophagus and the stomach or achalasia make swallowing difficult and painful. Intrasphincteric botulinum toxin (80 U) appears to provide better results than balloon dilation for pregnant Thai women for swallowing and adequate nutrition (Wataganara et al., 2009).

Stomach Toxicity

Ulcers are known to originate from chronic use of NSAIDs and from *H. pylori* infections. NSAIDs have been used therapeutically to relieve pain (analgesia), decrease inflammation, and prevent strokes and heart attacks via its anticoagulant properties. Other factors influencing gastric toxicity are age- and sex-related or determined by use of tobacco products and ethanol as noted earlier for other sections of the GI tract. One of the most serious NSAID interactions is the age-related decrease in gastric

mucosal prostaglandin synthesis (PGE_2 and PGI_2 produced by the cytoprotective constitutively expressed cyclooxygenase enzyme COX-1) with age, when these medications are used more intensively for arthritis relief (anti-inflammatory role more of a function of inhibition of the inducible COX-2 enzyme) and prevention of myocardial infarctions. Because most NSAIDs have pKas from 3–5, their acidity directly induces cellular dehydration and mortality. Normally, acid does not penetrate well into cells with pH values close to neutral or slightly higher. However, the stomach pH makes NSAIDs with a pKa of 4–5 easily ionized and enhances their entry into gastric mucosal cells. In the cells, the slightly alkaline pH makes them exist primarily in the nonionized state. NSAIDs intracellularly become ionized and less hydrophobic, making them accumulate in what has been referred to as the "chemical greenhouse effect." Their accumulation leads to focal mucosal pallor followed by hemorrhagic foci and ulceration simultaneously with decreased mucosal blood flow. The chemical association with acidic derivatives of NSAIDs and surface phospholipids appears to explain a decline observed in mucosal lipophilicity. Acutely, these medications inhibit cell proliferation but have the opposite effect chronically to cells of fundic and duodenal mucosa. NSAIDs also initiate or exacerbate stomach inflammation despite their systemic anti-inflammatory activity through upregulation of adhesion molecules (increased cytokine TNF-α, leukotriene LTB_4, and intracellular adhesion molecule-1 [ICAM-1]) with leukocyte adherence to the vascular endothelium in the microcirculation of the stomach. NSAIDs also uncouple mitochondrial oxidative phosphorylation leading to decreased adenosine triphosphate (ATP) and increased cell death. The endoplasmic reticulum (ER) also experiences stress by induction of glucose-regulated protein-78 (adapts to accumulation of unfolded proteins with some stress) and C/EBP homologous transcription factor (induces apoptosis with overwhelming stress). If the stress is increased to the ER, apoptosis can be initiated via activating transcription factor 6 (ATF6), ATF4, and X-binding protein. Increased intracellular calcium is also caused by celecoxib ("safer" on GI system COX-2 inhibitor), which activates the ER stress response.

Another reaction of interest in the stomach (and in the brain, cardiovascular system, liver, and kidney) is the generation of hydrogen sulfide. H_2S can be damaging (pro-inflammatory, vasodilatory) or protective (anti-inflammatory, atherosclerotic) depending on the concentration generated—as are other signaling molecules such as NO or CO. The gastric mucosa expresses two enzymes that mediate H_2S generation, cystathionine β-synthase, and cystathionine γ-lyase. H_2S protects endogenously to mucosal injury, but contributes to the inflammation produced by *H. pylori*. H_2S induces anti-inflammatory and cytoprotective genes in the presence of NSAIDs including heme oxidase-1 (HO-1), vascular endothelial growth factor, insulin-like growth factor receptor, and several genes associated with the transforming growth factor (TGF)-β receptor signaling pathway. It is of interest that the induction of HO-1 may produce more CO, which is cytoprotective and anti-inflammatory at endogenous concentrations via inhibition of NF-κB and inducible nitric oxide synthase. NSAIDs also increase the amplitude and frequency of gastric contractions/motility. This increases microvascular permeability and promotes cellular damage (Lim et al., 2009). NSAIDs also induce matrix metalloproteinases (MMPs), especially MMP-9 and MMP3, in a dose-dependent manner along with infiltration of inflammatory cells and disruption of the gastric mucosa. Melatonin downregulates both MMPs and heals acute gastric ulcers. Melatonin also provides antioxidant activities that protect against NSAID-induced gastric damage (inhibits protein oxidation, lipid peroxidation, hydroxyl radical formation, and SOD-2 expression [Ganguly and Swarnakar, 2009]).

The gram-negative rod *H. pylori* is another major contributor to stomach ulcers. However, alone it is not the threat that a cursory examination might indicate. In almost all infected people, a chronic gastritis develops starting with introduction by 10 years of age (90% of children). The progression of the disease to ulcers or other clinical outcomes depends on the genotype of the infection, host health status, and exposure to environmental factors. This

last portion is the focus of toxicology interest. Adequate nutritional status (high consumption of fruit, vegetables, and vitamins) appears to prevent the pathology associated with infection. Chronic *H. pylori* infection may be accompanied by normal, decreased, or increased acid secretion (via direct inhibition by the bacterial vacuolating cytotoxin, lipopolysaccharide, or acid-inhibiting factor or indirect inhibition of parietal cell function via cytokines, hormonal, paracrine, and neural control mechanisms), all of which may progress to gastric ulcer. Type I ulcers occur in the gastric body and appear to occur in people with low night acid secretion. Type II ulcers occur in the antrum and appear irrespective of acid secretion. Type III ulcers are found within 3.0 cm of the pylorus, associate with duodenal ulcers, and occur with high acid secretion. Type IV ulcers are observed in the gastric cardia and low acid secretion. Antral-predominant gastritis correlates with duodenal ulcer development. Corpus-predominant gastritis increases the risk of development of gastric ulcers, gastric atrophy, intestinal metaplasia, and gastric adenocarcinoma. Oddly, a systemic infection with *H. pylori* increases vascular inflammation as well and is correlated with increases in coronary artery disease, atherosclerosis, and stroke. This organism has also been connected to idiopathic Parkinson's disease or Alzheimer's disease. Glaucoma, especially open-angle glaucoma and pseudo-exfoliation glaucoma, may also involve *H. pylori* infection. The reason for mentioning these diseases is due to the generation of ROS and other inflammatory mediators. Toxic substances share some of these mechanisms as a key to their impact. For example, *H. pylori* infection stimulates macrophages through L-arginine/nitric oxide pathways. In the stomach, the generation of NO will yield the nitrosating compounds N_2O_3 and N_2O_4. These compounds in turn can produce nitrosamines and yield DNA damage. Nitrosating organisms in the GI tract can also catalyze the reaction between nitrite and organic nitrogen compounds in the stomach contents to form genotoxic *N*-nitroso compounds. The nitrite is formed in the stomach during hypochlorhydria occurring from *H. pylori*–induced atrophic gastritis. Stomach bacteria convert dietary nitrate to nitrite. Nitrate absorbed in the upper GI tract can also be concentrated by the salivary glands, and buccal bacteria convert the secreted nitrate to nitrite. This explains why diets high in fruits and vegetables high in antioxidant carotenoids, vitamin C, and vitamin E avoid nitrosamine formation and prevent the toxic action of nitrosamides. Similarly, polyphenols or catechins from the popular green tea brews also inhibit intragastric nitrosation. These protective mechanisms yield less cancer of the GI tract in individuals with *H. pylori* infection. Regarding noncancer diseases of the stomach, the high-salt diet of Americans consuming processed food destroys the mucosal barrier and favors *H. pylori* colonization. Salt has been found to cause gastritis and increase the genotoxic action of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine. The combination of high salt and low antioxidants (fresh fruits) aids the progression of atrophic gastritis. The combination of bacterial colonization, increased nitrite concentrations, and depletion of vitamin C yield more formation of *N*-nitroso compounds. Vitamin E also protects the tract from *H. pylori* (in male Mongolian gerbils) by reducing the accumulation of activated neutrophils as indicated by reduced myeloperoxidase activity and mouse keratinocyte-derived chemokine in gastric mucosal cells compared with a tocopherol-deficient group. Although antioxidants do not protect against chronic gastritis from *H. pylori*, cruciferous vegetables contain phytochemicals that are converted to anticancer isothiocyanates such as sulforaphane by the GI flora, which in turn protect gastric mucosal cells from genotoxicity related to *H. pylori* infection. A source of antioxidants and cytotoxic chemicals that may aid in reducing infection is a combination of wild blueberry and other berry derivatives. It is also important to note that the atrophic gastritis caused by *H. pylori* also decreases vitamin B_{12} and folic acid absorption reducing methylation reactions, including the homocysteine to methionine reactions. This increases plasma homocysteine in infected patients (Izzotti et al., 2009).

Ethanol damages the stomach; this is found to be most problematic in chronic alcoholism. It is worth looking at the protective effects of a natural pentacyclic triterpene, lupeol, and

N-acetylcysteine (NAC) on ethanol-induced gastric damage to elucidate ethanol toxicity. Lupeol and NAC restore nonprotein sulfhydryl depletion/oxidation caused by ethanol. Lupeol's gastroprotection was decreased by indomethacin, a potent COX inhibitor, and L-NAME, an NO-synthase inhibitor, indicating key roles for these mechanisms on ethanol toxicity or increased ethanol toxicity with COX-inhibiting NSAIDs. Interfering with Ca^{2+} channels (verapamil) profoundly affected lupeol protection, indicating similar action in ethanol's disruptive effects. However, presynaptic adrenergic (α_2) antagonism by yohimbine or K(ATP)-channel blocker glibenclamide had weak activity in undoing lupeol's protection, indicating little sympathetic activity or K^+ channel activity in ethanol's gastric toxicity (Lira et al., 2009).

It is of importance that three different models of stomach ulceration exist for testing the effects of agents that might prevent those ulcers. One includes giving 0.2 mL of 0.3 M HCl/60% ethanol solution to mice. A proton pump inhibitor (omeprazole) is given to indicate a protection control group for comparison purposes. Another mouse model is 100 mg/kg indomethacin (strong NSAID) given *per os* (PO) and 5 mg/kg bethanechol intraperitoneally (IP; muscarinic agonist). Stress induced by restraining mice for 4 hours in a cold environment (4°C) is a third model. The human equivalent toxicities were discussed earlier. Because toxicology is also the study of antidotes, it is of interest that an alkaloid extract of the bark of the Bolivian plant *Galipea longiflora* (Rutaceae family) was more potent in preventing stomach ulcers in the three murine models due to decreased acid secretion and increased gastric mucus content than was 2-phenylquinolone, which was also isolated from this plant (Zanatta et al., 2009).

Cancer of the stomach can result from exposure to the agents mentioned earlier or from the classic toxicants such as polycyclic aromatic hydrocarbons (PAHs). Road workers or roofers who work with asphalt are exposed to it via the skin and to bitumen fumes via the lung, stomach, and circulation to the bone marrow. This increases the risk of cancers to all these regions. Bitumen fumes contain PAHs. Nitrogen-, sulfur-, and/or oxygen-containing PAHs or their alkyl-substituted analogues may also lead to the mutagenicity and carcinogenicity of bitumen fumes. Polar adducts of DNA are observed in rats treated with coal-tar fume condensates. Thiophenes also appear to play an important role in the carcinogenicity of the fumes with some less mutagenic than the corresponding isomeric PAH and others potent carcinogens. It is of interest that sulfur analogues of PAHs in bitumen fumes have a higher concentration than the PAH of similar molecular weight, where the opposite is true for coal-tar fumes. This explains why more polar adducts of DNA are found in animals exposed to bitumen fumes (Binet et al., 2002). Road pavers show increased activation of the intrinsic pathway apoptosis–regulating proteins BAX and BCL-2 due to exposure to bitumen fumes from the hot asphalt. Their skin cells activate the extrinsic pathway for apoptosis with overexpression of tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) and its death receptor, DR5, and caspase-3 as well as enhanced terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) in chronically bitumen-exposed skin (Rapisarda et al., 2009).

Some "stomach" poisons are actually metabolic inhibitors and are used to control ants and similar pest populations (distinct from insect growth regulators and neurotoxins). These agents include hydramethylnon, sulfuramid, and sodium tetraborate decahydrate. Boric acid has also been used to poison rodents. In feeding studies in mice, boric acid causes hyperkeratosis and acanthosis (precancerous thickening) with hyperplasia and/or dysplasia of the stomach at high doses (25,000–100,000; National Toxicology Program, 1987). Plaster of Paris mixed 1:1 with sugar, cornmeal, cornstarch, oatmeal, and cocoa powder has been used as a homemade rodenticide by hardening in the stomach of the rodent and not passing through, causing starvation. An interesting compound that is really an anticoagulant was first used to disrupt the stomach and GI tract of rodents. Sweet clover (*Melilotus* sp.) was a good food source for milk cattle in Wisconsin at the beginning of the 20th century. However, spoiled sweet clover caused internal hemorrhaging and the death of dairy cattle. This is due to the release

of plant enzymes that, when the clover is cut, act on the glycoside component melilotoside, liberating a sugar moiety and coumarin. *Penicillium* and *Aspergillus* contamination results in metabolism to dicoumarol. This agent inhibits vitamin K epoxide reductase, which prevents clotting. Karl Link of the University of Wisconsin synthesized warfarin as a more potent derivative (the WARF name comes from his source of funding from the Wisconsin Alumni Research Foundation) and a rodenticide. After many years of use, rats and mice apparently were selected for spontaneous mutations in the gene synthesizing the vitamin K epoxide reductase (VKORC1) that conferred resistance to this "stomach" toxicant (hemorrhaging ulcer formation [Rost et al., 2009]).

Not everything that affects the GI tract has a known mechanism of action. For example, *Panicum maximum* cultivars Mombaça, Tanzânia, and Massai cause severe colic and death in horses and mules through severe hemorrhages and some mucosal erosions and ulcerations but the cause of this toxicity is unknown (Cerqueira et al., 2009).

Duodenal Toxicity

The specialized cells for absorption are found in the duodenum with villi and other sensitive structures. This is similar to the brush border also associated with the proximal tubule of the kidney where similar mechanisms occur for reabsorption of important nutrients that are filtered through the glomerulus. Problems with the duodenum or other sections of the small intestine (jejunum and ileum) may also involve pancreatic damage, because the pancreatic duct provides digestive enzymes into the proximal duodenum. Liver involvement may also prevent the formation or release of the bile salts into the proximal duodenum. Other internal organ toxicities may also impair absorption as certain transport molecules may not be formed. Additionally, changes in the microflora and nutritional deficiencies can augment or decrease absorption or nutrients or toxic substances. Also, inflammatory reactions may alter intestinal absorption. Even water absorption is crucial here, because toxicity may result in dehydrating diarrhea. One of the substances recognized early for many of these influences is ethanol. Acute ethanol administration increases the absorption of lipids, while chronic ethanol abuse yields decreased lipid absorption. Ethanol also diminishes ATP content and the activities of various catabolic enzymes (hexokinase, fructose-1-phosphate aldolase, fructose-1, 6-diphosphate aldolase, and fructose-1, 6-diphosphase). However, in the jejunum the activities of adenylate cyclase (producing signaling molecule cAMP) and pyruvate kinase are increased. Also, the small intestine as a whole has less amino acid, carbohydrate, vitamin B_{12}, and vitamin B_1 absorption. There has always been a controversy as to whether the caloric intake of alcoholics due to the alcohol itself and poor nutrition were both contributors to their poor nutritional status or whether alcohol toxicity affected absorption of nutrients. All appear to contribute to the problems of chronic alcohol administration. Ethanol also interferes with sodium and water absorption—possibly related to its effects on ATP or ATPase content—and causes less pituitary secretion of antidiuretic hormone, allowing further dehydration. Ethanol increases magnesium absorption but decreases calcium absorption. The calcium picture is complex as calcium absorption is related to vitamin D absorption. The hydrophobic vitamin D absorption is decreased in steatorrhea (or fatty stool production) or poor fat absorption. Steatorrhea may be related to pancreatic damage, liver damage/bile salt formation, cholestasis, intestinal bacterial overgrowth, small intestinal lesions caused by nutritional deficiencies (folic acid), or toxic insults. Ethanol might be that toxic insult, because giving high doses of ethanol by intragastric but not intraperitoneal administration reduces calcium absorption while causing necrosis of the villus epithelium and infiltration of lymphocytes and plasma cells in the remaining crypts (Krawitt, 1977).

Cytotoxic cancer chemotherapy agents cause cells with a high turnover rate to be affected. Mucositis is the key reaction that results in diarrhea from either anticancer medications or ionizing radiation (Sonis, 2009a). The five-stage process leading to mucositis involves initiation by DNA strand breaks, clonogenic death of basal epithelial cells, and the generation

of ROS. The primary damage response is the next phase involving signal transduction pathways, especially NF-κB, Wnt, p53, and associated canonical pathways, triggered by the DNA strand breaks and lipid peroxidation. Cytotoxic agents and radiation can activate NF-κB directly or indirectly via ROS. Two hundred genes are expressed as a result of this signal transduction molecule including cytokines, cytokine modulators, COX-2, inducible NO-synthase, SOD, and cell adhesion molecules. Normal cells will become apoptotic due to NF-κB formation. Radiation and chemotherapy also stimulate the ceramide pathway. This produces cell death via activation of matrix metalloproteinases from macrophages that respond to the fibrinolysis due to connective tissue damage from radiation or cytotoxic agents. Signal amplification can occur in a third phase as TNF has a positive feedback response on NF-κB, initiating MAPK signaling. This is followed by a signaling overload response that progresses to the next phase of ulceration. Bacteria may then invade, stimulating more pro-inflammatory cytokines or producing sepsis. If the worst does not occur, there is spontaneous ulcer healing from signaling produced by the submucosa's extracellular matrix and involving the activation of the intrinsic tyrosine kinase (Sonis, 2009b). One cancer chemotherapeutic antimetabolite to folic acid is methotrexate. Its toxicity appears to be manifested through nitrosative stress. In the rat, nitrate was elevated fivefold 12 hours following methotrexate administration. Also, nitrotyrosine was elevated in all parts of the small intestine, with the most found in the duodenum (Kolli et al., 2008).

NSAIDs still induce gastroduodenal damage, but even low-dose acetylsalicylic acid for prevention of cardiovascular disease appears to be toxic via its inhibition of COX-1, aspirin-specific alterations of the gastroduodenal mucosa, and reduction in platelet aggregation (Yeomans et al., 2009). As mentioned earlier, other medications can also cause GI disturbances. The immunosuppressive medication mycophenolate mofetil causes ulcerative esophagitis, reactive gastropathy, duodenal and ileal graft-versus-host disease with crypt architectural disarray, lamina propria inflammation, dilated damaged crypts, and crypt epithelial apoptosis (Parfitt et al., 2008).

Heavy metals also cause GI disturbances to differing degrees. Metals compete for absorption and damage, so localization of the sites of metal absorption and the presence of other metals have profound influences on toxicity. Metal absorption across the intestinal wall involves two steps. The first step involves transport over the luminal membrane into the epithelial cytoplasm. The second step is important as the timing of the transport over the basolateral membrane into the serosal fluid determines the metal's residence time in the mucosal epithelial cytoplasm. Slow movement out of this area makes the rate of mucosal sloughing a key factor in damage versus absorption. All metals are not absorbed in the same area of the intestine equally. For example, divalent cadmium is absorbed mainly in the duodenum, Zn^{2+} in the jejunum and ileum, Hg^{2+} in the proximal jejunum, and selenomethionine in the entire intestinal tract (Andersen et al., 1994). Acute heavy metal poisoning of the GI tract varies based on the metal. Lead causes tissue desiccation and mucosal damage to the GI tract, but death results from neurological damage. At lesser concentrations, lead affects hematopoiesis or the formation of blood cells. Mercury binds more strongly to proteins, and acute oral toxicity results in sloughing of the intestinal mucosa to a degree that pieces can be found in the stools. Significant water is lost in this way. Hg also induces edema via damage to the capillary walls. Shock and peripheral vascular collapse can occur; however, for those doses of Hg that do not cause shock or renal failure, neurological damage usually defines mercury poisoning (Iino et al., 2009). Certain metals also cause cancer. Cr(VI) in drinking water appears to be carcinogenic to the cells of the oral cavity in rats, but appears to shift to sensitivity to oncogenesis in the small intestine of mice (Stout et al., 2009). Another carcinogenic metal is arsenic and its metabolites monomethylarsonous acid and dimethylarsinous acid. Usually, metals are not considered to be CYP inducers that may mediate carcinogenic mechanisms. Also, the

intestine is rarely considered to be a large source of metabolism, because the portal circulation to the liver usually has profound effects on metabolism. Sodium arsenite and its metabolites induce CYP3A4 via increasing the pregnane X receptor (forms a heterodimer with retinoid X receptor alpha) in the small intestine and Ub-protein conjugates tempering the induction mechanism (Medina-Díaz et al., 2009). Iron toxicity may be noted in the liver, but its transport/absorption in the duodenum is mediated by the intramembrane divalent metal transporter 1. Iron export from a variety of cells is mediated by ferroportin, which is regulated by a circulating hormone hepcidin. This combination, along with plasma membrane transferrin and iron regulatory proteins 1 and 2 receptor, influences iron regulation from nutrient to toxic concentrations (Valerio, 2007).

Oxidative damage can also ulcerate the proximal GI tract as occurs due to ingestion of the plastic hardener methyl ethyl ketone peroxide; this has occurred accidentally in humans. Death usually results from liver necrosis, which can be prevented by the free radical scavenger N-acetylcysteine. Severe metabolic acidosis also occurs in response to the formation of formic acid similar to methanol intoxication and can cause optic nerve lesions (van Enckervort et al., 2008).

Bacterial toxins also can cause fatal reactions via intestinal damage. *Clostridium perfringens* type B and C isolates produce beta-toxin that causes significant hemorrhagic luminal fluid in the small intestine. Jejunum and ileal damage are more severe than damage to the duodenum (Vidal et al., 2008). *Staphylococcus aureus* produces toxins A–E and toxic shock syndrome toxin-1, causing food poisoning and toxic shock syndrome; it also produces enterotoxins G and I (scarlet fever and toxic shock). These enterotoxins produce villous atrophy with abnormal brush border. More detailed analysis implicates these toxins in microvilli destruction, mitochondrial damage (dilation), and lysosomes with cellular debris (Naik et al., 2008). Fungus-contaminated feed may be toxic to various organs or at least reduce feed efficiency in animals via intestinal alterations. For example, feed contaminated with *Fusarium* mycotoxins decreased the villus height in the duodenum of turkeys (Girish and Smith, 2008).

Immune responses to intestinal contents can also affect nutrient transport as a more subtle toxic reaction but is species and segment related. Lipopolysaccharide (LPS; gram-negative bacteria membrane component) infusion into pig intestines causes decreased ileal glucose transport in Yorkshire breeds but increased in the Meishan breed. Duodenal and ileal proline transport is decreased by LPS in Meishans but unaffected in Yorkshires. Glycylsarcosine transport is increased by LPS in both pig breeds. Resistance of the paracellular pathway between cells (barrier function) is increased in Yorkshires but not Meishans by LPS (Albin et al., 2007). Damage caused by flavonoids, diterpenes, and terpenes in *Ginkgo biloba* extract to the calciform cells in the duodenum may be responsible for less uptake of these cells of a diagnostic radiobiocomplex sodium pertechnetate ($^{99m}TcO_4Na$). This reduced uptake may also be due to oxidative stress generating inflammatory cell infiltration (Moreno et al., 2007). Certain food substances also prevent absorption of important nutrients. For example, in the original *Rocky* movie with Sylvester Stallone, there is a training scene where he eats a number of raw eggs prior to his morning run. There is a substance known as avidin in raw egg whites that has a high affinity for biotin binding, making this nutrient unavailable (White et al., 1992).

Jejunal Toxicity

Many of the toxicants that affect the tract proximal to the jejunum will also affect this more distal structure. Its length and the completion of the digestion process in this area make transport of nutrients and toxicants more important in this region. For example, it is important to note that certain mineral deficiencies increase the absorption of other toxic minerals, as is the case for Fe deficiency and Ni absorption due to less Ni export from the jejunal/intestinal mucosa (Müller-Fassbender et al., 2003). Also, studies using the chicken intestine indicate that addition of addition of D-glucose to

the mucosal side of voltage-clamped intestinal sections exhibited the highest current increases in the jejunum. This makes Na^+-glucose symport most important in this region. Any toxin that interferes with this transporter mainly affects duodenal and jejunum sections of the small intestine, as does deoxynivalenol or vomitoxin (type B trichothecene is especially prevalent in *Fusarium* fungal species; Awad et al., 2007). Cd is found to inhibit D-galactose transport in the presence of Ca in the rabbit jejunum (Mesonero et al., 1996). Unfortunately, this sodium-linked cotransport of sugars can also be utilized to transport toxic glycosides, such as prunasin (D-mandelonitrile-beta-D-glucoside), which is the primary metabolite of amygdalin (Strugala et al., 1995). Poisoning the $3Na^+,2K^+$-ATPase by ouabain or vanadium (vanadate) also has the effect of altering amino acid and water absorption (Hajjar et al., 1989). It is of interest that the jejunum on its own produces more ROS and RNS prior to insult than many other organs (jejunum > duodenum > kidney > ileum > blood > cerebellum > brain > heart > liver) as detected by electron paramagnetic resonance imaging of a nontoxic spin probe, 1-hydroxy-3-carboxy pyrrolidine. Endotoxin challenge as a model of toxic shock increases radical formation in the rat liver, heart, lung, and blood, but actually decreases ROS and RNS in the jejunum. This indicates that radical formation shifts result in the expected toxicity, decreased blood pressure due to NO formation or $ONOO^-$ production, and extensive oxidative damage (Kozlov et al., 2003). Oxidative injury may be important also due to the requirement for glutathione for prevention of severe degeneration of epithelial cells of the jejunum and colon as occurs with buthionine sulfoximine (Mårtensson et al., 1990). This protective function of reduced glutathione will become extremely important in maintaining liver function against toxic metabolites. Another aspect of small intestinal function is nerve-mediated toxicity via Na and fluid secretion. The rat jejunum, for example, responds to 8% ethanol perfusion by a net secretion of fluid and sodium. This is prevented by ganglionic blockade by hexamethonium, but does not interfere with ethanol absorption (Hallbäck et al., 1990).

Ileal Toxicity

The ileum is the last chance of the small intestine to absorb many nutrients. The jejunum and ileum succumb to the toxicity of *Clostridium perfringens* type A. The energy-rich, protein-rich, wheat- and barley-based diets of chickens causes growth of this pathogenic organism as opposed to diets rich in corn. Toxins such as alpha toxin and NetB may play a role in the development of jejunal and ileal necrotic enteritis. This disease is characterized by lesions that are focal-to-confluent, often with tightly adhered pseudomembrane (Cooper and Songer, 2009). *Clostridium difficile* produces toxins A and B that are responsible for its pathogenesis. Toxin A causes the antibiotic-induced diarrhea and pseudomembranous colitis. A selective A2A adenosine receptor agonist, ATL 313, appears to prevent some of the most damaging influences of toxin A on the ileum by reducing secretion and edema, myeloperoxidase activity (neutrophil infiltration), TNF- α production, adenosine deaminase activity, and cell death (Cavalcante et al., 2006). Where endotoxin and LPS decrease jejunal radical formation, they also activate phospholipase A_2 and increase the permeability of the ileal/intestinal wall to bacterial invasion and sepsis by cleaving the phosphatidylcholine protective layer of the surface of the GI tract. Specific inhibitors of phospholipase A_2 protect against this permeability change (Zayat et al., 2008). Permeability increases are also caused by other toxicants, such as the widely used herbicide glyphosate (at > 10 mg/mL), as determined by reductions in transmembrane electrical resistance and increased permeability to [^{3}H]-mannitol. Glyphosate also disrupts the actin cytoskeleton of ileal cells at the same high concentrations (Vasiluk et al., 2005). Cd^{2+} disrupts the paracellular barrier, increasing its toxicity to and absorption between ileal cells (Duizer et al., 1999). Bacteria can also activate a pro-drug as is the case of the antiproliferative cancer medication irinotecan. Carboxylesterases of the gastrointestinal cells, liver, serum, and cancer cells activate this drug to the toxic SN-38. The liver conjugates this metabolite to a nontoxic glucuronide. Bacteria flora β-glucuronidase is capable of releasing large amounts of active SN-38.

Germ-free mice had a lethal dose of ≥ 150 mg, while holoxenic mice succumbed to a dose range of 60–80 mg irinotecan. Normal mice with a good bacterial flora also had diffuse small and large intestinal damage, while germ-free mice had significantly less damage, mostly centered in the ileum. Diarrhea was found in 19 of 20 normal mice given 60 mg of the anticancer medication, while holoxenic mice had no diarrhea at that dose and sporadic diarrhea between 80 and 100 mg (Brandi et al., 2006). Diarrhea is also produced by ingestion of other toxins, such as that produced by the black sponge *Halichondria okadai*. Okadaic acid, responsible for diarrhetic shellfish poisoning, is a tumor promoter that is a specific cell-permeating inhibitor of protein phosphatases. It also induces micronuclei formation indicating genotoxicity. Also, apoptosis was observed by the TUNEL assay in mouse ileum, liver, and kidney (Le Hégarat et al., 2006). Secretory diarrhea is responsible for death from cholera toxin exposure. Inhibition of the cystic fibrosis transmembrane conductance regulator by 2-thioxo-4-thiozolidinone inhibits intestinal fluid loss and may be a useful toxicant for use in an animal model of cystic fibrosis (Ma et al., 2002). Zymosan, a yeast cell wall preparation consisting of protein–carbohydrate complexes, is used as a mouse model of multiple organ dysfunction syndrome of nonseptic origin. Zymosan IP injection causes peritoneal exudation and migration of neutrophils, pancreatic and ileal injury, an increase in myeloperoxidase activity of the ileum and the lung, and the formation of IL-1β and TNF-α. The mediation of injury by TNF-α formation is indicated by reversal of these effects by a specific TNF-α–soluble inhibitor, etanercept (Malleo et al., 2008).

Chronic cocaine use can cause significant intestinal damage. For example, in one case report, ischemia/infarction and hemorrhage were noted in the distal ileum on autopsy (Lingamfelter and Knight, 2010). Metabolism in the ileum may not parallel that in the liver by CYP3A isoenzymes. For example, vitamin D receptor is known to induce the formation of CYP3A1 and CYPA2 by 1,25-dihydroxy-vitamin D_3. However, this activated form of vitamin D induces vitamin D receptor expression in the ileum of the rat or the human, but only in the rat liver. This indicates differences in CYP regulation for human ileum enzymes. What role does this play in toxicity? The bile acids from human liver are represented by chenodeoxycholic acid, while the more toxic forms from bacterial metabolism are represented by lithocholic acid. It appears that vitamin D promotes the metabolism of the toxic lithocholic acid. However, chenodeoxycholic acid appears to "short-circuit" this mechanism in the rat ileum by preventing the induction of the CYP3A isoenzymes by higher vitamin D receptor expression and may increase the toxicity of lithocholic acid (Khan et al., 2010). Motility is also important in this portion of the small intestine. Isoliquiritigenin, a flavonoid extract of licorice (*Glycyrrhiza glabra*), inhibits charcoal meal travel at low doses and increases the travel speed at high doses. It is of interest that the section of the GI tract is also important in the effects of this flavonoid. Isoliquiritigenin produces an atropine-sensitive concentration-dependent spasmogenic action in rat stomach fundus, but a spasmolytic/opposite action on the rabbit jejunum, guinea pig ileum, and atropine-treated rat stomach fundus. The spasmogenic effect appears likely via muscarinic receptor agonism, while antagonizing calcium channels produce the spasmolytic effect (Chen, Zhu, et al., 2009). Aflatoxin B_1 produces ileal spasms via release of acetylcholine as indicated by atropine antagonism, although death occurs via liver damage (Luzi et al., 2002). Changes in the immune response in the small intestine also occur in the ileum. Depleted uranium ingestion in drinking water in rats causes increased responses of the inflammatory pathway (increased COX-2 expression for formation of prostaglandins, increased IL-1β and IL-10 cytokines, induced neutrophils) while decreasing other parameters associated with macrophage function (the previously mentioned changes in interleukins accompanied by decreased expression of CCL-2 mRNA) or the NO pathway (reduced expression of endothelial NO synthase mRNA, inductive NO synthase activity, and NO_2^-/NO_3^- concentrations). This indicates possibilities for chronic poisoning of the immune response or alternatively producing short-term hypersensitivity (Dublineau et al., 2007).

Colon Toxicity

Colon cancer is a scourge of well-fed carnivorous humans. It has been suspected that red meat consumers have an iron-rich diet that increases the risk of colon cancer. A feeding study of iron-fortified diets to rats at levels appropriate for human consumption indicated an increase in free radical–generating capacity and lipid peroxidation with the cecum/proximal colon as the site of highest risk (Lund et al., 2001). Difluoromethylornithine and NSAIDs prevent colorectal cancer development in mice. Polyamine synthesis is stimulatory of inflammation and colorectal cancer. The first enzyme in the synthesis of polyamines is ornithine decarboxylase, which is inhibited by difluoromethylornithine. NSAIDs by definition are anti-inflammatory agents. The role of polyamine synthesis is confirmed by recurring adenomas in colon polyps accompanied by a single nucleotide polymorphism of the ornithine decarboxylase promoter (Rial et al., 2009). Dimethylhydrazine is a selective colon cancer–causing agent. Its metabolism to azoxymethane (Likachev et al., 1978) has made the metabolite a modern model for DNA methylation and cell-signaling changes indicative of cancer development. The discoveries that various plant flavonoids released into the colon during digestion, such as quercetin, reduce various cancer formations, including colon cancer, yielded mechanistic questions (Deschner et al., 1991). Gene expression analyses via high-density microarrays indicate that Wnt-beta catenin, phospholipase A_2-eicosanoid, and mitogen-activated protein kinase appear to be upregulated during development of colon cancer from azoxymethane (Davidson et al., 2009). Mutations in *APC*, *Ras*, *DCC*, and *p53* genes are also associated with precancerous tumors or polyps that may develop into malignant tumors. As mentioned previously, consumption of red meat can increase colorectal cancer risk by 12–20%, as opposed to a diet rich in fish, which may lower the risk by 40%. The heme iron may play a role as indicated previously, but also cholesterol, fatty acids, and products formed from preservation and cooking, including *N*-nitroso chemicals and heterocyclic amines, are possible contributors to these cancers. Increased protein in the diet increases the amount of *N*-nitroso compounds, which is true of meat eaters. DNA-alkylation is catalyzed by the intestinal microflora, inducing colitis-linked colorectal cancer. These changes may be monitored by fecal water biomarker analysis. Bile acids are produced from cholesterol metabolism. The secondary bile acids produced by bacteria stimulate the proliferation of colonic cells and induce apoptosis. These bile acids also cause single-stranded DNA breaks and base oxidation and alter the barrier function. This may lead to mutations that are resistant to apoptosis, forming tumors that metastasize to other areas. Fortunately, consumption of foods rich in phenolic compounds (tea, red wine, chocolate) are antioxidant, anti-inflammatory, and anticarcinogenic by preventing promotion of tumorigenesis and inducing apoptosis. Calcium also is thought to protect against heme iron and colorectal cancer. Probiotics and prebiotics affect the microflora to decrease the risk of cancer and promote host health (Pearson et al., 2009).

Conditions that may lead to cancer formation but are problematic on their own involve colon inflammation. Bacterial infections, ischemia, and autoimmunity can yield ulcerative colitis or Crohn's colitis. Supplementation of drinking water with low-molecular-weight dextran sodium sulfate yields epithelial damage and enhanced colonic inflammation in mice, which can be visualized in a journal devoted to exhibiting videos (Whittem et al., 2010). A truly opposite approach is to use a weekly enema or intrarectal administration of 2,4,6-trichlorobenzene sulfonic acid. This colitis type yields an initial T helper type 1 response in BALB/c mice, including cytokines IL-12p70 and interferon-γ. After 3 weeks, this reaction decreases and is replaced by an increase in IL-23/IL-25 at 4 to 5 weeks. This is followed by higher IL-17 and interleukins normally associated with a T helper type 2 response, especially an IL-13 peak between 8 and 9 weeks. IL-13 induces the IL-13Rα_2 receptor key to synthesis of TGF-β_1 and fibrosis (Fichtner-Feigl et al., 2008).

Trimethyltin chloride increases chloride secretion regulated by the basolateral Ca^{2+}-sensitive K^+ channels (Yu et al., 2009). Nanotechnology

is a new source of potential toxicants, especially those coupled to metals. Cobalt-doped tungsten carbide nanoparticle suspensions induced highest toxicity to astrocytes and colon epithelial cells due to the ionic cobalt content of the particles (Bastian et al., 2009).

Clinically, a number of medications and chemicals cause common colonic toxicity. Alosetron, amphetamines, cocaine, ergotamine, estrogen, pseudoephedrine, sodium polystyrene, and vasopressin have been linked cases of colonic ischemia due to effects on mesenteric vessels (shunting of blood away from mesentery, thrombogenesis, and vasospasm). Colonic pseudo-obstruction has been found in patients taking atropine, various narcotic agents, nifedipine, phenothiazines, tricyclic antidepressants, and vincristine by either antagonizing neurotransmitters that increase intestinal motility, stimulating neurotransmitters that decrease motility, binding to receptors that cause dysmotility, relaxing smooth muscle, and increasing toxicity to enteric neurons. Antibiotics can lead to pseudomembranous colitis. If people use cathartics over a long period of time, lower colonic motility may occur along with abdominal distention. There is an association between ampicillin and hemorrhagic colitis. Anticancer medications may cause neutropenic colitis. The iron-chelating agent deferoxamine has been linked to patients with *Yersinia* enterocolitis. These diseases result from either from altering the bowel flora and allowing pathogenic microorganisms to develop or become more virulent, or reduced immune system function or altering the mucosal barrier to bacterial invasion. Cytotoxic colitis occurs with α-methyldopa, gold compounds, and NSAIDs due to an allergic reaction, antimetabolite action, or mucosal cytotoxicity. A toxic colitis is found in people who administer corrosive chemicals intrarectally. Lymphocytic colitis is associated with an activated or attenuated immune response due to the use of cyclo 3 fort, flutamide, lansoprazole, NSAIDs, or ticlopidine (Cappell, 2004).

Forensic Analysis of Pancreatic Damage

This sections returns to dietary causes of toxicity. For example, lectins from beans (such as *Phaseolus vulgaris*) may cause toxicity via damage to cells of the GI tract. In the Upper Midwest, corn and soybeans are the key crops. Soybeans are toxic if not processed with significant heating, which people in this area know well from the smells of soybean plants. Soybeans produce trypsin inhibitors that can cause lethality by significant damage to the pancreas (Liener, 1983). Damage to the pancreas by agents listed in **Table 16-2** may affect exocrine (most of the

TABLE 16-2 Forensic Chart of Pancreatic Toxicity

Pancreatic Toxicity	Toxic Agents
Increased HCO_3 and water secretion/decreased protein	Oleic acid (Laugier and Sarles, 1977)
Mitochondrial membrane dysfunction	Oleate (Frigerio et al., 2006)
Pancreatic cancer	Alcohol (Apte et al., 2009), azaserine (Povoski et al., 1993), 7,12-dimethylbenzo[*a*]anthracene (Harris et al., 1977), 4-hydroxyaminoquinoline-1-oxide (Imazawa et al., 2003), *N*-nitrosamines (Scarpelli et al., 1984)
Pancreatitis	Alcohol (Apte et al., 2009), ethionine (Yamaguchi et al., 2009), medications (Trivedi and Pitchumoni, 2005)
Reactive oxygen species	Alloxan, streptozotocin (Szkudelski, 2001)
Trypsin inhibition	Soybean trypsin inhibitors (Liener, 1983)

digestive enzyme functions of the GI tract) and endocrine (insulin and glucagon and diabetes mellitus) functions of this key small organ. Usually, the exocrine function is more sensitive to these agents, especially via the oral route of administration. Alcohol has been mentioned earlier for other GI disturbance, and it plays a role in acute and chronic pancreatitis with acinar atrophy and fibrosis. The increase in the permeability of the intestinal wall leads to endotoxinemia, necroinflammation, and progressive deterioration of the pancreas via activation of stellate cells. This environment is then facilitatory for production of cancer stroma (Apte et al., 2009). A good article to help start thinking about toxicity to the pancreas beyond these more likely toxicities was published in the 1980s (Scarpelli, 1989). Severe necrotic pancreatitis develops from diets that are deficient in choline and supplemented with ethionine. Initial observations of changes in S-adenosylmethionine and methionine adenosyltransferases did not give a clear linkage between toxicity/lethality and observed decreases in these parameters (Lu et al., 2003). However, it appears that IL-6 is a good indicator of the inflammatory reaction that precedes the toxicity from an ethionine-rich diet, and the serotonin 5-HT_{2A} receptor may mediate the damage as indicated by the protection offered by an 5-HT_{2A} antagonist risperidone (Yamaguchi et al., 2009).

Medications can also cause pancreatitis. Inflammatory bowel disease has higher occurrences of acute pancreatitis with azathioprine/mercaptopurine immunosuppressants (Bermejo et al., 2008). Estrogens can increase hypertriglyceridemia by increasing very low-density lipoprotein (VLDL) and reducing triglyceride lipase in the liver. Severe hyperglyceridemia leads to pancreatitis (Lee and Goldberg, 2008). Diuretics such as furosemide and hydrochlorothiazide (with the angiotensin-converting enzyme [ACE] inhibitor lisinopril) can cause an acute or fatal necrotizing pancreatitis (Bedrossian and Vahid, 2007). The list of medications based on risk of pancreatitis has been separated into classes. Class I is reserved for those medications with > 20 reported cases and includes didanosine (antiretroviral), asparaginase (anticancer), azathioprine, valproic acid (antiseizure), pentavalent antimonials (antiparasitic for leishmaniasis), pentamidine (antiprotozoal), mercaptopurine, mesalamine (anti-inflammatory), estrogens, opioid analgesics, tetracycline (antibacterial), cytarabine (anticancer), steroids, trimethoprim-sulfamethoxazole (antibacterial), sulfasalazine (anti-inflammatory), furosemide (loop diuretic), and sulindac (analgesic). Class II medications indicate > 10 cases of pancreatitis and include rifampin (antibacterial), lamivudine (antiretroviral), octreotide (somatostatin analog), carbamazepine (antiseizure), acetaminophen (analgesic), phenformin (banned in U.S. antidiabetic medication due to fatal lactic acidosis), interferon alfa-2b (immunomodulator/anticancer), enalapril (ACE inhibitor), hydrochlorothiazide (diuretic), cisplatin (anticancer), erythromycin (macrolide antibacterial), and cyclopenthiazide (another thiazide diuretic). Class III represents the rest of medications with any reported incidence of pancreatitis. Considered together, it appears that many medications taken orally and causing toxicity may yield pancreatitis in patients prone to this disease (Trivedi and Pitchumoni, 2005). The fatty acid oleate was shown in the 1970s to induce the exocrine pancreas to secrete more water and bicarbonate on an acute infusion. Protein output first increased then was inhibited below basal values. An anti-cholecystokinin-pancreozymin factor was proposed (Laugier and Sarles, 1977). A 3-day treatment with oleic acid causes mitochondrial membrane dysfunction and low insulin response to a stimulatory concentration of glucose. These lipotoxic effects appear to be counteracted by topiramate, an antiseizure medication that increases expression of the nutrient sensor PPARα and the mitochondrial fatty acid carrier CPT-1, which is associated with an increased β-oxidation rate of the lipid (Frigerio et al., 2006). ROS can be generated to damage the pancreas as a whole and the B cells more specifically that produce the insulin. Alloxan is reduced to dialuric acid, forming a redox cycle that generates superoxide radicals. Hydrogen peroxide is then generated by SOD, and hydroxyl radicals form via the Fenton reaction. These ROS and a large increase in cytosolic Ca^{2+} concentrations rapidly damage the B cells. Streptozotocin gains

entrance to the pancreas via the GLUT2 glucose transporter. It alkylates DNA and induces activation of poly ADP-ribosylation. The poly ADP-ribosylation depletes cellular NAD^+ and ATP. Subsequent ATP dephosphorylation provides a substrate for xanthine oxidase, which generates superoxide and, via dismutation and Fenton reaction, hydrogen peroxide and hydroxyl radical. Streptozotocin also increases nitric oxide, which inhibits aconitase activity and produces more DNA damage. B cells then undergo necrosis (Szkudelski, 2001). DNA damage results in the exocrine pancreas carcinomas that are so difficult to treat. There are questions as to factors that mediate the progression from damage to cancer. Acidophilic atypical acinar cell foci and nodules represent preneoplastic lesions that are generated by azaserine (α-diazoketone). Their growth appears to be mediated by cholecystokinin-A receptors (Povoski et al., 1993). 7,12-Dimethylbenz[*a*]anthracene (DMBA) and other similar PAHs can be metabolized by cells of the pancreatic duct to active DNA-reactive compounds found as adducts. The reason this compound has been employed is that the amount of adducts formed is higher with DMBA than benzo[*a*]pyrene (Harris et al., 1977). It is clear that some agents such as 4-hydroxyaminoquinoline-1-oxide (a metabolite of 4-nitroquinoline-1-oxide) produce cancer via adduct formation, increased apoptosis, and the induction of proliferative genes, particularly p53 (Imazawa et al., 2003). The model system used for these studies is important. For example, the rat pancreas responds to carcinogens with acinar adenomas and adenocarcinomas, but not with ductal adenocarcinomas as occurs in humans. *N,N*-dipropylnitrosamine undergoes β-oxidation to *N*-nitrosamines that cause pancreatic ductal adenocarcinomas in the Syrian golden hamster (Scarpelli et al., 1984).

Forensic Analysis of Liver Damage

The liver is the filter and processing organ for nutrients and toxicants that are absorbed by the intestinal tract through the portal vein. It is under low oxygen tension because it filters venous blood. It can metabolize the toxic components for excretion by the kidney or pass them out through the bile duct. The liver should be viewed as the biochemical anatomy organ due to the distribution of important metabolic enzymes and cofactors in the liver acini. **Figure 16-1** shows the biochemical anatomy of the liver moving from portal vein or hepatic artery to central vein (terminal hepatic vein). The zones and the formation of a hypoxic zone by the central vein (zone 3) are important for toxic reductive mechanisms and the increased presence of CYPs for bioactivation. The high glutathione concentration in the liver (10 mM) lessens into zone 3, which could manifest the toxicity of metabolites. However, metals would be expected to be highest coming in from the intestine into zone 1 and exert their largest influence there. Bile outflow also occurs in zone 1. Kupffer cells, stellate cells, and sinusoidal endothelium should also be considered in addition to hepatocytes in zones or bile duct cells. Liver damage is the reason many people succumb to an overdose of many over-the-counter medications, prescription medications, and/or drugs of abuse. The relationship between alcoholism and cirrhosis of the liver is a well understood phenomenon. Moldy feed has caused fatalities in animals (aflatoxin) as well as ingestion of blue-green algae–contaminated water (microcystins). This section focuses first on the areas or sites of the liver susceptible to toxicants and then the toxicity associated with a variety of toxicants due to specific types of injury as listed in **Table 16-3**.

Anatomical Localization of Liver Injury

Periportal Toxicity (Zone 1)

Periportal (zone 1) damage occurs more often than people care to consider based on the availability of iron-supplemented children's vitamins. Children who are not mature enough to understand the difference between medicines, vitamins, and candy should only have access to vitamins not supplemented with minerals. Consuming an entire bottle of Fe-supplemented vitamins would be fatal. The liver can store iron in ferritin and in an insoluble toxic hemosiderin form causing centrilobular (zone 3)

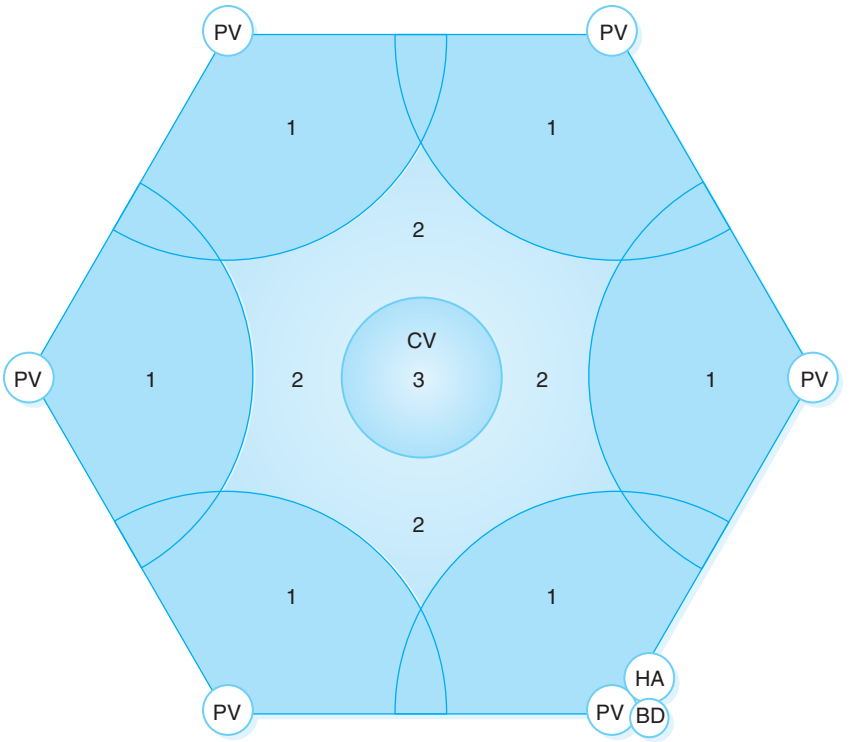

FIGURE 16-1 Biochemical Anatomy Schematic of a Liver Lobule in a Hexagon (Variable in Size)

Note: A liver acinus would stretch from one central vein (CV) or terminal hepatic vein to another CV. The flow direction is from portal vein (PV) or hepatic artery (HA) to central vein, so the oxygenation of the tissue decreases into the hexagon (zone 1àzone 3). The bile duct (BD) is there to remove primary bile acids and conjugated compounds into the duodenum possibly for enterohepatic recirculation. Zone 3 has the highest CYP activities for activation and least glutathione for conjugation/detoxication.

fibrosis as indicated by hepatic hydroxyproline content (collagen deposition). Hepatocellular necrosis is also involved as indicated by plasma alanine aminotransferase activity (leaked out of liver cells). Lipid peroxidation also results from ROS generated by excess iron (Valerio and Petersen, 2000). The oxygen-rich environment of the periportal region also is responsible for copper-induced hepatocellular apoptosis. Cu catalyzes the generation of oxidized glutathione (GSSG) from the reduced form (GSH) and superoxide. Degradation of the Cu-Zn SOD causes decreased dismutation of $O_2^{-\bullet}$. The mitochondria in zone 1 show decreased Mn SOD function, reduced thiol/disulphide ratio, and increased superoxide resulting in loss of ATP, collapse of the mitochondrial membrane potential, and induction of the mitochondrial permeability transition (Roy et al., 2009). Anticancer agents damage here as well for a variety of reasons. Doxorubicin is activated by P-450 reductase, generates ROS consistent with the oxygenation of zone 1 but also interferes with synthesis of macromolecules, binds covalently to and cross-links DNA, inhibits topoisomerase II, arrests cells in G_2 phase, and induces apoptosis. It also attracts the accumulation of inflammatory cells. Cisplatin is activated by metabolism and forms DNA adducts, also causing G_2 phase arrest and apoptosis. These two anticancer agents induce periportal fibrosis, focal inflammation, and degeneration of hepatic cords in addition to apoptosis. 5-Fluorouracil causes less damage than the other agents but still can lead to apoptosis, invasion by inflammatory cells, and damaged cytoplasmic organelles with collagenous fibrils in necrotic cells. This agent is first metabolized to 5-fluoro-deoxyuridine-monophosphate prior to inhibition of thymidylate synthase

TABLE 16-3 Forensic Chart of Hepatic Toxicity

Hepatic Toxicity	Toxic Agents
Anatomical	
Periportal (zone 1)	Fe (Valerio and Petersen, 2000), Cu (Roy et al., 2009), anticancer (El-Sayyad et al., 2009), allyl alcohol (Campion et al., 2009), signs of N-acetylcysteine-induced delayed acetaminophen recovery (Yang et al., 2009), ethanol metabolism and effects on gluconeogenesis and oxygen uptake—lactate decreasing ketogenesis (Lopez et al., 2009), aflatoxin (Kiran et al., 1998), dexamethasone (Micuda et al., 2007), α-naphthyl isothiocyanate (Kodali et al., 2006)
Centrilobular (zone 3)	Acetaminophen overdose (Yang et al., 2009), atenolol-induced inflammation + prednisone (Dumortier et al., 2009), tienilic acid enhanced by buthionine-(S,R)-sulfoximine (Nishiya et al., 2008), alcohol cirrhosis (Tipoe et al., 2008)
Kupffer cells	Lipopolysaccharide-induced cholestasis, clodronate (Sturm et al., 2005)
Disease	
Sex-linked	Estrogen-induced intrahepatic cholestasis (Leuenberger et al., 2009), estrogen-induced steatosis (Elias et al., 2007), formamide-induced hemangiosarcoma (National Toxicology Program, 2008), PCBs, PBBS, and hexachlorobenzene induction of uroporyphyria development via CYP1A1 induction (Smith et al., 1990)
Apoptosis/necrosis	Acetaminophen (Holt et al., 2008), CCl_4 (Weber et al., 2003)
Steatosis	Obesity, ethanol, CCl_4 puromycin (Pan et al., 2007)
Fibrosis/cirrhosis	Ethanol, CCl_4, bile duct ligation/destruction, α-naphthyl isothiocyanate, dietary 3,5-diethoxycarbonyl-1, 4-dihydrocollidine, thioacetamide, dimethylnitrosamine, infection (Henderson and Iredale, 2007)
Neoplasms	Aflatoxin, ethanol (Cha and Dematteo, 2005), dioxins, PCBs, phenobarbital (Oliver and Roberts, 2002), vinyl chloride (Bolt, 2005), CCl_4 (Weber et al., 2003), di(2-ethylhexyl)phthalate (Butterworth et al., 1987), sex hormones (Kalra et al., 2008), tamoxifen (Brown, 2009)
Cytoskeleton toxicity	Ethanol (Shepard and Tuma, 2009), pectenotoxins (Espiña and Rubiolo, 2008), As (Bernstam and Nriagu, 2000), vinca alkaloids, paclitaxel (Hruban et al., 1989), microcystins (Dawson, 1998)
Sinusoidal injury	Acetaminophen augmented by ethanol binge (McCuskey, 2006)
Immune/inflammatory	Isoniazid, ketoconazole, troglitazone, pyrazinamide, halothane, tienilic acid, penicillamine, propylthiouracil, procainamide, minocycline, α-methyldopa, hydralazine, propylthiouracil, phenytoin, statins, zafirlukast, CCl_4, acetaminophen, ethanol, Con A, α-naphthyl isothiocyanate (Adams et al., 2010)
Cholestasis/bile duct injury	Indomethacin, statins, digoxin, enalapril, midazolam, tamoxifen, diclofenac, methotrexate, troglitazone, lisinopril, itraconazole, verapamil, bosentan, glyburide, flucloxacillin (Grattagliano et al., 2009)

(El-Sayyad et al., 2009). Methapyrilene (N,N-dimethyl-N′-pyridyl-N′[2-thienylmethyl]-1,2-ethanediamine) has become a model hepatotoxin for observing the effect of S-oxidation of the thiophene group. It depletes the rich concentration of GSH in the periportal region while increasing reduced glutathione in the centrilobular region. Heme-oxygenase 1 and glutamate cysteine ligase catalytic subunit are increased as a cellular defense mechanism. Cytotoxicity progresses via apoptosis and is followed by necrosis and hepatic cancer development if the animal survives the initial liver damage (Mercer et al., 2009). Allyl alcohol is a model periportal toxicant dependent on oxidative mechanism for activation. Along with the toxicity to periportal cells, a marked upregulation of multidrug resistance–associated protein 4 is noted in protected centrilobular hepatocytes (Campion et al., 2009). Ethanol is extensively metabolized in the periportal region and inhibits gluconeogenesis and decreases oxygen uptake more in the periportal region. Lactate decreases ketogenesis more in this

region as well (Lopez et al., 2009). The moldy feed aflatoxin produces diffuse and severe hydropic degeneration, bile duct hyperplasia, and periportal fibrosis in broiler chicks. This was reduced to slight or moderate hydropic degeneration by inclusion of polyvinylpolypyrrolidone in the diet (Kiran et al., 1998).

Because fibrosis is a precursor to cirrhosis, it is worth investigating how model hepatotoxicants affect the regional manifestation of fibrotic damage. Thioacetamide, dimethylnitrosamine, and carbon tetrachloride were reinvestigated for the damage they cause when administered orally or by IP injection. Thioacetamide caused little fibrosis. Instead, it resulted in inflammatory infiltration in the periportal region and bile duct proliferation. The Histological Activity Index indicated fibrosis 6 weeks after administration. Dimethylnitrosamine produced piecemeal necrosis with little necrosis and 100% lethality 5 weeks after administration. Carbon tetrachloride resulted in a periportal fibrosis at 8–10 weeks and a septal fibrosis at 12–14 weeks. This study indicated a concern about the reproducibility of the toxic results of these chemicals between laboratories and individual researchers (Jang et al., 2008). Fat accumulation in the periportal region is induced by dexamethasone treatment. p-Glycoprotein is expressed primarily in the periportal region. The corticosteroid dexamethasone may cause enlargement of the liver at least in part by increased excretion of p-glycoprotein substrates in the bile and microvesicular steatosis (Micuda et al., 2007). Another periportal susceptibility is the attraction of neutrophils via beta2-integrin CD18. Alpha-naphthyl isothiocyanate causes periportal inflammation, widespread hepatic necrosis, and acute cholestatic hepatitis in wild-type or in partially CD18-deficient mice, but not in CD18-null type mice (Kodali et al., 2006). From these examples, it is apparent that oxygen-mediated toxicity, the unequal distribution of metabolic enzymes and transporting moieties, the proximity to the circulation and inflammatory cells, the highest concentrations of toxicants entering from the portal circulation, and the presence of the bile ducts in the periportal region all lead to damage in this region.

Centrilobular Toxicity (Zone 3)

Many women succumb to liver damage caused by using acetaminophen for pain relief, supposing it to be a safe analgesic. At high concentrations, the N-acetyl-p-benzoquinone imine metabolite conjugation with GSH depletes glutathione concentrations, forms covalent adducts, and initiates mitochondrial damage (increased membrane permeability transition and collapse of mitochondrial membrane potential). The reduction in ATP results in massive centrilobular necrosis (high CYP and low GSH) and extensive inflammatory cell infiltration. N-acetylcysteine (NAC; precursor to GSH) is the antidote early in this process to support the glutathione-based protection. However, if given later, NAC actually delays recovery by impairing glucose metabolism and endangering the endogenous GSH recovery. The liver is an odd organ in that it is known to regenerate tissue even following partial liver surgical removal. The cells that are least affected by acetaminophen and may serve as sources for recovery are found in the periportal region. That is also the source of extended toxicity if NAC is given too late as indicated by periportal hepatocyte vacuolation (Yang et al., 2009).

The other big looming disease of the liver that has brought down many people is chronic alcohol-induced cirrhosis. In a voluntary feeding model, rats show increased damage if ethanol is accompanied by fish oil as the source of dietary fatty acids. Ethanol does generate steatosis or fatty liver. It also causes necrosis, inflammation, and centrilobular collagen deposition indicating fibrosis. The extent of liver damage and modulation of metabolism by toxic metabolites are observed as increased endotoxin, alanine aminotransferase in plasma, CYP2E1, and lipid peroxidation. NF-κB and proinflammatory TNF-α, iNOS, and COX-2 increase on ethanol toxicity as does procollagen-I, especially in the centrilobular regions (Tipoe et al., 2008). Part of the reason for the centrilobular damage induced by ethanol is the hypoxia generated by the metabolism to acetaldehyde and beyond in an area that is already oxygen poor. An increase in intracellular Na^+ is observed in hepatocytes

exposed to ethanol and hypoxia. Na^+ influx does not occur when the metabolic inhibitors are used, during the incubation of cells in a bicarbonate-free buffer, or in the presence of 5-(*N*,*N*-dimethyl)-amiloride, a Na^+/H^+ exchange antagonist (Carini et al., 2000). Ethanol metabolism decreased the hepatocyte pH. Inhibition of ethanol metabolism by 4-methylpyrazole and acetaldehyde oxidation by cyanamide prevented this toxic effect. The immune system can also be involved in centrilobular damage as well. A patient on the beta-blocker atenolol exhibited an acute hepatitis with portal and centrilobular inflammatory lesions. The immunosuppressant steroid prednisone was given and resolved the periportal lesions but worsened the centrilobular damage (Dumortier et al., 2009). Carbon tetrachloride is another model of centrilobular necrosis. The CYP2E1 enzyme initiates the process in the hepatocytes near the central vein. Mice deficient in this enzyme are resistant to the liver damage associated with CCl_4. Downregulation of the gene responsible for expression of this enzyme is a protective mechanism against CCl_4 hepatotoxicity. However, it is interesting that age may increase the toxicity of this compound, possibly by influencing circadian rhythms. Not only are there sex-differences in toxicity, but also periods of the day/night cycle when animals and humans are more susceptible to toxicity. Period 2 is an important part of the core clock oscillator and plays a protective role in CCl_4-induced liver damage through inhibiting uncoupling protein-2 gene expression in mice via a PPARα signaling pathway. The presence of Period 2 gene expression keeps ATP levels higher and decreases production of toxic metabolites (Period 2–null mice have decreased ATP concentrations and increased toxic metabolites; Chen, Li, et al., 2009). This last study is not only important to consider for the site specificity of the liver damage but to encourage young researchers to consider that the rodent model uses an animal during the light period that normally sleeps during the daylight hours and may not be the best toxicity model for a human during waking hours.

A diuretic medication tienilic acid was withdrawn from the marker based on fulminant hepatic failure. Tienilic acid is metabolized by CYP2C9 to electrophilic reactive intermediates that bind covalently to macromolecules including the CYP enzyme. It decreases hepatic GSH content and causes lipid peroxidation. When hepatic gene expression was analyzed by microarrays, glutathione synthase and glutamate-cysteine ligase were upregulated indicating increased glutathione synthesis. Oxidative stress was indicated by increased expression of heme oxygenase-1 and NAD(P)H dehydrogenase quinone 1. Phase II drug metabolism was also elevated as indicated by glutathione *S*-transferase and UDP glycosyltransferase 1A6 expression. When an inhibitor of glutamate-cysteine ligase, buthionine-(S,R)-sulfoximine, was given with tienilic acid, extensive centrilobular necrosis was observed (Nishiya et al., 2008).

Kupffer cells produce cytokines that mediate the hepatic acute phase response and cholestasis to a circulating lipopolysaccharide known as endotoxin during sepsis. The sensitivity to transport of bile acids resides in the regulation of basolateral Na^+-taurocholic acid cotransporting polypeptide (Ntcp) by retinoid X receptor-retinoid acid receptor nuclear heterodimer and the liver-enriched transcription factor hepatocyte nuclear factor 1α. Cytokine release by the Kupffer cells inhibits the regulatory factors binding and transactivation of the Ntcp gene. The studies used to identify Kupffer cells as the cause of certain toxicities such as hepatic acute phase response use the phagocytic nature of the Kupffer cells to accumulate the toxin dichloromethylene-bisphosphonate, known as clodronate in liposomes, which depletes the liver of these cells (Sturm et al., 2005).

Toxicity Associated with Liver Damage

Sex-Linked Damage

It is important to address why female humans and animals display higher rates of certain liver toxicities. This is an important feature in women's health. For example, women activate STATs genes in the liver by continuously secreting growth hormone from the pituitary, as opposed to the stochastic (more

random pulses) distribution of male growth hormone production. Sexually dimorphic liver gene expression occurs for processes such as androgen metabolism, energy production, and inflammation. Males involve DNA methylation and methylation-sensitive transcription factors in hepatic gene expression (*Slp* and *Cyp2d9* promoters). CYP7B1 (oxysterol 7α-hydroxylase) has increased expression in males and represses androgen biosynthesis by decreasing the availability of DHEA, the key precursor to testosterone. Clearance of the estrogen receptor antagonist hydroxycholesterol by CYP7B1 metabolism activates the estrogen receptor. Women who use estrogen-containing medications for birth control of menopausal symptoms activate the estrogen receptor, which also regulates *Cyp7b1* expression. This can lead to estrogen-induced inflammation and hepatotoxicity, including intrahepatic cholestasis. However, if PPARα (peroxisome proliferator-activated receptor) is stimulated, this activates heteromeric transcription factors GABPs (especially GABPα) bound to the *Cyp7b1* promoter. This represses expression of the enzyme and decreases intrahepatic cholestasis, which is the most frequently observed liver toxicity of pregnancy (Leuenberger et al., 2009). Changes in liver enzyme induction and resultant toxicity are also sex-dependent. Development of uroporphyria in response to polychlorinated biphenyls (PCBs), polybrominated biphenyls, or hexachlorobenzene treatment appears to be a significant factor for female rats. Ethoxyresorufin O-deethylase associated with CYP1A1 is higher in female rats, and the induction of CYP1A1 and CYP1A2 is highly induced in the centrilobular region. The association between CYP1A1 and uroporphyria development is consistent with the metabolic activity and induction of this enzyme in female rats (Smith et al., 1990).

Conversely, males can be very sensitive to the effects of environmental estrogens as the liver is called to make vitellogenin (egg yolk protein) with essentially no place to transport it. For example, male fish exposed to high concentrations of 17α-ethynylestradiol show gross hypertrophy of the liver (accumulation of fat and disruption of acinar organization) and kidney and high mortalities (Elias et al., 2007). Male mice (B6C3F1), but not female mice or either sex of rats (F344/N), exhibit hemangiosarcoma of the liver in response to formamide (National Toxicology Program, 2008).

Apoptosis/Necrosis

Apoptosis is probably the likely form of cell mortality becoming necrosis only in severe damage. CYP metabolites are the usual chemical toxic species that may yield problems, especially from pharmaceuticals (Gómez-Lechón et al., 2008). However, the death of liver cells can come from diverse mechanisms such as cholestasis, viral hepatitis, ischemia or reperfusion injury, liver preservation for transplantation, and direct toxicity of medications or industrial chemicals. Both apoptosis and necrosis occur due to mitochondrial permeabilization and dysfunction. Apoptosis still requires sufficient ATP synthesis or stores to initiate a death program through Fas ligand with Fas, leading to stimulation of the caspase activation cascade. Necrosis requires acute ATP depletion as a result of metabolic damage consistent with the perturbations that occur with ischemia or reperfusion injury or medication-induced toxicity (Malhi et al., 2006). The model medication for apoptosis or necrosis is still the over-the-counter analgesic acetaminophen. Acetaminophen or paracetamol (European generic name for the same drug) is the leading cause of acute liver failure in the United States and the widely used model for hepatic toxicity in the mouse. As discussed previously, the quinone imine metabolite is responsible for the depletion of glutathione and adduct formation. A centrilobular necrosis results with extreme invasion of neutrophils. It appears that the toxic species and the infiltration of the polymorphonuclear leukocytes participate in the progression of acetaminophen hepatotoxicity. The benefit of removal of apoptotic neutrophils from inflammatory sites stems from the leaking of their proinflammatory and toxic intracellular contents that harm surrounding less damaged or healthy tissue. However, circulating monocyte infiltration appears to lead to phagocytosis of apoptotic cells and helps resolve inflammation while promoting tissue repair. This dichotomy of white blood cell function in toxicity and repair

is important in understanding mechanisms that lead to either apoptosis/necrosis or recovery. Hepatic macrophages, natural killer cells, and neutrophils can all contribute to cellular injury via TNF-α, IL-1β, and NO. The macrophages conversely provide protection to liver cells via Il-10, IL-6, and IL-18. The way this may be possible is that macrophages can be divided into two populations. Classically activated M1s generate proinflammatory cytokines. Alternatively activated M2s downregulate inflammation, participate in tissue remodeling, remove tissue debris and apoptotic cells, and induce new blood vessel formation (Holt et al., 2008).

A former medication and industrial solvent is carbon tetrachloride. The haloalkanes are toxic based on how well they form a stabile carbon-centered radical. The more halide groups there are, the more electron-withdrawing capacity from the carbon center that becomes more electropositive. This makes a radical formation more likely. CCl_4 is dependent on oxygen in an interesting manner for lipid peroxidation. Too high pressures (> 100 mm Hg) prevents lipid peroxidation, as does the anoxic state. However, hypoxic states between 5 and 35 mm Hg favor lipid peroxidation and the reductive dehalogenation of carbon tetrachloride by CYP2E1, CYP2B1, CYP2B2, and possibly CYP3A to form a trichloromethyl radical, $CCl_3^{\cdot}$. This reactive intermediate binds to macromolecules (lipid, nucleic acids, protein) and disrupts lipid metabolism/fatty degeneration leading to steatosis (fatty liver). DNA adducts lead to hepatic cancer. Reactions with oxygen lead to trichloromethylperoxy radical $CCl_3OO^{\cdot}$, which initiates lipid peroxidation of polyunsaturated fatty acids (especially phospholipids). This alters the permeability of mitochondrial, endoplasmic reticulum, and plasma membranes. Calcium sequestration is lost and leads to apoptosis and necrosis. Additionally, the fatty acids degrade to toxic reactive aldehydes (particularly 4-hydroxynonenal) that inhibit enzymes. Hypomethylation of cellular components due to CCl_4 toxicity inhibits protein synthesis (RNA hypomethylation) or lipoprotein secretion (phospholipid hypomethylation). Together these processes lead to cell death. Activation of TNF-α leads to apoptosis, while TGF-α proceeds to fibrosis. The formation of IL-6 and IL-10 helps the cells not too damaged toward recovery. CYP inducers increase toxicity, while inhibitors decrease CCl_4 toxicity (Weber et al., 2003). Ketones and ketogenic compounds (alcohols) potentiate CCl_4 hepatotoxicity (Pilon et al., 1988). Because carbon tetrachloride can damage membranes in the cell, the damage to the ER is assumed from the location of the CYPs; however, the ribosomes are part of the rough ER. While CCl_4 inhibits protein synthesis, it does not do so by damaging the membranes of the ER. Actually, it appears that the damage to protein synthesis occurs via disruption of the ribosomes, because cycloheximide protects against the protein synthesis inhibition by CCl_4. An antioxidant N,N′-diphenyl-*p*-phenylenediamine protects against membrane and ribosomal injury (Farber et al., 1971).

Steatosis

Fatty liver, or steatosis, can be generated by a simple process, such as overfeeding geese to make a fatty liver, which is used in the French cuisine pâté de foie gras. Obesity, insulin resistance, and ethanol can lead to triglyceride accumulation that is the basis of steatosis. CCl_4 can also lead to steatosis, especially in the presence of puromycin. CCl_4 3 hours after administration in rats decreases the secretion of triglycerides as part of VLDL. The failure of the Golgi has been implicated in this accumulation of fat. Why would a protein synthesis inhibitor such as puromycin increase steatosis yet decrease necrosis? There are a number of transporting proteins that are important for export of triglycerides. Triglycerides are secreted due to lipoproteins, which depend on the function of apolipoprotein B (apoB) and microsomal triglyceride transfer protein (MTP). ApoB is a structural protein, while MTP is a required chaperone for assembling the triglyceride-lipoprotein complexes. That is why protein synthesis inhibition by either carbon tetrachloride or specific agents that bind to ribosomes cause steatosis (Pan et al., 2007). A further elucidation shows that there are hyperlipidemic stages for rats given CCl_4 and experiencing acute liver damage or given puromycin amino nucleoside

and developing nephrotic syndrome. In both pathological states, the hyperlipidemic state is associated with an increase in high-density lipoprotein (HDL) and steady-state levels of apo A-1 mRNA. Early stages of these diseases show increases in total cholesterol and triacylglycerols but no induction of HDL and apo A-1 mRNA. Further examination of these secondary hyperlipidemia models shows that small viscosity changes below the physiological range induce apo a-1 gene expression at the mRNA level, but inhibit apo A-1 gene expression when viscosity returns to physiological values (Nuño et al., 1997). Mice also lacking phosphatidylinositol transfer proteins also lead to spinal cerebellar degeneration, intestinal and hepatic steatosis, and hypoglycemia (Alb et al., 2003).

Fibrosis/Cirrhosis

Many of the mechanisms already mentioned can lead to fibrosis and cirrhosis of the liver. The chief cause is clearly ethanol. However, the chronic nature of the disease may give some hint that mechanisms other than direct toxicity play key roles in the development of fibrosis and its progression to cirrhosis. The infiltration of immune cells appears to be vitally important in the development of liver fibrosis. However, not all immune cells play a vital role in models of liver fibrosis development. First, consider innate immunity. Bile duct ligation causes the infiltration of neutrophils. This would appear on first glance to implicate neutrophils in fibrosis. However, if neutrophils are depleted or faulty transgenic ones lacking IL-8 expression are present, fibrosis is little affected. Similarly, the α-naphthyl isothiocyanate model of liver fibrosis is little affected by neutrophils with a poor chemokine receptor (CXCR2[–/–]) for recruitment to the site of toxic injury. Mast cells also do not play a role in fibrosis as indicated by CCl_4 or by a pig serum challenge in mast-cell-deficient mutant Ws/Ws rats or W/Wv mice. As indicated for carbon tetrachloride, there are at least two populations of macrophages—one that is engaged in injury that leads to fibrosis (M1s) and one that leads to repair (M2s). For example, a 3-day dimethylnitrosamine treatment of rats resulted in activated hepatic stellate cells and marked fibrosis thereafter. However, when a mutated form of monocyte chemoattractant protein 1 was given, the infiltration by macrophages was reduced considerably and activated stellate cells were not found (no fibrosis). In support of these findings of macrocyte involvement in fibrosis, thioacetamide-induced fibrosis is reduced in rats by treatment with gadolinium chloride ($GdCl_3$). $GdCl_3$ inhibits ED1-immunolabelled cells (exudates macrophages) and ED2-immunolabelled cells (Kupffer cells). If the stellate cells are important in fibrosis, then natural killer cell (lymphocyte subclass) activation should eliminate activated stellate cells and decrease fibrosis. This is exactly what occurred in mice fed a 3,5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC) diet or injected with carbon tetrachloride to generate liver fibrosis. Addition of a Toll-like receptor 3 ligand, polyinosinic-polycytidylic acid, activated natural cells and interferon gamma as part of stimulation of innate immunity.

Next, consider adaptive immunity and B and T lymphocytes. The T cells comprise the cell-mediated immunity and the B cells provide antibody-producing or humoral immunity. CCl_4- or thioacetamide-treated mice, which were genetically modified to express rat IL-10, had decreased fibrosis. IL-10 reduces the CD8+ T cell activation of stellate cells. In a parasite (schistosomiasis) model of hepatic fibrosis mediated by T-helper type 2 cytokine release, an inhibitor of IL-13 (sIL-13Ralpha2-Fc) reduced procollagen I and procollagen III mRNA expression by fibroblasts and the development of fibrosis. Another researcher found that in CCl_4-treated mice B cells appeared to play more of a role in the laying down of collagen in the development of fibrosis and did so independent of any T cells. A schistosomiasis model conversely caused more liver fibrosis in B cell–deficient mice. Thus, it appears that inflammation and white blood cells play a role in hepatic fibrosis and necrosis, but the immune cells and cytokines responsible for these effects may vary based on the nature of the development of hepatic damage (Henderson and Iredale, 2007).

Neoplasms

As previously examined, genotoxic agents, both activation-independent and metabolically

activated, play a role in the development of cancer, as do similar epigenetic mechanisms of cancer formation. However, what is unique to liver cancer? Is it simply its location as the filter of food-borne toxicants? Is it the liver's metabolic activity? Perhaps it is a function of its stem cell population and regeneration capacity. On closer examination, numerous mechanisms lead to the formation of various primary liver tumors. Hepatocellular carcinoma clearly can come from either a severe event of DNA modification/damage or multiple insults. CCl_4 reveals the carbon-centered radical that initiates hepatic cancer (Weber et al., 2003). Mutations in tumor suppressor genes, proto-oncogenes, and DNA mismatch repair genes all produce cancers. The most widely studied tumor suppressor gene is p53. Mutations in this gene are present in 30–60% of patients with liver cancer. Point mutations in one allele or deletion in another one in the p53 gene help the promotion of cancers, but not initiation. Here is where a contaminant of spoiled (moldy) food has a role in hepatocarcinogenesis. Aflatoxin B_1 from *Aspergillus flavus* contamination of stored food items (peanuts, corn, rice) forms an epoxide, which forms a p53 mutation in codon 249 (G → T; arginine → serine). The aflatoxin B_1 mutation is also considered a tumor initiator because it is found in normal patients without any signs of cirrhotic damage. Hepatitis B virus acts synergistically with aflatoxin B_1 in hepatic cancer. On its own, this virus is associated with chronic liver cancer development. Hepatitis B integrates into the host DNA 80% of the time and may cause direct effects. It may activate promoters of several oncogenes (c-jun and c-fos) and inhibit apoptosis (inhibits p53 protein) through a protein of 154 amino acids that it produces. This viral protein increases expression of the epidermal growth factor receptor and potentiates TGF-α. Liver fibrosis is also stimulated by upregulating TGF-β signaling. The inflammatory reactions to the virus also appear to be proportional to the development of cancer. Inflammatory reactions are now thought to play a role in cancer as well as fibrosis and cirrhosis, as hepatitis C generates more liver inflammation and more frequent cirrhosis than hepatitis B. Cirrhosis increases dramatically the frequency of liver cancer in chronically hepatitis-infected patients. Cirrhosis indicates that alcohol is a key factor in chronic liver cancers. Another sign that a tumor suppressor gene has aided in cancer development is loss of heterozygosity.

Proto-oncogenes may be overexpressed in a variety of cancers. However, in liver cancers, Ras, c-fos, and c-erbB-2 mutations are not frequent in hepatic tumors. c-Myc is overexpressed in < 50% of liver cancers, but signals intrahepatic metastases and shorter survival. One classic agent that is a known human carcinogen that directs its activity toward hepatic endothelial/sinusoidal cells and parenchymal cells is vinyl chloride. It is oxidized to a chloroethylene oxide and then exocyclic etheno adducts form with DNA. These adducts are pro-mutagenic and affect proto-oncogenes and tumor suppressor genes and the gene and gene product levels. Lipid peroxidation and oxidative stress also play a role here (Bolt, 2005).

Although signaling pathways are not in common to all hepatic cancer development, mutations in the Wnt/beta-catenin pathway are seen as an early marker for 25% of liver cancer cases. Other important signaling pathway alterations are those involved in interferon response and TGF-β/IGF2R/Smad pathway (Cha and Dematteo, 2005).

Some liver cancers develop from stimulating the proliferation of liver cells and inhibition of apoptosis. These events can be modified by the aryl hydrocarbon receptor (dioxins and PCBs), constitutive androstane receptor (phenobarbital) without resorting to gene mutation (Oliver and Roberts, 2002). Peroxisome proliferators such as the ubiquitous plasticizing agent di(2-ethylhexyl)phthalate cause liver cancers in female rats and male and female mice at high doses. These agents also are known as hypolipidemic carcinogens. Although these chemicals may produce ROS as a result of peroxisome proliferation, it is their ability to act as mitogenic stimulators that correlates with their carcinogenic potency (Butterworth et al., 1987).

Obesity alone appears to be a risk factor for development of various organ cancers including liver tumors. The role of food intake may be linked to carcinogenic food ingredients,

excessive calories, loss of protective factors by reduced exercise, signaling factors from the adipose tissue itself, and other injurious conditions such as the development of a fatty liver or gallstones (Percik and Stumvoll, 2009). The role of sex hormone receptors in hepatocellular carcinoma is not well known despite the understanding of a male predominance in chronic liver diseases such as hepatitis B, hepatitis C, alcoholic liver disease, and alcoholic steatohepatitis progressing to cirrhosis and further on to liver cancer. The increase in hepatic neoplasms and oral contraceptives indicates a role for the estrogen receptor, and so does the presence of androgen receptors in hepatic cancer, which indicates a stimulatory role for testosterone, especially in the intrahepatic recurrence of tumors (Kalra et al., 2008). A note of interest is that tamoxifen, a weak estrogenic medicinal compound used to treat breast cancer, causes liver tumors in rats. This appears to involve metabolism rather than hormonal action, as tamoxifen is α-hydroxylated and then conjugated to a sulfate in the rat liver. This is a reactive species of this compound that binds to DNA at the N(2)-position of guanine, yielding pro-mutagenic lesions. It is unclear whether this is species-specific or occurs in female patients (Brown, 2009).

Cytoskeleton Toxicity

Again, ethanol plays a role in liver injury. Ethanol induces hyperacetylation of proteins including histone H3 (one of five main histone proteins), p53 (tumor suppressor), PGC-1α (energy metabolism regulator), SREPB-1c (lipid homeostasis), AceCS2 (soluble mitochondrial matrix protein), and α-tubulin (constitutive protein of microtubules). This includes cytoskeletal proteins such as α-tubulin and affects microtubule stability (Shepard and Tuma, 2009). Marine toxins of the macrolactone chemical group target the actin cytoskeleton in liver causing severe hepatotoxicity (pectenotoxins of dinoflagellate genus *Dinophysis*; Espiña and Rubiolo, 2008). Another water-borne toxin that is becoming a large problem in lakes contaminated with phosphorus runoff from fertilization of farm fields and lawns is the freshwater cyanobacterium *Microcystis aeruginosa*, which is released into the water and causes toxicity upon ingestion. Microcystins are heptapeptides with a unique structure that are potent inhibitors of phosphatases 1 and 2A. Inhibition of these enzymes disrupts the cytoskeleton and results in gross hepatic hemorrhage (Dawson, 1998). Arsenic disrupts the cytoskeletal structures responsible for structural soundness of cells, their shape, and movement. Changes in liver and skin have been studied but have not provided a molecular mechanism as of yet (Bernstam and Nriagu, 2000).

Cytoskeleton toxicity is not just the mode of agents known to damage the liver with little therapeutic value. It is also the mechanism used in the treatment of metastatic cancers as cell division is impaired. The anticancer drug paclitaxel was isolated from the bark of the Western yew tree in 1971. It is a diterpenoid compound that binds to the β-tubulin subunit of microtubules and prevents their disassembly, resulting in mitotic arrest of cancer cells and normal cells of the esophagus, stomach, small intestine, colon, liver, and bone marrow. Another set of antimitotic medications for treating neoplasms are the vinca alkaloids from the Madagascar periwinkle plant. They also bind to β-tubulin, but prevent polymerization with α-tubulin (Hruban et al., 1989).

Sinusoidal Injury

A topic related to cytoskeletal injury is the early damage to sinusoidal endothelial cells due to acetaminophen toxicity. This is especially apparent following binge ethanol intake. Sinusoidal epithelial cells swell and lose their ability to endocytose FITC-FSA (scavenger receptor ligand). Gaps in the cells form by damage to fenestrae prior to any signs of observed histological changes to parenchymal cells. Red blood cells penetrate into the space of Disse and may be followed by sinusoidal collapse and decreased blood flow. The gaps observed are larger with acetaminophen plus ethanol—similar to hepatic veno-occlusive disease caused by pyrrolizidine alkaloids of the toxic *Crotalaria* plant species. NO donor administration or inhibition of iNOS decreases toxicity, while inhibitors of eNOS increase toxicity to these cells. That suggests a protective role for constitutive NO originating from sinusoidal epithelial cells. Inhibitors of

MMP-2 and MMP-9 also minimize toxicity to these cells. Taken together it appears that the cytoskeleton is the target of the toxicity, because this structure aids in the formation and maintenance of the fenestrae (McCuskey, 2006).

Immune and Inflammatory Damage

Immune mechanisms that can lead to fibrosis and ultimately to cirrhosis have already been examined. Acute and idiosyncratic drug-induced liver injuries are important contributors to sickness and death of patients. The idiosyncratic responses are more prone to develop into fulminant liver failure (25% of intensive care unit cases). It is easy in toxicology to focus on the formation of active metabolites and their direct toxicity to macromolecules or organelles. Idiosyncratic drug-induced liver injury of this type is classified as metabolic. Medications such as isoniazid (anti-tuberculosis), ketoconazole (antifungal), troglitazone (banned antihyperglycemic agent), and pyrazinamide (anti-tuberculosis) fit into the metabolic group. However, it appears that the direct toxicity may initiate the immune response. Idiosyncratic reactions that generate a fever, rash, a 1- to 4-week onset of symptoms, and a rapid onset on another challenge dose are classified as immune. Drugs that yield antimedication or autoimmune antibodies include halothane (anesthetic) or tienilic acid (diuretic). However, some medications that yield autoimmunity in brown Norway rats, such as the anti-arthritis medication penicillamine or propylthiouracil (treats Graves' disease–induced autoimmunity in cats), do not have a rapid onset on a subsequent challenge. Similarly, heparin-induced, antibody-mediated thrombocytopenia does not reemerge once the antibodies are no longer present. Those that develop clear autoimmune antibodies such as procainamide-induced lupus-like syndrome usually resolve rapidly if the reaction is not severe and no rechallenge occurs. Some medications, such as the antibiotics minocycline and nitrofurantoin and discontinued anti-hypertensive α-methyldopa, develop a condition resembling idiopathic autoimmune hepatitis. Development of idiopathic drug-induced liver injury may take as long as a year or more and involve autoimmune reactions such as lupus-like syndrome, autoimmune-hemolytic anemia, or vasculitis. Isoniazid, minocycline, α-methyldopa, hydralazine, propylthiouracil, phenytoin, statins, and zafirlukast are representative of this group.

There are 10^{10} resident lymphocytes in the human liver that can initiate an innate immune response. The total lymphocyte population is represented by the T cells, the B cells, the natural killer (NK) cells, and the natural killer T (NKT) cells. Inflammation and intrahepatic localization of lymphocytes increase lymphocyte recruitment. The degree of damage to hepatocytes and cholangiocytes is associated with the scale of lymphocyte infiltration and inflammatory response. The liver's two sources of blood supply (portal vein and hepatic arteries) make it more prone to immune-mediated damage. The immune cells migrate across the sinusoidal epithelial cells just discussed. These sinusoidal cells lack the P-selectin adhesion molecule and have very low levels of E-selectin expression. The portal vascular endothelium expresses selectins when inflamed. ICAM-1 is a constitutively expressed adhesion receptor in the sinusoidal epithelium that mediates leukocyte adhesion to hepatic sinusoids. VAP-1 is another sinusoidal adhesion receptor that mediates lymphocyte recruitment. VAP-1 has an enzymatic activity that, on provision of a substrate, leads to NF-κB-dependent upregulation of VCAM-1 and ICAM-1. This enhances lymphocyte adhesion from the blood flow. CD44 is an adhesion molecule that causes the sequestration of neutrophils in sinusoids during sepsis due to deposition of hyaluronan (HA)-associated protein on sinusoidal cells. During inflammatory bowel disease, MAdCAM-1 is expressed as an adhesion molecule that is normally confined to the mucosal endothelium of the GI tract. This adhesion molecule can also be induced in the liver resulting in T cell infiltration that leads to immune cell–mediated toxicity. Chemokine secretion by liver cells leads to lymphocyte recruitment. CCR5 ligands CCL3–5 are expressed in high amounts in portal vascular endothelium. This leads to graft-versus-host disease, immune-mediated liver disease, and graft rejection. The parenchymal infiltration occurs in the periportal area in active hepatitis and in the hepatic lobules in lobular hepatitis. Viral infection and autoimmune

liver injury are associated with expression of CXCR3 ligands CXCL9–11 on sinusoidal epithelium. Chemokines can also be expressed by cholangiocytes, hepatocytes, and stellate cells during inflammation and transported from the basolateral to luminal endothelial surface by transcytosis from hepatocytes and stellate cells or captured by the proteoglycan-rich endothelium glycocalyx from the flow from cholangiocytes. Stellate and Kupffer cells are activated by ROS and innate immune signaling pathways triggered by toxicants. Once the lymphocytes infiltrate, they can establish tertiary lymphoid structures for continued recruitment in the liver, including neovessels in the portal tracts that exhibit features of endothelial venules in secondary lymphoid tissue. In autoimmunity, the cytokine IL-17 appears to be produced by a subset of the helper T cell, known as the Th17 cell due to the secretion of this cytokine. The role of the macrophages in fibrosis due to various agents including acetaminophen has already been covered. Osteopontin (OPN) is an acidic member of the small integrin-binding ligand N-linked glycoprotein family and appears to be responsible for macrophage chemotaxis in a number of diseases. OPN is expressed in Kupffer cells exposed to CCl_4. This macrophage chemoattractant factor appears to be involved in the concanavalin A (Con A)–induced hepatitis model in the mouse. OPN appears in the Con A model to involve lymphocytes and neutrophils. OPN appears to be involved in alcoholic liver disease causing increased neutrophilic inflammation and necrosis. The neutrophils mentioned here and previously cause additional damage via generation of hypochlorous acid, leading to increased ROS and ultimately cell mortality. The drugs most associated with these mechanisms are α-naphthyl isothiocyanate and halothane (Adams et al., 2010).

Cholestasis and Bile Duct Toxicity

Cholestasis, or retention of toxic bile salts in the liver cells, is enhanced by damage to proteins on the hepatocyte canalicular membrane that transports drugs. Examples of drug substrates for these transport proteins are indomethacin, statins, digoxin, enalapril, midazolam, tamoxifen, diclofenac, methotrexate, and troglitazone. Inhibition of ATP-dependent bile salt transport proteins makes coadministration of other medications more likely to cause cholestasis. Pairs of medications that become problematic are troglitazone plus lisinopril, itraconazole plus verapamil, and bosentan plus glyburide. Cholangiocytes, epithelial cells of the bile duct, may sustain direct injury by medications such as flucloxacillin, an isoxazolylpenicillin (Grattagliano et al., 2009). Usually, the liver cells are able to sense toxic products caused by metabolism and enhance their elimination. Xenobiotic receptors CAR and PXR are key members of the NR1I nuclear receptor family. A ligand for these receptors is phenobarbital, which has been used to treat cholestatic liver disease. A Chinese herbal medicine, Yin Zhi Huang, treats or prevents neonatal jaundice and is a CAR ligand. This action increases the speed of bilirubin clearance (Kakizaki et al., 2009).

GI Toxicity Tests

GI toxicity tests as indicated by research publications are listed in **Table 16-4**. DNA analysis of humans has been performed on cells on the inside of the cheek. Buccal cells are also used in a minimally invasive test for those who use chewing tobacco or are exposed systemically to mutagens. The micronucleus assay in exfoliated buccal cells has been used since the 1980s to determine cytogenetic damage caused by environmental and occupational exposures to toxicants. The human micronucleus project (www.humn.org) is an international project to validate the methodology (staining), scoring (inter- and intra-individual differences), and interpretation of this test. Its variability currently limits its usefulness (Holland et al., 2008).

The buccal test is truly noninvasive, as is the ^{13}C-sucrose breath test for small intestinal sucrase activity. Mucositis can be assessed by the evolution of $^{13}CO_2$ in the breath as a measure of enzymatic activity. This has been found to correlate well with damage of chemotherapy and repair and jejunal sucrase activity (Butler, 2008). Exposing rats is a classic way of assessing

TABLE 16-4 GI Toxicity Tests

Testing For	Individual Assays
Mouth/systemic DNA damage	Buccal cell micronucleus assay (Holland et al., 2008)—exfoliated buccal mucosal cells are collected by a tongue depressor, spatula, or cytobrush (preferred) moistened with water. Cells are shaken in a saline solution to release cells and then centrifuged to wash the cells in a buffer solution. Pipette or cytocentrifugation transfer to slides is followed by fixation in methanol-glacial acetic acid. Staining (Feulgen-Fast Green preferred for specificity of DNA specificity) is followed by light microscopy and scoring.
Complete GI toxicity based on rat mechanistic model	Cell models—ultrastructural and biochemical models Intact animals—nutrient and toxicant absorption, microscopic examination (check liver and pancreas as well) Permeation, mitochondrial DNA, and COX-2 analyses (Yáñez et al., 2003) Toxicant models—MPTP-induced ulcers (Deng and Zheng, 1994), trinitrobenzene sulfonic acid-induced colitis (Fitzpatrick et al., 2010), 1,2-dimethylhydrazine-induced colon cancer (Moreira et al., 2009), enterohepatic recirculation in animal that has a gall bladder (Shou et al., 2005)
Noninvasive small intestinal damage/mucositis	^{13}C-sucrose breath test (Butler, 2008)—intestinal sucrase activity is determined by ingesting ^{13}C-sucrose and determining breath $^{13}CO_2$ levels.
Intestinal motility—alternative	Zebrafish (*Danio rerio*; Eimon and Rubinstein, 2009)

toxicity. Because NSAIDs and chemotherapy agents are a key group of toxicants of the GI tract and affect GI permeability, oxidative phosphorylation, generation of ROS and mitochondrial DNA, GI permeability probes, mitochondrial DNA analysis, and COX-2 mRNA expression may give many of the parameters that need to be evaluated. In the human patient, symptoms of diarrhea frequency, stomach and abdominal pain, and visualization of gross abnormalities of the upper and lower GI through a flexible scope give some information, such as the development of precancerous polyps or ulcers. However, mechanistic analyses indicate that other techniques mentioned earlier may be beneficial to understanding the cause of the inflammation that can result in mucositis (Yáñez et al., 2003). Clearly there are different levels of analysis. Cell or tissue preparations can be used to look for damage to organelles and biochemical markers of damage (e.g., keratin in epithelial cells, enzyme markers for brush border, lysosomes, peroxisomes, mitochondria, and ER). Intact animals can be used to assess absorption of nutrients and the toxicant itself. The liver and pancreas can also be indicated as organs for assessing puzzling effects not directly related to cellular damage in the tract. Microscopic examination is also important in these models. Results should be compared with known toxicants such as ulcers produced by 1-methyl-4-phenyl-1,2,3,6-tetrahydopyridine (MPTP destruction of mucus bicarbonate via dopaminergic neuron damage; Deng and Zheng, 1994) in the rat; bile acid-, ethanol-, or trinitrobenzene sulfonic acid (immunologically; Fitzpatrick et al., 2010)–induced colitis; 1,2-dimethylhydrazine-induced colon cancer (Moreira et al., 2009); and enterohepatic recirculation in a species that has a gall bladder (such as monkeys [Shou et al., 2005] but not deer, rats, and horses).

An alternative approach to GI toxicity *in vivo* is through the use of zebrafish (*Danio rerio*) to evaluate toxic effects on GI motility, because the clear view of development and ease of use of adults makes drug toxicity evaluation relatively easy (Eimon and Rubinstein, 2009).

Pancreas Toxicity Tests

Organ damage is usually assessed by leakage of enzymes normally sequestered in the organ into the plasma as indicated in **Table 16-5**. For the pancreas, exocrine enzymes should not be found in appreciable amounts/activities in the plasma. Amylase and lipase are clinically relevant markers of pancreatic damage (Arafa et al., 2009). Inflammatory reactions that may lead to (cerulein-induced) pancreatitis may be examined by cytokines IL-6 and TNF-α, and histological grading of vacuolization, inflammation, lobular disarray, and edema (Jo et al., 2008). When inducing pancreatic cancer using agents such as 2,3,7,8-TCDD, it is also useful to examine apoptotic bodies, immunohistochemistry of CYP1A1, CCK, AhR, CCKAR, amylase, and proliferating cell nuclear antigen, and looking for the development/incidence of lesions (Yoshizawa et al., 2005). Toxicants that damage the acinar cells affect the exocrine pancreas. Chemicals that damage the endocrine pancreas may be monitored by plasma hormones insulin (beta-cells) or glucagon (alpha-cells) and blood/urine glucose levels as diabetes mellitus is manifested. A glucose tolerance test may also be done to see similar dysfunctions. Additionally, direct damage to the Islets of Langerhans indicates endocrine dysfunction and can be measured by β-cell mass (point counting method) anti-insulin antibody immunohistochemical staining (Kim et al., 2009).

Liver Toxicity Tests

Enzymes leaking out of cells again has served the clinical community well in assessing liver damage in human patients as shown in **Table 16-6**. Liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have served has indicators of liver damage. Increasing serum ALT levels indicate hepatocyte necrosis. AST may be elevated in muscle damage, heart injury, body mass alterations, blood diseases, or pancreatic injury and so may not be the best indicator of liver dysfunction alone. Hy's law should be considered as a good indicator of severe hepatotoxicity. Transaminases should be three times normal activity coupled to increases in serum bilirubin concentrations at two times normal levels. However, this threshold is too high for treatment, because irreversible changes may have already occurred. Leakage of other enzymes may also be good biomarkers for liver damage. α-Glutathione-*S*-transferase (GST-α) is a conjugation enzyme protective against activated metabolites such as those derived from acetaminophen. It is found primarily in the centrilobular region of the liver, so it indicates necrosis close to the hypoxic central vein region. γ-Glutamyl transpeptidase indicates hepatobiliary damage, particularly cholestasis, as it is localized in the bile ducts. It is also expressed in the kidney and the pancreas. Paraoxygenase-1 (PON-1) is an HDL-associated esterase that metabolizes organophosphates. It is mostly a

TABLE 16-5 Pancreas Toxicity Tests

Testing For	Individual Assays
Plasma (exocrine)	Amylase and lipase (clinical; Arafa, 2009)
	Glucose tolerance test, serum insulin levels (Kim, 2009)
Organ damage	Pancreatic myeloperoxidase, glutathione-*S*-transferase, nitric oxde, malondialdehye (Arafa, 2009)
	Pancreatic IL-6,TNF-α, histological examination/grading of vacuolization, inflammation, lobular disarray, edema (Jo, 2008)
	Apoptotic bodies in pancreatic sections, immunohistochemistry of CYP1A1, CCK, AhR, CCKAR, amylase, proliferating cell nuclear antigen, incidence of lesions (Yoshizawa, 2005)
	Anti-insulin antibody immunohistochemical staining of β-cells, point counting β-cell mass determination, insulin mRNA expression, TUNEL staining for apoptosis, caspase-3 activity (Kim, 2009)

TABLE 16-6 Liver Toxicity Tests

Testing For	Individual Assays
Plasma	Alanine aminotransferase (3x normal) + bilirubin (2x normal; hepatocyte necrosis according to Hy's law), aspartate aminotransferase, α-glutahione-*S*-transferase (centrilobular necrosis), γ-glutamyl transpeptidase (hepatobiliary/cholestasis), paraoxygenase-1 (decreased = liver damage), purine nucleotide phosphorylase (hepatic necrosis released to sinusoids), malate dehydrogenase (necrosis/cirrhosis; Marrer, 2010), sorbitol dehydrogenase, glutamate dehydrogenase, alkaline phosphatase (cholestatic), albumin, glucose, coagulation factors, ammonia, urea nitrogen (Ramaiah, 2007)
Organ damage	Histological determination of periportal, midzonal, and centrilobular, or random damage, microvesicular and macrovesicular steatosis, glycogen accumulation, CYP induction accompanied by centrilobular hypertrophy, hepatocellular alterations, hepatic hyperplasia/adenoma, carcinoma (Ramaiah, 2007)

liver enzyme, although the brain, kidney, and lung express this enzyme. It is not leaked but is released in conjunction with HDL. Its reduction in serum is consistent with liver injury in conjunction with other markers, but can also signal atherosclerosis and vasculitis. Purine nucleotide phosphorylase is located in the cytoplasm of endothelial cells, Kupffer cells, and hepatocytes. Its leakage into hepatic sinusoids is a marker for necrosis, which occurred prior to ALT leakage in endotoxin-challenged rat livers. Malate dehydrogenase of the tricarboxylic acid cycle is found at highest activity in the mitochondria of the liver and less in the heart, skeletal muscle, and brain. It is an indication of necrosis and cirrhosis. Enzyme data should be interpreted carefully and in combination with other data that are consonant with the liver toxicity to avoid embarrassment associated with finding that a genetic population expresses certain polymorphisms that may be mistaken for injury or lower normal activities that mask damage (e.g., PON-1 and GST-α; Marrer and Dieterle, 2010). Other leakage enzymes are sorbitol dehydrogenase and glutamate dehydrogenase for necrotic livers and alkaline phosphatase for cholestatic hepatobiliary injury. Other factors that can indicate liver injury include a reduced ability to produce albumin for the plasma oncotic pressure, coagulation factors such as prothrombin and fibrinogen, and waste conversion functions as monitored by plasma ammonia and urea nitrogen concentrations (Ramaiah, 2007). According to the Society of Toxicology, organ weights may be helpful in assessing certain types of organ damage (Sellers et al., 2007). More specific parameters, such as the accumulation of fatty tissue, glycogen, or bile acids in the liver, may yield more specific information. A set of reference histology slides on various types of liver damage are available from the Internet Pathology Laboratory for Medical Education, Mercer University School of Medicine (Klatt, 2015). Examination should be able to determine periportal, midzonal, and centrilobular necrosis or random damage. Steatosis can be microvesicular or macrovesicular. CYP induction may be accompanied by centrilobular hypertrophy. Cancer or precancerous lesions may be indicated by hepatocellular alterations, hepatic hyperplasias/adenoma, or primary hepatocyte carcinoma (Ramaiah, 2007).

Questions

1. Bisphosphonates taken orally with little or no water do what kinds of damage to the mouth and esophagus? Why?
2. How do NSAIDs and *H. pylori* lead to stomach damage?
3. Why does chronic ethanol ingestion lead to the opposite of acute ingestion in the duodenum?
4. Why is GSH so important in the jejunum (importance in liver is understood based on metabolic role)?

5. Why is phospholipase A_2 activity important to pathogenesis of the ileum?
6. Why is chronic red meat consumption a problem for the colon?
7. How is alloxan destruction of pancreatic cells mediated?
8. Indicate which kinds of mechanisms mediate damage in zones 1 versus 3 in the liver.
9. Which toxicant appears to be sex-linked; leads to steatosis, cirrhosis, and cytoskeletal changes; and is associated with liver cancer?
10. Why is taking acetaminophen for a headache following binge drinking similar to poisoning oneself with pyrrolizidine alkaloids from eating toxic plants of the *Crotalaria* species?
11. What factors makes liver cells prone to immune infiltration-induced damage?
12. What would be a test for an intact human with mucositis?
13. What are tests for intact animals or humans of exocrine and endocrine pancreatic damage?
14. Why is liver damage indicated by someone's eyes and skin turning yellow?

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