Response to Liver Injury

In response to liver injury, hepatic tissues are often infiltrated by cells of the immune system such as macrophages and neutrophils. Although these cells help to remove foreign materials and cell debris, they also produce chemicals that may be toxic to surrounding healthy cells, such as nitric oxide. Thus, as in many tissues, inflammation may be either helpful or damaging, depending on the degree and circumstances of the response.

Hepatocytes also have a significant regenerative capacity. If a portion of the liver is lost due to physical or chemical injury, the remaining portions will increase in mass until the approximate original mass of the liver is regained. Regeneration of lost or damaged tissue is primarily due to the replication of the remaining hepatocytes; however, there are stem cells in the liver called oval cells that can serve as precursors of hepatocytes. Following chemical injury (as opposed to surgical removal of tissue), regeneration is more likely to include proliferation of oval cells. Replication of hepatocytes appears to be triggered by cytokines including TNF-α and growth factors HGF and TGF-α.

Evaluating Liver Injury and Treating Disease

Several methods, both clinical and experimental, are used to test for injury to the liver. Serum enzyme tests look for activity of enzymes in the blood that are normally found in hepatic cells. Increased serum activities of these enzymes may indicate damage to hepatocytes and subsequent leakage of the enzymes. Enzymes that are typically assessed may include *aminotransferases* such as serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT), serum *alkaline phosphatase* (AP), serum *lactate dehydrogenase* (LDH), and many others. Some of these enzymes are more specific for liver injury than others (which may be elevated when other tissues are also injured). On the other hand, some are specific enough not only to indicate liver injury, but also to actually aid in diagnosing the type of injury.

The damaged liver is of great interest as a model in the development of transgenic cell therapy. This is in part due to the natural regenerative capacity of hepatocytes (vs. neurons, for example). A *transgene* (DNA that is incorporated into the cell from another source) might encode, for example, the normal sequence of a protein missing from the diseased liver. One approach would be to introduce a therapeutic transgene directly using an engineered virus as a vector. Another approach would be to culture cells from a patient, modify the cells using the virus, and then introduce the transgenic cells into the liver.

Prospects for curing liver diseases have also risen due to several fortunate properties observed in the early phases of experimental cell therapy. *Hematopoietic stem cells* are the current workhorses of experimental cell therapy and have been used in attempts to treat lymphoma and other cancers. They are harvested from bone marrow and are capable of differentiation into various types of blood cells. Injected hematopoietic stem cells also migrate into liver and a few other organs in a type of homing response, a property that has been exploited in experimental therapy of liver disease in mice. Interestingly, hematopoietic stem cells have also been observed to differentiate into hepatocytes. As cell therapy technology develops to employ other types of stem cells, diseases of the liver will be among the most promising candidates for treatment.

Case Study: Reye's Syndrome

In 1963, a doctor in Australia named Ralph Reye published his observations of a number of children who developed a condition characterized by a combination of encephalopathy (neurological problems) and hepatic dysfunction. This condition, which came to be known as Reye's (or Reye) syndrome, carries with it a high level of mortality, particularly if it is not recognized and treated promptly.

From the beginning, Reye's syndrome has been puzzling. First of all, the disease occurs almost exclusively in children and young adults under the age of 18. It also tends to occur following a viral illness, most commonly chicken pox or influenza. However, a single common etiologic (causative) infectious agent cannot be identified. Symptoms include vomiting, listlessness, and drowsiness progressing to aggressive behavior, delirium, and coma. Reported pathology includes swelling of the brain, as well as fatty changes in the liver. In fact, diagnosis of Reye's relies not only on the combination of history of viral illness and unexplained vomiting, but also on the elevation of serum liver enzymes (which, as you may recall, indicate hepatic damage). On the histological level, liver cells show proliferation of smooth endoplasmic reticulum and peroxisomes, and enlarged mitochondria. This involvement of mitochondria is one of the factors that helps distinguish Reye's from some of the inherited metabolic disorders that may mimic it.

Although it became clear early on that prior viral infection was strongly associated with the development of Reye's syndrome, the fact that most children with viral illnesses do not develop Reye's led researchers to search for additional factors that might be contributory agents. Because Reye's is relatively rare, establishing a link between the disease and causative factors is difficult. However, multiple studies have shown a strong association between development of Reye's and use of aspirin during the viral illnesses that typically precede its onset. In fact, the evidence was strong enough that

in 1986 the FDA required a warning on products containing aspirin. This warning states that children and teenagers who have viral illnesses should not be treated with products containing aspirin. There remained some controversy in the medical community about whether aspirin is really a contributing factor, but the facts are that since the publication of this warning, the incidence of Reye's in the U.S. has dramatically declined. While there were 555 cases reported in children in 1980, this had dropped to fewer than 2 cases per year in the mid- to late 1990s.

Recent research in hepatotoxicity may be able to provide some answers as to the molecular mechanism behind Reye's syndrome, and one hypothesis developed by Trost and Lemasters focuses on mitochondria as the key. Salicylate, the active metabolite of aspirin, has been shown to induce the mitochondrial permeability transition (see Chapter 4) in hepatocytes, leading to mitochondrial uncoupling. This disruption of hepatic metabolism would be expected to lead to metabolic changes such as those seen in Reye's, including hypoglycemia, increase in fatty acid levels, and increase in levels of ammonia. Excess ammonia, in turn, has been shown to lead to brain edema and other neurological effects. Thus, most of the symptoms of Reye's correlate well with salicylate toxicity.

At least one question, though, remains: Why does Reye's almost invariably follow a viral illness? There may be a molecular answer to this. First, there is evidence that viral infections may also act on mitochondria, disrupting calcium metabolism, resulting in increased calcium levels in hepatocytes. Other studies have then shown that increased calcium levels significantly enhance the ability of salicylate to invoke the MPT. Thus, the viral infection may sensitize hepatocytes to salicylate toxicity, setting the stage for the development of Reye's syndrome.

Still, Reye's syndrome is rare, even in individuals exposed to the combination of the two factors of viral infection and aspirin. This implies that there must be other factors also involved in its development. Perhaps only individuals with a particular genetic predisposition are susceptible, or perhaps there are other environmental factors that have not yet been identified. Nonetheless, over the past 40 years the combination of epidemiological and laboratory research has done a great deal to advance the understanding of this once totally mysterious syndrome.

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12

Renal Toxicology

Function of the Kidneys

In general, the kidneys play a major role in the maintenance of a constant internal environment within the body. This dynamic process, known as *homeostasis*, allows the body to maintain optimal conditions within its cells, even in the face of external changes in the environment.

One specific function of the kidneys is to excrete waste (including soluble xenobiotics and conjugates) from the blood through formation of urine. The kidneys also act to regulate levels of water and salts such as potassium and sodium in the body. In addition, hormones and enzymes produced by the kidney are important in the regulation of blood pressure, the maintenance of stable pH levels in blood and body fluids, the regulation of calcium metabolism, and the production of red blood cells. Therefore, toxicant-induced kidney damage has the potential to effect significant physiological changes that extend well beyond the boundaries of the organ itself.

Anatomy and Physiology of the Kidneys

The paired kidneys are located in the abdominal area, near the posterior wall. The structure of a kidney is defined by several morphological features (Figure 12.1). Each kidney is covered by an outer *capsule*. Underneath the capsule is a layer of tissue called the *cortex* and an inner zone known as the *medulla*. Blood enters the kidney through the *renal artery* and leaves via the *renal vein*. The cortex receives the bulk of the blood flow to the kidney and has a much higher rate of oxygen utilization than the medulla. (As we will see, most of the energy-intensive processes in the kidney occur in the cortex.) *Urine*, the waste-containing fluid formed in the tissues of the kidney, is collected and passes through the renal pelvis and out the *ureter*.

The functional unit of the kidney is the *nephron* (Figure 12.2), with each kidney containing around a million nearly identical nephrons. Each nephron

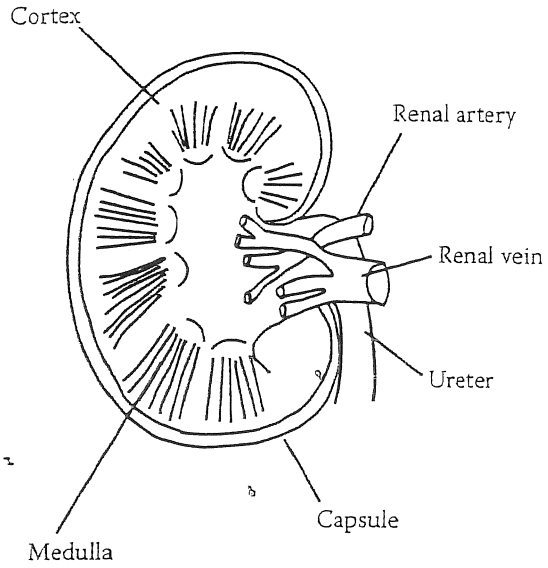

FIGURE 12.1
The anatomy of the kidney.

is composed of a *glomerulus*, which is a knot of capillaries and which is surrounded by a structure called *Bowman's capsule*. Leading out of Bowman's capsule is a tubule consisting of a *proximal portion*, a loop (*loop of Henle*), and a *distal portion*, which empties into a *collecting duct*. The glomerular portion of all nephrons is located in the cortex, but while the tubules of some nephrons are found only in the cortex, the tubules of other nephrons (those located deep in the cortex) extend far down into the medulla.

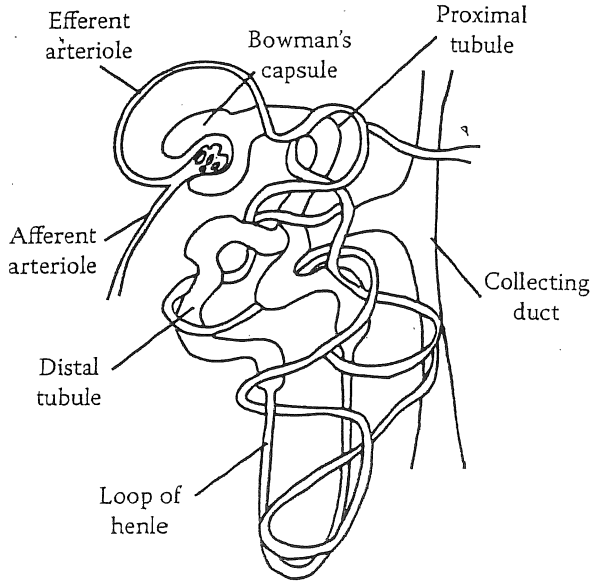

FIGURE 12.2
The nephron, showing Bowman's capsule, the proximal tubule, the loop of Henle, the distal tubule, and the collecting duct, as well as the afferent and efferent arterioles and glomerulus.

The kidney basically acts as a biological filter. Fluid from the blood is filtered out of the glomerulus (the glomerular capillaries are quite permeable), driven by a combination of hydrostatic pressure (blood pressure) and osmotic pressure. Approximately 20% of blood volume is filtered out in a single pass through the kidney. Blood cells, however, as well as larger molecules such as albumin do not typically pass out of the glomerulus (in fact, if they do, it is often an indication of kidney dysfunction).

The fluid that leaves the vascular system to enter the kidney tissues at this point is now termed the *filtrate* and is then routed directly into the proximal tubule. As the filtrate passes through the proximal tubule, many substances in the filtrate are *reabsorbed* by the epithelial cells that line the tubule. In fact, 60 to 80% of the water and solutes that make up the filtrate will be reabsorbed by these cells. In addition, some substances that were not originally filtered out of the glomerulus are picked up from surrounding blood vessels and *secreted* into the filtrate by the proximal tubule cells. The filtrate is further concentrated in the loop of Henle, and final adjustments to concentrations of water and solutes are made in the distal tubule and collecting duct. The filtrate that exits the nephron is then eventually routed to the ureter and excreted as urine.

Effects of Toxicants on the Kidney: General Principles

Toxicant-induced damage to the kidney may be mild or severe, reversible or permanent, depending on the toxic agent and the dose. The kidney is particularly susceptible to the effects of toxicants for several reasons. First, blood flow to the kidneys is high (25% of cardiac output), so blood-borne toxicants will be delivered to the kidneys in large quantities. Second, as the kidney removes salts, water, and other substances from the filtrate through the process of reabsorption, any toxicant that is not reabsorbed may become highly concentrated in the remaining filtrate. Finally, even if a toxicant is reabsorbed, it still may accumulate to high concentrations within the epithelial cells that line the tubule themselves. Thus, kidney tubule cells may be exposed to concentrations of a toxicant that are many times higher than the concentration of that toxicant in the plasma. In addition, many cells in the proximal tubule possess cytochrome P450 activity, so if bioactivation of a toxicant occurs, those cells may be affected.

Damage to the Glomerulus

One site at which nephrotoxicants may act is the glomerulus (shown in Figure 12.3). The glomerulus itself is a network of capillaries arising from

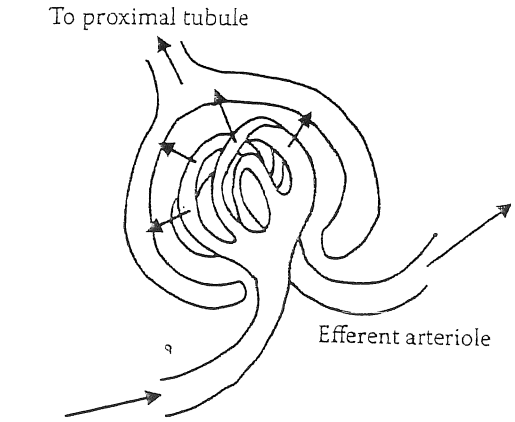

FIGURE 12.3
The glomerulus.

an *afferent arteriole*, a branch of the renal artery. The walls of the glomerular capillaries are very porous. Blood enters the glomerulus at relatively high pressure (around 60 mmHg). This pressure, which is regulated in part by specialized cells of the afferent arteriole called *juxtaglomerular cells*, forces blood fluids out of the pores, across a basement membrane, and through filtration slits between the *podocytes* (the epithelial cells that are part of Bowman's capsule). The capillaries reunite upon exiting Bowman's capsule, forming an *efferent* arteriole that then branches into a second network of capillaries that wrap around the rest of the tubule. Efferent arterioles eventually empty into the renal vein.

Thus, the glomerulus acts as a filter, allowing the passage of plasma fluids and small molecules into Bowman's capsule. Not only size (remember, blood cells and most plasma proteins are too large to fit through the filter) but also net electrical charge of a molecule affects filtration, with neutral molecules more likely to pass through the glomerular membrane (which is itself negatively charged). In a normal adult, a total of around 125 ml of fluid per minute is filtered by the two kidneys. This number is called the *glomerular filtration rate* (GFR).

There are a number of ways in which toxicants may affect the glomerulus. First of all, toxicants may increase glomerular permeability, resulting in *proteinuria*, the leakage of large-molecular-weight proteins into the filtrate, and thus into the urine. Other toxicants may damage podocytes, increasing leakage through increasing filtration slit size. One compound that produces this effect is the antibiotic *puromycin*, which may alter podocytes through effects on expression of proteins such as podocin and nephrin that play a role in slit morphology.

Some toxicants (such as *gentamicin*) can reduce the negative charge of the glomerular membrane, leading to the increased excretion of large anions. Additional toxicants (*amphotericin*, for example) may decrease GFR by causing vasoconstriction of glomerular capillaries. Finally, heavy metals and