

Kevin E. Bove

## 13.1 Introduction

Genetically determined metabolic liver disease results from an inherited defect that causes malfunction of an enzyme, structural protein, or organelle that is critical for normal liver function. Metabolic diseases may affect the liver alone or in concert with manifestations in other organs. The category genetic metabolic liver disease may be extended to include cellular derangements that are transient and precipitated by exogenous factors such as drugs or pregnancy that may stress existing suboptimal or partly compromised genetic pathways.

Diagnostic liver biopsy is performed to investigate the precise cause of biochemical abnormalities in blood tests related to liver injury or function, usually in conjunction with liver enlargement. Previously unrecognized metabolic disease is more likely if the patient has any of the findings listed in Table 13.1. In some metabolic diseases, morphological findings in liver tissue are specific. In others, the histology and electron microscopy are suggestive of a particular group of metabolic diseases, significantly narrowing the differential diagnosis. The microscopic findings often guide selection of appropriate biochemical and/or genetic and molecular tests. Modern laboratory methods

K.E. Bove, MD  
 Department of Pathology,  
 Cincinnati Children's Hospital,  
 3333 Burnet Ave., Cincinnati, OH 45229, USA  
 e-mail: [boveke@ucmail.uc.edu](mailto:boveke@ucmail.uc.edu)

**Table 13.1** Clinical presentations in genetic metabolic liver disease

|                                                                                                                            |
|----------------------------------------------------------------------------------------------------------------------------|
| 1. Nonimmune fetal hydrops                                                                                                 |
| 2. Antecedent history in a newborn infant of pregnancy complications such as HELLP 3. syndrome or fatty liver of pregnancy |
| 3. Unexpected deterioration and death of a normally developed newborn baby                                                 |
| 4. Failure to thrive in an infant with abnormal facies and/or brain and skeletal anomalies                                 |
| 5. Sudden infant death syndrome (crib death)                                                                               |
| 6. Unexplained metabolic crisis precipitated by a mundane viral infection with or without a history of a previous episode  |
| 7. Unexplained organomegaly/organ dysfunction                                                                              |
| 8. First-degree relative with unexplained serious illness, single organ dysfunction or sudden death                        |
| <i>Metabolic crisis: features</i>                                                                                          |
| 1. Hypoglycemia, nonketotic or hypoketotic                                                                                 |
| 2. Normoglycemic ketoacidosis                                                                                              |
| 3. Metabolic acidosis, not otherwise specified                                                                             |
| 4. Hyper- or hyponatremia                                                                                                  |
| 5. Liver dysfunction with or without hyperammonemia                                                                        |
| 6. Encephalopathy (lethargy, stupor, or coma)                                                                              |
| 7. Vomiting                                                                                                                |
| 8. New onset seizure                                                                                                       |
| 9. Rhabdomyolysis                                                                                                          |
| <i>More common causes of acute metabolic crisis in infants</i>                                                             |
| 1. Beta oxidation defects, especially MCAD                                                                                 |
| 2. Mitochondriopathies                                                                                                     |
| 3. Lipid transport defects, e.g., systemic carnitine deficiency and carnitine palmitoyltransferase deficiency              |
| 4. Glycogen storage diseases, especially types I and III                                                                   |

increasingly provide alternative approaches to a definitive diagnosis of metabolic disorders such as identification of diagnostic metabolites in body fluids or molecular analysis of leukocyte or fibroblast DNA. This process can be significantly focused by the clinical presentation or laboratory findings, thereby obviating the need for a liver biopsy. The aim of this chapter is to provide an approach to pathologists who evaluate liver biopsy specimens in patients who are suspected to have a metabolic disease. For more exhaustive coverage of the subject, the reader is encouraged to consult other sources (Gilbert-Barness and Barness 2000; Gilbert-Barness et al. 2007; Clayton 2002, 2003; Stocker et al. 2011; Bove 2011).

---

### **13.2 Handling a Liver Biopsy Specimen for Suspected Metabolic Disease**

At least two cores of liver tissue should be obtained. Cautery of surgical specimens must be avoided, or enzyme studies may be useless. Freeze one core of liver tissue or one slice of a surgical biopsy specimen in anticipation of need for biochemical or genetic analysis. Delay the decision about use of frozen liver tissue until light and electron microscopy are completed unless clinical data clearly indicate a particular class of metabolic disease. The value of electron microscopy is severely limited, if a sample of liver tissue is not placed in a suitable fixative such as glutaraldehyde without delay; 2 mm of a typical needle core is sufficient and need not be processed beyond embedding in resin if deemed unnecessary. If fixation for electron microscopy was overlooked, flash frozen tissue thawed in glutaraldehyde is superior to paraffin-embedded tissue for study of ultrastructure. When clinical suspicion of a metabolic disease/storage disease is supported by light microscopic findings, the decision of how to proceed is aided by assessment of ultrastructure of all cell types and organelles and evaluation for storage material and its location. For example, when light microscopy identifies features of glycogen storage disorder, and study of liver ultrastructure confirms that excess nonlysosomal glycogen has displaced structurally normal organelles in most hepatocytes,

it is then appropriate to commit the frozen sample to measurement of the glycogen concentration and to screen for glycolytic enzyme defects.

---

### **13.3 Analysis and Reporting of Liver Biopsy Specimens for Suspected Metabolic Disease**

Conventional stains of paraffin sections of a diagnostic liver specimen are hematoxylin and eosin, Masson trichrome, reticulin, and periodic acid-Schiff (PAS) with prior diastase digestion. Stains that prove useful only in certain contexts, but are nonetheless often routinely employed, are PAS, Prussian blue for hemosiderin deposits, and rhodanine for copper complexes in secondary lysosomes. Stains that help identify a stored material are Sudan black and Ziehl-Neelsen for ceroid and Alcian blue and colloidal iron for mucopolysaccharide or polyglucosan. Specific immunostains may help identify a particular protein such as alpha-1-antitrypsin or fibrinogen or a specific organelle such as mitochondria or lysosomes.

Preparation of a useful written report of a liver biopsy is a skill that can be acquired through practice. An appreciation of normal liver histology combined with orderly enumeration of histological features, both normal and abnormal, is essential. When chronic hepatitis of any kind is present, activity and stage should be assessed. Interaction with the clinician who performed the procedure is required to understand why it was done and what information is desired. The objective, whenever possible, should be diagnostic interpretation rather than mere description.

---

### **13.4 Histological Patterns of Metabolic Liver Disease**

#### **13.4.1 Normal or Near Normal Liver Histology in Metabolic Disease**

Rarely, metabolic diseases that affect the liver are pure functional disorders with minimal or absent histological changes (Table 13.2). In certain

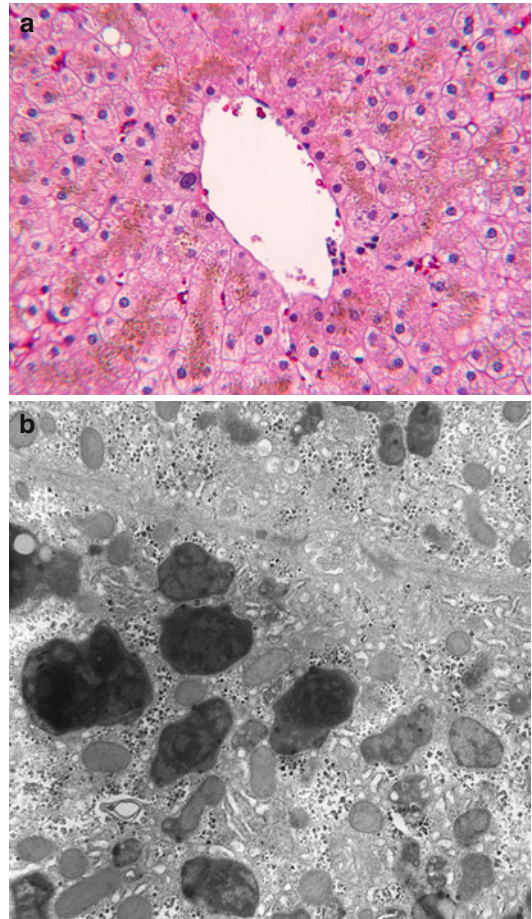
**Table 13.2** Hepatocentric metabolic disease without chronic liver disease

|                                                        |
|--------------------------------------------------------|
| 1. Oxalosis                                            |
| 2. Dubin-Johnson syndrome                              |
| 3. Crigler-Najjar syndrome                             |
| 4. Fabry disease                                       |
| 5. Urea cycle defects                                  |
| 6. Aminoacidemias                                      |
| 7. Organic acidemias                                   |
| 8. Disorders of glycosylation                          |
| 9. Alpha-1-antitrypsin phenotypes other than ZZ and SZ |
| 10. Smith-Lemli-Opitz syndrome                         |
| 11. Peroxisomal disease other than Zellweger disease   |

circumstances, such a liver may be utilized for transplantation (Popescu and Dima 2012). Examples of metabolic diseases due to abnormal liver metabolism that consistently lack morphological abnormalities are primary oxalosis, acute intermittent porphyria, maple syrup urine disease, and homozygous hypercholesterolemia. Also lacking progressive liver disease are disorders of bilirubin conjugation (Gilbert syndrome, Crigler-Najjar syndrome types I and II), in which diminished or absent glucuronosyltransferase activity results in indirect-reacting hyperbilirubinemia without hemolysis (Sampietro and Iolascon 1999). Bilirubin encephalopathy is a hazard in the severe form of this disease. In Dubin-Johnson syndrome, mutations in the MRP2 gene impair secretion of conjugated bilirubin across the canalicular membrane into bile causing mild conjugated hyperbilirubinemia without liver disease. A melanin-like lipofuscin pigment accumulates in the apical region of zones 2–3 hepatocytes and secondarily in Kupffer cells, without further histological or functional consequences (Fig. 13.1).

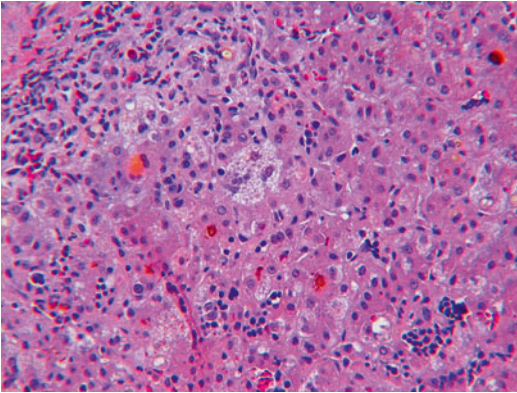
### 13.4.2 Liver Inflammation in Metabolic Disease

Idiopathic neonatal hepatitis (INH) is an umbrella term originally devised for transient nonobstructive cholestatic liver disease in the first several months of life. Histological features are lobular cholestasis, giant cell transformation of variable extent, persistent extramedullary hematopoiesis,

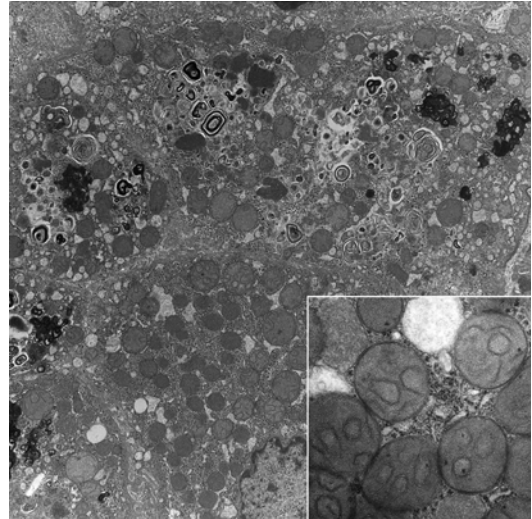


**Fig. 13.1** (a) Dubin-Johnson syndrome. Golden brown pigment accumulates in apical cytoplasm of zone 3 hepatocytes. H&E stain. (b) Bulky polymorphous residual bodies (secondary lysosomes) adjacent to canalicular. EM

mild inflammation, minimal or absent changes in bile ducts, and little or no fibrosis (Fig. 13.2). Modern advances in understanding of the etiology of cholestatic liver disease in infants with direct hyperbilirubinemia indicate the lesion of INH exists in numerous contexts and is often the initial histological diagnosis in an infant who develops progressive liver disease due to a specific metabolic disease (Table 13.3). Despite these advances, experience indicates that INH continues to have multiple etiologies including perinatal virus infection, immunopathy such as hemophagocytic lymphohistiocytosis (HLH), presumably undiscovered disorders of bile formation and transport, and as a transient expression of immaturity of the infant liver under stress.



**Fig. 13.2** INH. Canalicular and cytoplasmic cholestasis (cholesterol crystals), erythropoiesis in sinusoids, and myelopoiesis in portal area and focal giant cell transformation. H&E stain



**Fig. 13.3** INH. Hepatocytes contain abundant secondary lysosomes and autophagic bodies. Membranous profiles suggest phospholipid derived from bile or autophagy. *Inset:* mitochondria show internal rearrangements of cristae. EM

**Table 13.3** Conjugated hyperbilirubinemia in early infancy

|                                                        |
|--------------------------------------------------------|
| 1. Idiopathic neonatal hepatitis                       |
| (a) Nonspecific stress/liver immaturity                |
| (b) Infection                                          |
| (c) Parenteral nutrition related                       |
| (d) Endocrinopathy (thyroid, pituitary)                |
| (e) Unrecognized defect in bile formation or transport |
| 2. Biliary atresia                                     |
| 3. Alpha-1-antitrypsin storage disease                 |
| 4. Alagille syndrome                                   |
| 5. PFIC, especially PFIC 2                             |
| 6. Galactosemia                                        |
| 7. Fructose intolerance                                |
| 8. Mitochondriopathy                                   |
| 9. Bile acid synthetic defects                         |
| (a) 3-OH steroid dehydrogenase deficiency              |
| (b) 5-beta reductase deficiency                        |
| 10. Tyrosinemia                                        |
| 11. Niemann-Pick disease, especially type C            |
| 12. Zellweger syndrome                                 |
| 13. Smith-Lemli-Opitz syndrome                         |
| 14. Cystic fibrosis                                    |

Histological features that suggest that an INH-like disorder may have a specific, underlying metabolic etiology are prevalence of pseudoacini, excessive giant cell necrosis, excessive inflammation, intralobular (pericellular) fibrosis, steatosis, prominent vacuolation of Kupffer cells, and prevalence of injury to small bile ducts. Liver

ultrastructure in INH demonstrates nonspecific features of lobular cholestasis (Fig. 13.3) and may help identify specific metabolic diseases that cause direct hyperbilirubinemia in infants.

Portal and lobular inflammation, other than extramedullary hematopoiesis, is usually a minor feature in INH and may be absent in many metabolic disorders that affect the liver of infants. The explanation may be lack of metabolic by-products that are toxic to the affected cells; or a toxic effect may be easily compensated by replacement of injured cells or organelles without permanent injury to the liver. On the other hand, some metabolic diseases of the liver are commonly accompanied by inflammatory changes, such as alpha-1-antitrypsin deficiency, tyrosinemia, and several bile acid synthetic defects, that may mimic chronic viral hepatitis or drug-induced hepatitis or autoimmune hepatitis. The common denominator in metabolic disorders consistently presenting as “hepatitis” seems to be accumulation of toxic metabolites that stress hepatocytes or small bile ducts. An alternate explanation is that inflammation incidental to intercurrent events such as viremia, sepsis, systemic inflammatory disease, or drug exposure may be poorly

**Table 13.4** Metabolic diseases with chronic liver disease*Chronic inflammation a common feature*

1. Tyrosinemia
2. Bile acid synthetic defects
3. Alpha-1-antitrypsin storage disorder
4. Wilson disease

*Cirrhosis a common outcome*

1. Galactosemia
2. Fructose intolerance
3. Alpha-1-antitrypsin storage disease
4. Tyrosinemia
5. Glycogen storage disease, type IV
6. Wilson disease
7. Bile acid synthetic defects

*Increased risk for hepatic neoplasms*

1. Tyrosinemia
2. Glycogen storage diseases
3. Alpha-1-antitrypsin storage disease
4. PFIC2

tolerated in metabolic disease. Examples of metabolic liver diseases with propensity to inflammation and/or cirrhosis and long-term liability to neoplasia are listed in Table 13.4.

### 13.4.3 Metabolic Disease with Prominent Lobular Cholestasis

Canalicular cholestasis and cytoplasmic cholestasis regularly occur in INH and in certain metabolic diseases. Disruption of the canalicular network as a result of necrosis or extensive giant cell transformation may contribute to nonobstructive cholestasis. Examples are tyrosinemia, alpha-1-antitrypsin storage disease, perinatal iron storage disorder, galactosemia, and some mitochondrialopathies. Another group includes disorders in which the synthesis or transport of bile acids is abnormal. In both, lobular cholestasis is severe, but in synthetic defects, toxic hydrophobic monohydroxy bile acids may injure bile canaliculi and ductules as well as hepatocytes. Bile ducts are spared and serum gamma glutamyl transferase is typically normal. A third group with cholestasis are genetic metabolic diseases that are characterized by progressive cholangiopathy

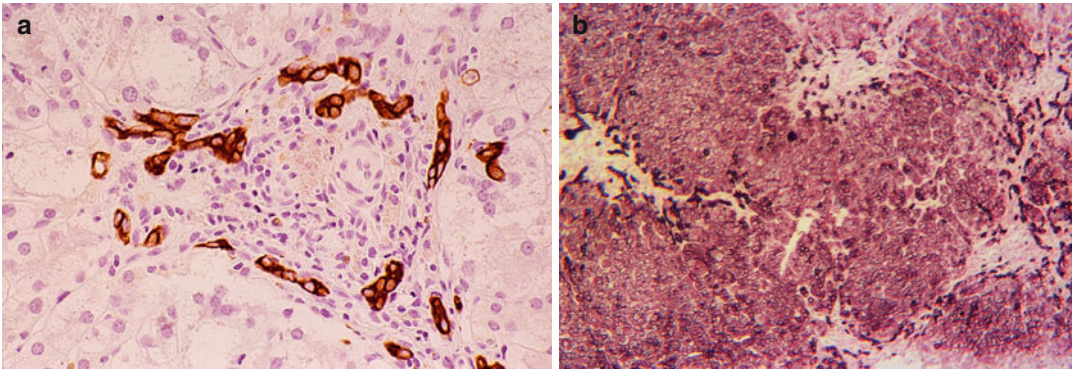
affecting bile ducts such as Alagille syndrome, Zellweger disease, cystic fibrosis, and one form of progressive familial intrahepatic cholestasis, PFIC3. In this group are those few infants with alpha-1-antitrypsin storage disease who have early-onset cholestasis with progressive small bile duct injury potentially resulting in paucity. Serum gamma glutamyl transferase is typically elevated in cholangiopathies.

Paucity of interlobular bile ducts is by definition a feature of Alagille syndrome. Nonsyndromatic paucity has been reported rarely in a host of other metabolic, genetic, and acquired diseases such as alpha-1-antitrypsin deficiency, PFIC2, bile acid synthetic defects, congenital panhypopituitarism, or conditions such as Zellweger disease, Down syndrome, and arthrogryposis-renal-cholestasis syndrome. Paucity may be due to bile duct destruction with or without sclerosis or delays in small bile duct formation (Kahn et al. 1986; Sinha et al. 2007).

### 13.4.4 Bile Ductules Versus Ducts in Metabolic Disease

Recognition of the difference between proliferation of interlobular bile ducts and reactive bile ductules is important for distinguishing a true cholangiopathy such as biliary atresia from the common periportal ductular reaction (DR). The latter occurs in diverse liver diseases (viral, autoimmune, drug induced, and metabolic) that are not primary diseases of bile ducts (Fig. 13.4a–c). Persistent ductular reaction is a sign of injury to bile ductules and zone 1 hepatocytes. Metabolic diseases such as tyrosinemia, galactosemia, alpha-1-antitrypsin deficiency, and the more hepatotoxic bile acid synthetic defects are regularly accompanied by ductular reaction and progressive periportal fibrosis. Ductular reaction in Alagille syndrome and Zellweger syndrome may hinder recognition of paucity of interlobular bile ducts.

True cholangiopathies are characterized by primary injury to intra- and/or extrahepatic bile ducts and are often accompanied by a persistent DR with progressive periportal fibrosis. DR and



**Fig. 13.4** (a) Ductular reaction. Interlobular bile duct inapparent though paucity was absent. 3-beta OH steroid dehydrogenase deficiency (bile acid defect). Cytokeratin

immunostain. (b) Ductular reaction. Paucity of interlobular bile ducts is striking. Zellweger disease. Cytokeratin immunostain

proliferation of interlobular bile ducts often coexist in large duct cholangiopathies, e.g., extrahepatic biliary atresia, ABCB4 disease (PFIC3), Langerhans cell histiocytosis, and primary sclerosing cholangitis.

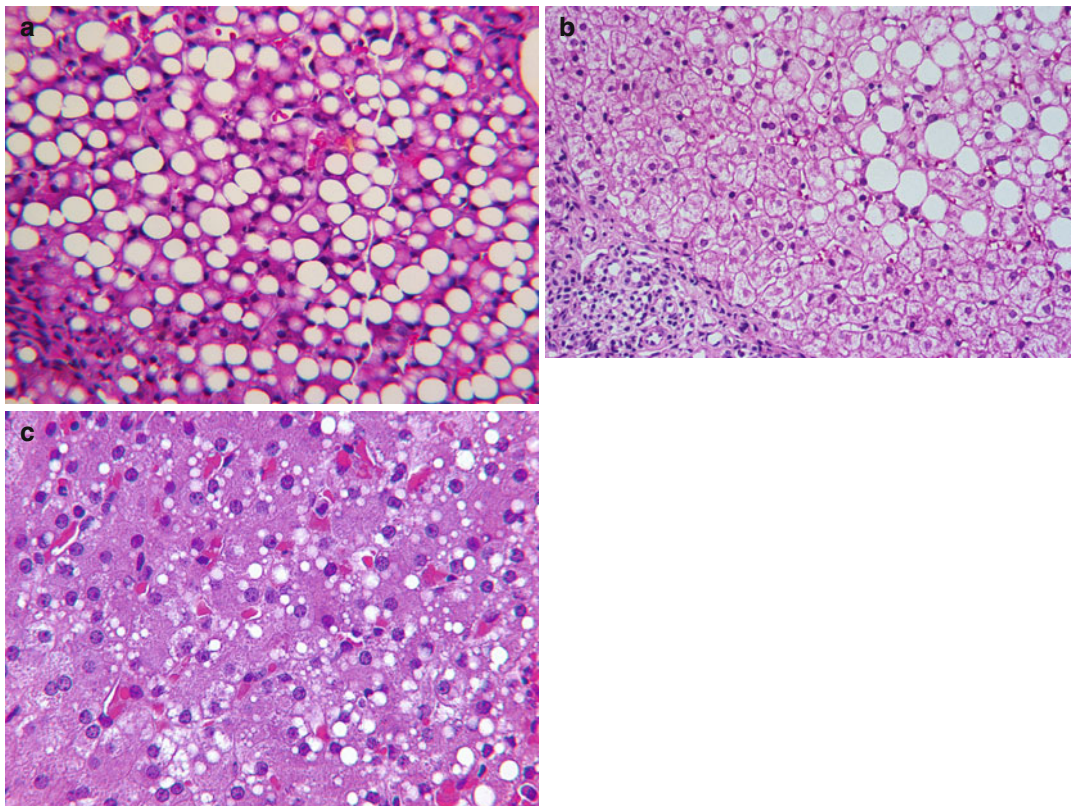
Bile duct epithelium usually is not involved in metabolic storage diseases that affect Kupffer cells and/or hepatocytes. Exceptions include some lysosomal storage diseases (type II glycogenosis, GM1 gangliosidosis, mucopolysaccharidoses) and cystic fibrosis. In perinatal hemochromatosis (PH), hemosiderin deposits in duct epithelium may be observed possibly reflecting the general deposition of hemosiderin in extrahepatic parenchymal cells.

### 13.4.5 Acquired Steatosis Versus Metabolic Disease with Steatosis

Fatty change in the liver of infants and children is usually acquired but may be a manifestation of an underlying rare genetic metabolic disease such as a defect in beta oxidation of lipids, mtDNA depletion, disorder of specific electron transport proteins, urea cycle defect (UCD), organic acidemia, or an aminoacidopathy. Accumulation of lipid in hepatocytes without inflammation, hepatocyte necrosis or other abnormalities of liver cells, or evidence of fibrosis, is simple steatosis (Fig. 13.5a). Simple steatosis may be seen with

cystic fibrosis, Wilson disease, celiac disease, protein avoidance in urea cycle defects, corticosteroid therapy, certain drug reactions, hypertriglyceridemia, after gastric bypass surgery and portocaval shunts, and with blind loop and short gut syndromes. Simple steatosis is characterized by large vacuoles of neutral lipid that displace the nucleus to the periphery (Fig. 13.5a). This change commonly is limited to periportal hepatocytes (zone 1) but may be panlobular, or limited to zone 3 in certain circumstances related to gastrointestinal dysfunction (Fig. 13.5b). A form of simple steatosis is readily acquired in infants and children during periods of temporary caloric deprivation. Simple histiocytosis is common at autopsy of critically ill children who die in hospital intensive care units and occurs in a minority of infants who are diagnosed with sudden infant death syndrome. In these circumstances, lipid droplets are of small to medium size, often panlobular, and tend not to displace hepatocyte nuclei (Fig. 13.5c). Because coalescence of lipid into visible medium- and large-sized vacuoles is a dynamic process, hepatocytes containing medium- and large-sized lipid droplets often coexist. Nonetheless, the predominant physical form of lipid vacuoles provides a clue, albeit an imperfect one, to the tempo and the underlying cause of lipid accumulation.

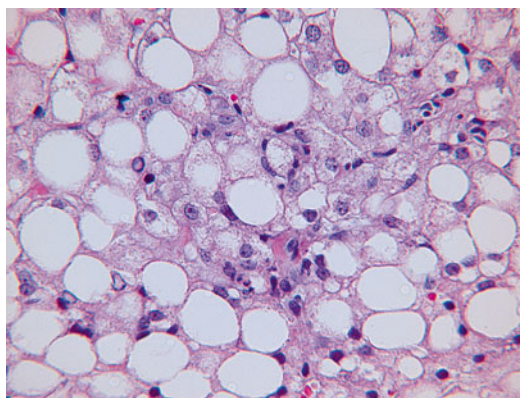
Inflammation and fibrosis typically are absent in simple steatosis except in cystic fibrosis where focal obstructive cholangiopathy may coexist.



**Fig. 13.5** (a) Simple steatosis. Cystic fibrosis with protein malabsorption. (b) Simple steatosis in GI tract disease. (c) Subacute simple steatosis at autopsy

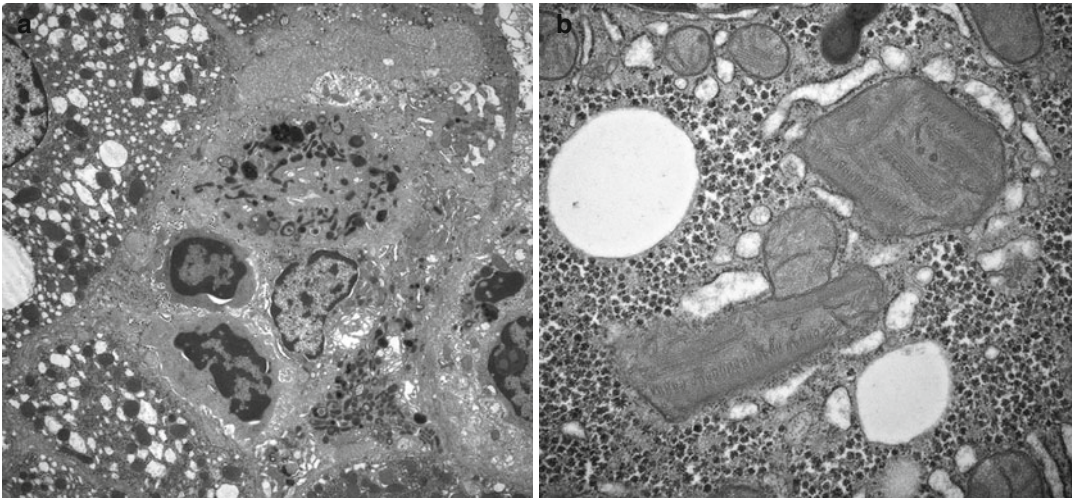
Because lipid has been extracted during processing, it can only be presumptively identified in paraffin sections based upon the sharp interface with surrounding hepatocyte cytoplasm. Storage lysosomes may have a similar sharp interface by light microscopy, but ultrastructure discloses a limiting membrane.

Simple steatosis may be the only change in nonalcoholic fatty liver disease (NAFLD), or these patients may have steatohepatitis (SH) (Schwimmer et al. 2005). NAFLD is linked to obesity, type 2 diabetes mellitus, and insulin resistance. Obese children who develop transaminasemia are commonly subjected to liver biopsy for the purpose of staging and grading the liver lesion (Kleiner et al. 2005; Brunt et al. 2011). SH usually can be distinguished from simple steatosis or steatosis due to a genetic defect in lipid metabolism by subtle lobular inflammation and active hepatocyte injury. Findings in SH in addition



**Fig. 13.6** Nonalcoholic steatohepatitis. Lobular inflammatory foci accompany fatty change. H&E stain

to macro- and microsteatosis include multifocal lobular inflammation, ballooned degenerate hepatocytes, and, especially in children, portal inflammation (Fig. 13.6). Mallory-Denk bodies



**Fig. 13.7** (a) Nonalcoholic steatohepatitis. Sinusoidal inflammatory cell aggregates are common. Hepatocytes exhibit mitochondrial pleomorphism and universal dilatation

of endoplasmic reticulum. EM. (b) Steatohepatitis. Stressed mitochondria contain paracrystalline matrix inclusions. EM

are more common in adults than children with SH. Ultrastructural changes in NAFLD are more commonly observed in SH than with simple steatosis. Excess smooth and rough endoplasmic reticulum are common but nonspecific. An array of focal mitochondrial changes presumably due to oxidative stress includes pleomorphism, matrix crystalloids, and occasional megamitochondria (Fig. 13.7). Persistent hepatocyte injury in NAFLD carries risk for progressive fibrosis but is reversible.

#### 13.4.5.1 Steatosis, a Confounding Variable

Hepatic steatosis may coexist as a confounding variable in many metabolic disorders, either because of poor nutrition, or because an underlying genetic defect secondarily interferes with lipid processing or utilization (Table 13.5). TPN initiated before a specific diagnosis is made may contribute to steatosis. In MCAD and in some aminoacidopathies such as isovaleric acidemia, hepatocyte lipid accumulation may be prominent at the time of a metabolic crisis. Large vacuolar lipid accumulation in hepatocytes is common in type I and type III glycogenosis and rarely is so extensive as to obscure the diagnostic histological features of glycogen excess (Fig. 13.8).

**Table 13.5** Metabolic disease and steatosis

##### *Steatosis a constant feature*

1. MCAD
2. LCAD
3. Galactosemia
4. Fructose intolerance
5. Citrin deficiency
6. Glycogen storage disease, types I and III
7. Abeta- and hypobetalipoproteinemia

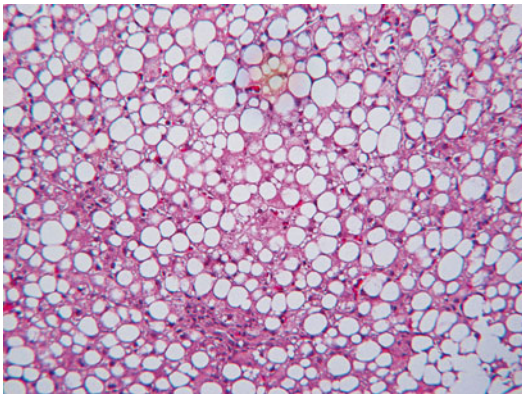
##### *Steatosis an inconstant feature*

1. Cystic fibrosis
2. Mitochondriopathy, especially congenital lactic acidosis
3. Urea cycle defects
4. Wilson disease
5. Aminoacidopathies
6. Glycogen storage diseases, other than types I and III

#### 13.4.5.2 Steatosis in Neonatal Hepatitis Syndrome

Many metabolic liver diseases that present in infants are routinely included in the lengthy differential diagnosis of direct hyperbilirubinemia due to “neonatal hepatitis,” but it is helpful to know that with the following exceptions prominent lipid vacuolation of hepatocytes is unusual in most disorders now recognized as specific causes of neonatal cholestasis with giant cell





**Fig. 13.8** Steatosis, a common feature in GSD I, may mask glycogen excess. H&E stain

transformation. Metabolic diseases presenting in young infants that often contain noteworthy amounts of lipid in hepatocytes include galactosemia; hereditary fructose intolerance; hepatic mitochondriopathies; urea cycle defects; Niemann-Pick A, B, and C; citrin deficiency; and lysinuric protein intolerance; in these disorders, lipid vacuoles of variable size may be accompanied by mild portal inflammation, progressive portal fibrosis, and liver failure. In all forms of Niemann-Pick disease, Kupffer cells are vacuolated as well as hepatocytes.

### 13.5 The Metabolic Disease Autopsy

When an infant or young child dies suddenly or after a brief hospitalization, without a specific diagnosis, and is suspected to have a metabolic disease, a common question is what investigations to pursue as part of the autopsy (Olpin and Evans 2004). Issues of cost and reimbursement eventually emerge, but the most essential component of any such investigation is availability of frozen tissue from heart, liver, and skeletal muscle and body fluid specimens such as urine, blood, bile, vitreous, and cerebrospinal fluid. Specimens collected during life and briefly retained in the clinical laboratory should be sequestered and frozen, along with those collected at autopsy. Additionally, a fibroblast

culture should be established pending a decision about further investigation. Cultured fibroblasts contain many enzymes that are deficient in metabolic disease and are an invaluable source of DNA. The final decision about how to proceed is best guided by the clinical and family history and by the histological findings. Microscopy of organs critical to the particular questions posed such as heart, lungs, liver, and skeletal muscle should be expedited to guide the investigation. If an alternate explanation for illness/death is not found, and if histological findings suggest the presence of a metabolic disease affecting one or more organs such as the liver or heart, urine carnitine and acylcarnitine profiles and serum amino acid profiles may be helpful. The results of neonatal screening should be reviewed. Consider using an autopsy blood spot on a Guthrie card to obtain a repeat screen at a government or private laboratory that has a comprehensive newborn screening program.

The most likely causes of lethal metabolic crisis are listed in Table 13.3. The yield for the metabolic autopsy has not been high except for cases of congenital lactic acidosis and fatty acid oxidation defects; only the latter are associated with sudden unexpected death. However, new applications such as targeted or whole exome sequencing may change the outcome by alerting caregivers to risk, should they become economical.

### 13.6 Fatty Acid Oxidation Defects

#### 13.6.1 Clinical Manifestations

More than a dozen disorders of fatty acid oxidation (FAO) have been recognized (Vockley and Whiteman 2002; Saudubray et al. 1999). Morbidity/mortality is highest during infancy. A metabolic crisis with hepatomegaly and nonketotic hypoglycemia is a typical presentation in medium-chain acyl-coenzyme A (CoA) dehydrogenase deficiency (MCAD), the most common FAO, but true hepatic failure is rare. Progressive cardiomyopathy and episodic rhabdomyolysis are less common presentations of FAO.

MCAD is the most common of the defects in FAO. The incidence is about 1/10,000 live births in Caucasians but is much less common in Asians based upon data from newborn screening programs (Horvath et al. 2008). Newborns with high C8-acylcarnitine levels have an increased frequency of the common 985A > G mutation. Early detection by screening improves outcome because it highlights the vulnerability to fasting and need for preventative measures, particularly during the first several years of life. However, genetic variants of uncertain significance have emerged from the screening data, and genotype-phenotype correlation remains uncertain (Lindner et al. 2010). In MCAD, an acute metabolic crisis with nonketotic hypoglycemia is precipitated by a febrile illness that causes loss of appetite, exposing the inability of affected children to metabolize lipid during brief starvation. MCAD is also associated with cardiac arrhythmias and sudden death, accounting for a small percentage of neonatal and unexplained infant deaths. FAO disorders may mimic epidemic post-viral Reye syndrome (RS) in clinical presentation, although the affected age groups substantially differ. Morphological manifestations may provide a basis for differentiation if liver tissue is available for both light and electron microscopy (Treem et al. 1986).

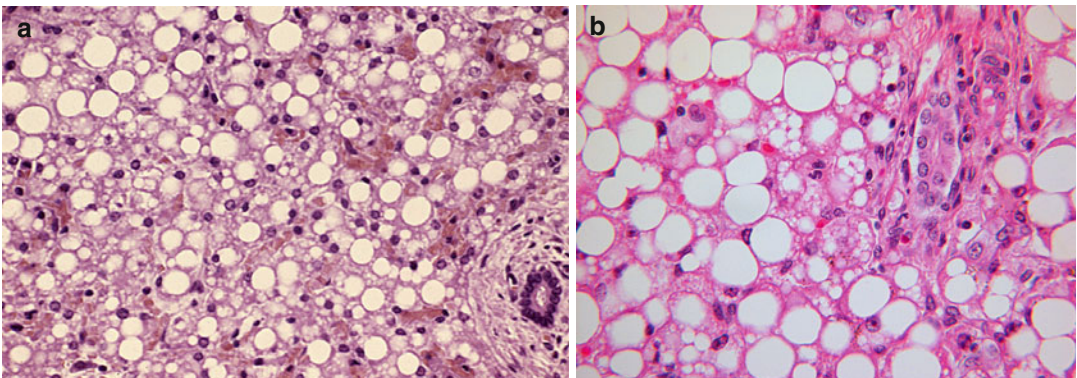
Long-chain acyl-CoA dehydrogenase deficiency results in progressive liver fibrosis, often with cardiomyopathy, myopathy, or pigmentary retinopathy. FAO defects or lipid transport defects

may result in a metabolic crisis in the immediate newborn period before feeding is initiated, as in the infantile forms of carnitine palmitoyltransferase two deficiency and carnitine-acylcarnitine translocase deficiency (Chalmers et al. 1997). Many cases of acute fatty liver of pregnancy are a manifestation of recessively inherited FAO defects in the fetus, the most common being mitochondrial trifunctional protein defect that impairs oxidation of long-chain fatty acids (Ibdah et al. 1999; Yang et al. 2002).

Several FAO defects, including medium-chain acyl-CoA dehydrogenase, long-chain acyl-CoA dehydrogenase, carnitine transporter defect, and carnitine translocase defect, have been implicated in a small number of sudden deaths in infancy and childhood.

### 13.6.2 Pathology

Based upon observations in liver biopsies and autopsy material, infants and children with defective FAO accumulate neutral lipid in organs most dependent upon FAO such as the liver, heart, proximal renal tubules, and type 1 skeletal muscle fibers. The lipid accumulation occurs predominantly in the form of microvesicular steatosis in which the lipid vacuoles indent but do not displace the nucleus (Fig. 13.9a, b); coexistent large droplet lipid is usually associated, but often a lesser component. In MCAD, the accumulation of lipid in hepatocytes may be transient



**Fig. 13.9** (a) Liver in MCAD with both micro- and macrovesicular steatosis. H&E stain. (b) Liver in LCAD with both micro- and macrovesicular steatosis. H&E stain

and may abate or disappear without permanent liver injury when homeostasis is restored. Analysis of urine for acylcarnitine compounds and establishment of a fibroblast culture are essential supplements to appropriate tissue samples when clinical suspicion is high. Limited published observations on antemortem liver samples suggest that ultrastructure of mitochondria in FAO are not distinctive in comparison to mitochondrial alterations in primary disorders of electron transport, mitochondrial DNA (mtDNA) depletion, and epidemic Reye Syndrome.

### 13.6.3 Diagnosis

Diagnosis is usually based upon a characteristic abnormal urine acylcarnitine profile determined by high-performance liquid chromatography (HPLC) and plasma acylcarnitine profile determined by fast atom bombardment mass spectrometry from Guthrie card bloodspots, organic acid profiles, and studies of fatty acid processing performed on cultured fibroblasts. Mutation analysis is available for the most common mutations in MCAD.

---

## 13.7 Mitochondriopathies

### 13.7.1 Clinical Manifestations

Mitochondriopathies are abnormalities of energy metabolism caused either by nuclear DNA mutations or mtDNA mutations/deletions that impair oxidative phosphorylation (OXPHOS) (Finsterer 2004; Dimauro 2011). Clinical manifestations in infants or children may involve one or more organs, often in clinically recognizable patterns, but the spectrum differs somewhat from those that affect adults (Bernier et al. 2002). Though enigmas remain, understanding continues to evolve as knowledge and experience expand and the difference between primary and secondary mitochondrial dysfunction due to other metabolic disturbances is clarified.

Mitochondriopathies first recognized, such as those characterized by ragged-red muscle fibers,

usually do not affect the liver. Most are caused by maternally inherited mtDNA mutations that typically affect one particular component of OXPHOS system. Clinical manifestations are usually delayed until the proportion of abnormal mitochondria in a given organ, usually skeletal muscle, exceeds a critical threshold that varies with metabolic demand. Large deletions in mtDNA cause Kearns-Sayre syndrome, a myopathy in which the liver is rarely involved, and Pearson marrow-pancreas syndrome. Pearson syndrome, the more common of the two in infants and young children, causes anemia, both exocrine and endocrine pancreas dysfunction, and liver disease that may be clinically significant (Williams et al. 2012). Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a multisystem disease that affects young adults but may begin in childhood; the liver is not usually involved (Garone et al. 2011).

Nuclear DNA (nDNA) mutations may affect one particular enzyme in the Krebs citric acid cycle, one subunit of the OXPHOS system, or generally impair mtDNA synthesis resulting in multiple defects in OXPHOS. The liver may be affected alone or in various combinations with brain, heart, kidney, skeletal muscle, or gastrointestinal tract.

Acute liver failure due to mitochondriopathy is rare and usually manifest in infants, many of whom have or will develop significant brain disease (Sarzi et al. 2007). One cause, deficiency of cytochrome oxidase (complex IV), associates with many phenotypes, one of which is congenital lactic acidosis, a systemic disease with devastating clinical manifestations that suggest multiorgan dysfunction. Another phenotype, GRACILE syndrome (intrauterine growth restriction, aminoaciduria, cholestasis, hepatic iron overload, and lactic acidosis), is due to mutation in BCS1L, a chaperon protein for complex III, that results in reduced levels of this electron transporter. Still another phenotype, Wolcott-Rallison syndrome, consists of neonatal-infantile diabetes mellitus, skeletal dysplasia, growth retardation, and intermittent liver failure. In these infants, complex I is deficient due to mutation in a nuclear gene, PRK-like ER kinase. Recent

studies show that infant liver failure is often caused by another mechanism, mtDNA depletion, that results from mutations in nuclear genes that control synthesis of most subunits of the OXPHOS system (Fellman and Kotarsky 2011). Examples of mutations in nuclear genes or nuclear-coded proteins that are reported to cause progressive hepatic or hepatocerebral mitochondriopathy in infancy are deoxyguanosine kinase, *POLG1*, *SUCLG1*, and *MPV17*. The phenotypic spectrum of *MPV17* mutation-related disease includes the Navajo neurohepatopathy (Holve et al. 1999; Karadimas et al. 2006).

Most, if not all, of the valproic acid-associated cases of acute liver failure in infants or children with seizures have an underlying mitochondriopathy that affects both liver and brain. Such patients are exceptionally vulnerable to valproic acid because it interferes with mitochondrial oxidation of fatty acids (Silva et al. 2008). Most cases of Alpers-Huttenlocher syndrome are mitochondriopathies in which brain involvement leads to early presentation as a seizure disorder (Gordon 2006). The well-known association of progressive liver and brain mitochondriopathy is the basis for the warning to avoid liver transplants in infants or children with liver failure unless brain involvement can reasonably be excluded.

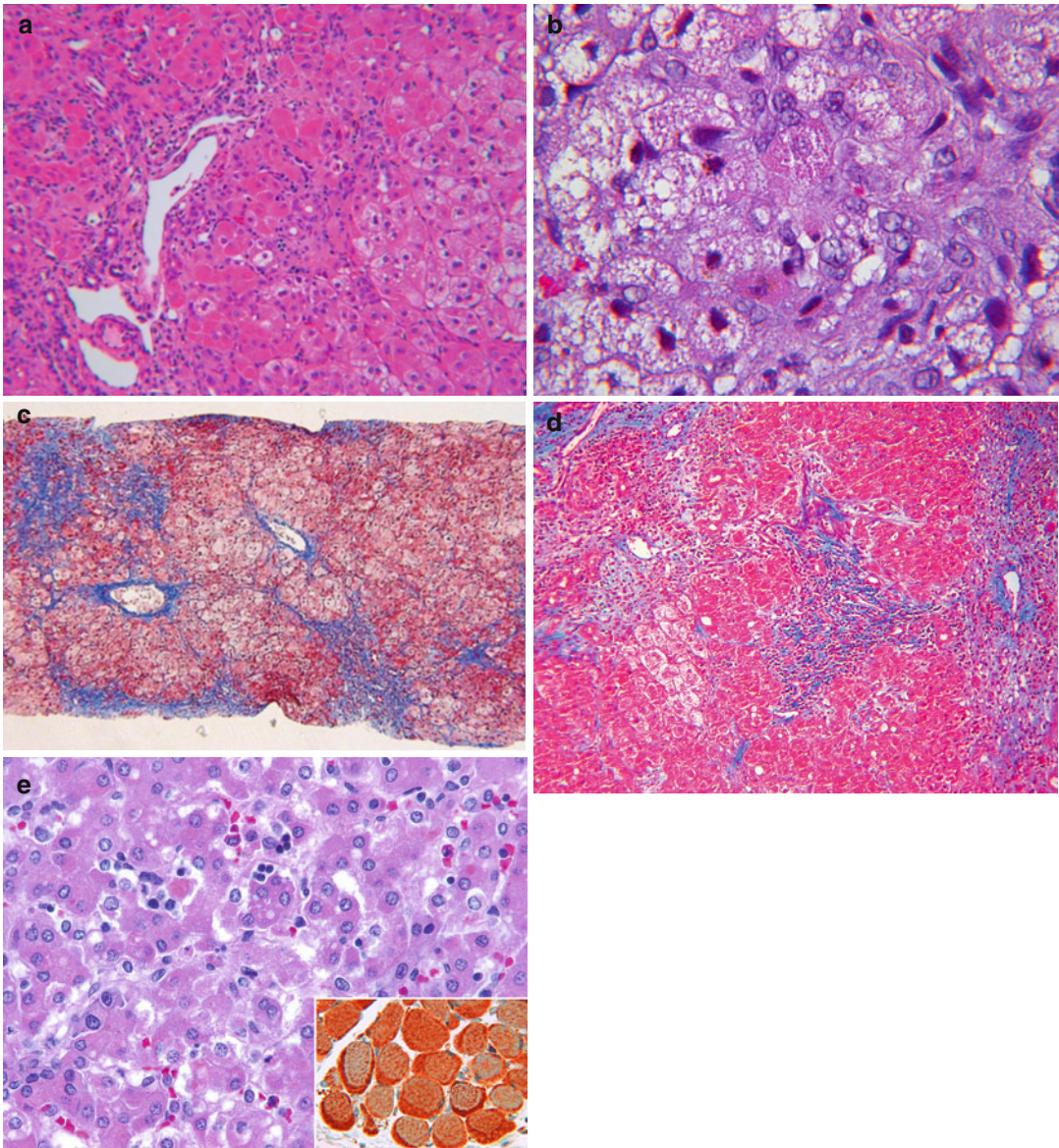
Mitochondrial hepatotoxicity of nucleoside reverse transcriptase inhibitors of DNA polymerase gamma may cause steatosis and lactic acidemia. These complications of therapy for chronic viral infections are rare in children perhaps because long-term exposure is necessary, but have been reported in infants exposed during pregnancy.

### 13.7.2 Pathology

Liver histological features at the time of liver failure due to a primary mitochondriopathy vary depending on the age of onset and stage of the disorder. Typical changes in older infants at the time of liver failure include patchy, sometimes extensive micro- and macrovesicular steatosis, intralobular cholestasis, finely granular bright

red hepatocytes isolated or clumped in groups that contain excessive numbers of mitochondria, swollen hepatocytes that lack lipid droplets, scattered necrotic hepatocytes, foci of intralobular regeneration and collapse, and mixed portal/lobular inflammation accompanied by progressive portal fibrosis (Fig. 13.10a–d). Oxidative enzyme histochemistry applied to cryostat sections of the liver may be helpful in recognition of mitochondriopathy. Selective reduction or hyperintensity of histochemical reactions for cytochrome oxidase and succinic dehydrogenase, normally uniform in cryostat sections of liver, may be observed (Ibdah et al. 1999). In the liver, as well as in organs such as the heart, mitochondriopathy may induce generalized proliferation of mitochondria despite functional deficiency. The generic histological phenotype described above may be absent in fulminant mitochondriopathies that cause lethal lactic acidosis in the newborn period (Fig. 13.10e). Immunohistochemical stains may be useful to demonstrate mitochondrial hyperplasia.

Distinctive ultrastructural mitochondrial abnormalities are reported in many infants and young children with proven mitochondriopathy (Mandel et al. 2001; Labarthe et al. 2005; Wong et al. 2007). These include pleomorphism of size and shape, increased or decreased numbers of mitochondria per hepatocyte, bizarre dilatation of cristae, and expansion of matrix that displaces cristae as it accumulates causing mitochondrial enlargement that may be spectacular. Matrix may be pale or dense (Fig. 13.11a–d). Similar morphological changes occur in acute liver failure associated with what formerly were called “idiosyncratic” drug reactions to valproic acid used to treat seizures in infants or in older children. Many of these patients have an unrecognized genetic mitochondriopathy that causes mDNA depletion. Curiously, intra-cristal paracrystalline inclusions of the type that are common in the ragged-red fibers of mitochondrial myopathy are rarely, if ever seen in mitochondrial hepatopathy. Whether mitochondrial ultrastructural changes may be specific for particular defects requires more observation.

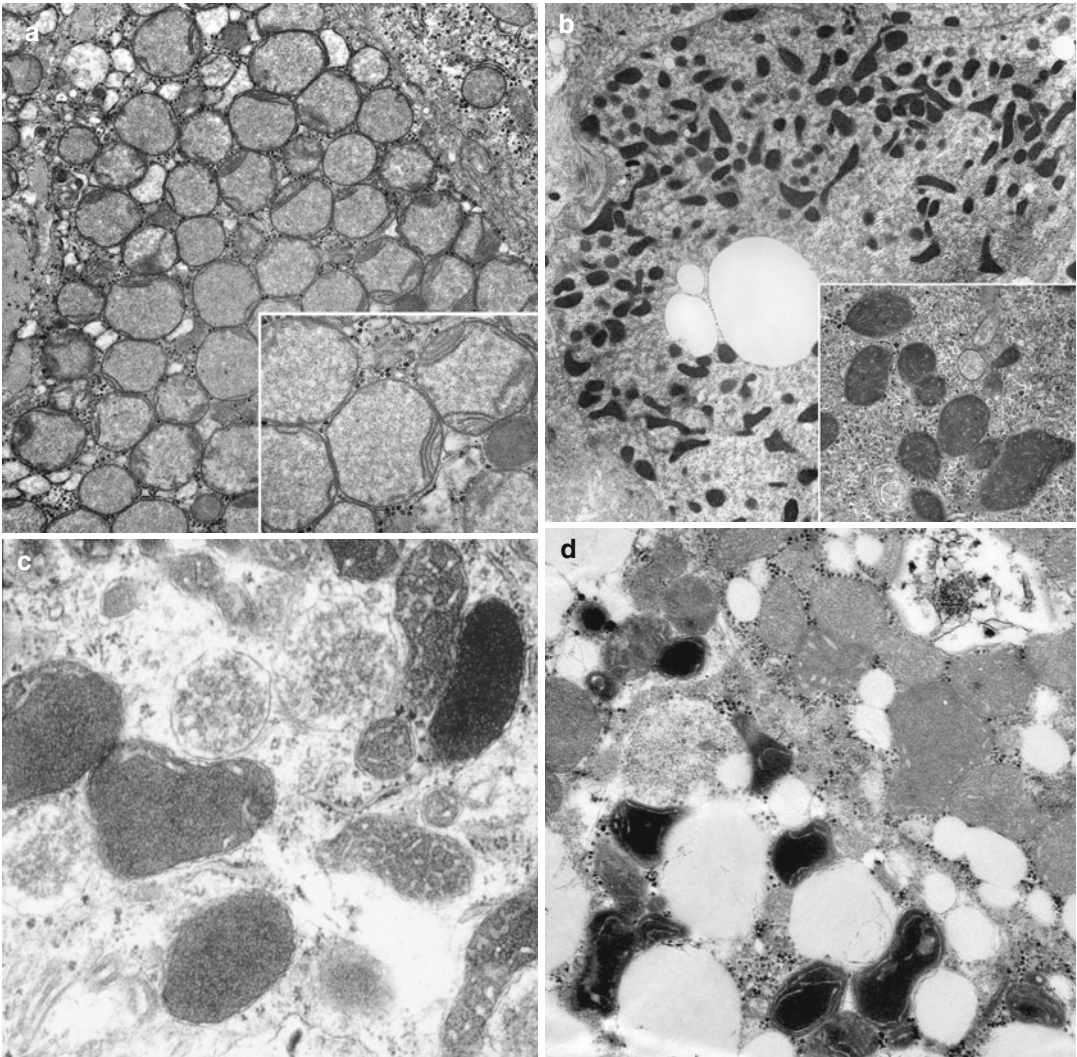


**Fig. 13.10** (a) Mitochondriopathy. mDNA depletion in late infancy. Patches of eosinophilic faintly granular hepatocytes are intermixed with swollen hepatocytes. Lipid is uncommon. Portal and lobular inflammation are mild. H&E stain. (b) Mitochondriopathy, MPV17 defect. Microvesicular steatosis is prevalent. Two isolated granular red hepatocytes contain excess mitochondria. H&E stain. (c) Mitochondriopathy, MPV17 defect with swollen hepatocytes and minimal steatosis. Note periportal fibrosis

with early bridging and pericellular fibrosis. Trichrome stain. (d) Mitochondriopathy, valproate-induced acute liver failure. Periportal fibrosis is flanked by areas of zone 3 collapse. Trichrome stain. (e) Congenital lactic acidosis. Universal fine granular hepatocyte cytoplasm is due to mitochondrial hyperplasia, present also in heart and skeletal muscle. Lipid vacuoles are a minor feature. H&E stain. *Inset*: massive accumulation of mitochondria in diaphragm muscle verified by immunohistochemical stain

The range of ultrastructural changes in primary mitochondriopathy appears to differ from changes due to nonspecific oxidative metabolic

stress that accompany ethanolism, nonalcoholic steatohepatitis, urea cycle disorders, bile acid synthesis defects, extrahepatic portal vein



**Fig. 13.11** (a) Mitochondriopathy in subacute liver failure in late infancy due to mtDNA depletion. Mitochondrial matrix is pale, flocculant, and expanded and displaces cristae to periphery. EM. (b) Mitochondrial pleomorphism in multiacyl-CoA dehydrogenase deficiency. *Inset:* pleomorphic mitochondria contain dilated cristae, coarsely granular matrix, and increased dense granules.

EM. (c) Mitochondriopathy in valproate-induced liver failure. Mitochondrial pleomorphism, variable accumulation of dense matrix and dilatation of cristae. EM. (d) Mitochondriopathy in acute liver failure due to MPV17 defect and mtDNA depletion. Wide range of mitochondrial abnormality includes pleomorphism, variable accumulation of abnormally dense matrix, and dilatation of cristae. EM

obstruction, and rarely in glycogenosis. These changes include normal mitochondria with mild pleomorphism, scattered pale swollen mitochondria, paracrystalline matrix inclusions, and megamitochondria (Figs. 13.7 and 13.14d).

### 13.7.3 Diagnosis

Criteria for clinical and laboratory diagnosis of mitochondriopathy in the context of neuromuscular disease with or without multiorgan

involvement are not perfect (Gordon 2006). These criteria are even less helpful when mitochondrial disease primarily affects one or more visceral organs such as the liver. Skeletal muscle is commonly affected in multiorgan mitochondrial pathologies but does not always exhibit diagnostic changes by light or electron microscopy. Nonetheless, muscle biopsy is convenient and, properly triaged and processed, has a reasonably high yield as a screening test for biochemical and molecular genetic disorders (nDNA or mtDNA). Muscle biopsy may be useful when clinical signs indicate primary liver involvement alone or in conjunction with central nervous system disease.

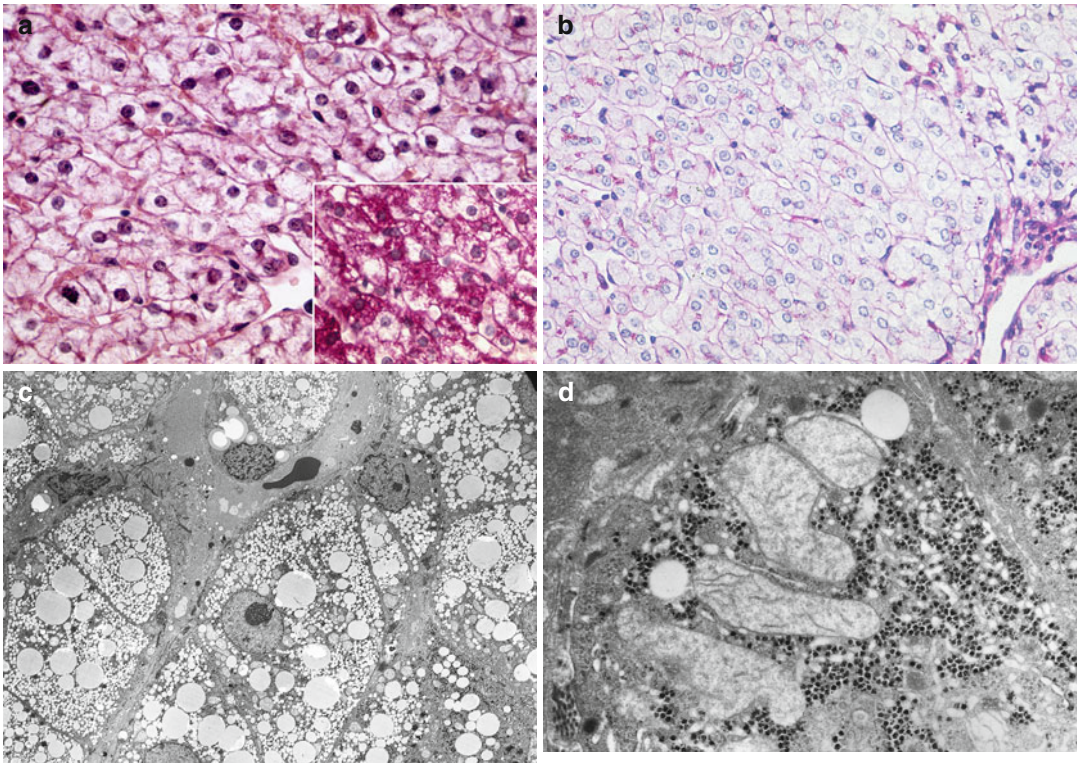
Light and electron microscopic findings in needle biopsy samples of the liver often create suspicion for mitochondrial hepatopathy and may occasionally be diagnostic though not specific for a particular genetic entity. mtDNA content may be assessed in a needle biopsy specimen. Specific mutations in nDNA that cause mtDNA depletion and liver failure in infants or young children can now be assayed in blood leukocyte DNA. However, open liver biopsy may be needed for a complete assessment that includes light and electron microscopy, measurement of electron transport activities, mtDNA content, and search for mtDNA deletions or point mutations. With the advent of mitoxome and whole genome sequencing, DNA from the liver, cultured fibroblasts and leukocytes will modify the role of liver biopsy.

#### 13.7.4 Reye Syndrome (RS)

RS is an acute liver dysfunction with encephalopathy and fatty degeneration of the liver. RS occurs in two clinical guises. One form affects older, previously well children, peaked between 1970 and 1985 and has almost disappeared. The other form mainly affects infants and young children, as a complication of an underlying metabolic disorder. The former was a transient post-viral acute encephalopathy associated with fatty degeneration of hepatocytes in which

microvesicular steatosis developed in the wake of a viral illness along with signs of brief transient failure of hepatic synthetic function, hyperammonemia, and mild to moderate elevation of serum aminotransferases. Clear evidence of association of RS with epidemics of chicken pox and influenza B was followed by epidemiological evidence linking the syndrome to low-therapeutic levels of salicylate. Mortality and long-term morbidity due to brain injury was about 5%. Both light and electron microscopy of liver tissue obtained early in the course of the disease were distinctive and established the basis as a hyperacute emergence (phanerosis) of microvesicular fat as a marker for acute liver failure due to a transient reversible mitochondrial pathology (Partin et al. 1971; Bove et al. 1975). Concomitant glycogen depletion was often severe, accounting for hypoglycemia. The hepatocytes in the early phase are swollen and contained few visible lipid droplets in paraffin sections (Fig. 13.12). However, abundant microvesicular lipid, apparently concealed during rapid evolution of the hepatopathy, was demonstrable in fat stains on frozen sections. Zonal necrosis was absent, but apoptosis was often seen. Histochemical studies showed depletion of succinic dehydrogenase and cytochrome oxidase. Ultrastructural changes in mitochondria were distinctive and remain so in retrospect, suggesting kinship to the “membrane permeability transition” pathway. The biology of the relationship of RS to low blood levels of salicylate remains mysterious, and the validity of the statistical link, though generally accepted, has been questioned.

With decline of aspirin usage as an antipyretic in children, RS has almost, but not completely, disappeared in the United States (Belay et al. 1999) except in the context of acute decompensation of an underlying metabolic disorder of energy metabolism typically in infants or very young children. In such cases, the distinctive ultrastructural changes of mitochondria in epidemic RS are absent (Garone et al. 2011). Skepticism about the existence of



**Fig. 13.12** (a) Reye syndrome. Transient encephalopathy. Swollen hepatocytes without obvious vacuolation contain concealed lipid. H&E stain. *Inset*: mild glycogen depletion. PAS stain. (b) Reye syndrome. Severe encephalopathy. Swollen hepatocytes contain massive amounts of concealed lipid and no stainable glycogen. PAS stain. (c)

Reye syndrome, with abundant microvesicular lipid visible in resin-embedded section. Toluidine blue-Azure blue stain. (d) Reye syndrome, enlarged swollen amoeboid mitochondria with pale watery matrix and displaced cristae coexist with preserved glycogen. EM

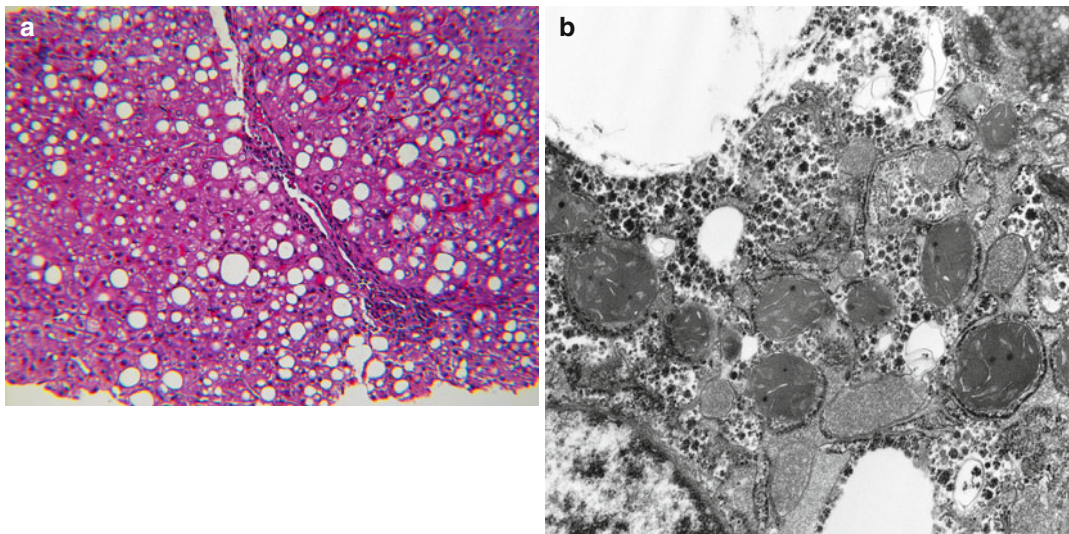
RS except in the context of a specific metabolic disorder is based upon advances over the past 30 years in diagnostic techniques that were not available at the height of the epidemic. However, this argument is unconvincing given the prevalence of epidemic RS in older children and rarity of second episodes in the vast majority who survived.

### 13.7.5 Wilson Disease (WD)

WD is an autosomal recessive disorder of copper transport due to mutations in the *ATP7B* gene that results in copper overload in the liver, progressive liver disease, and degeneration of central brain nuclei often with onset in childhood. Laboratory criteria for diagnosis are low

serum ceruloplasmin, elevated 24-h urine copper, and high hepatic tissue copper levels, but may not be definitive (Nicastro et al. 2010). Early signs in children such as elevated serum transaminases and/or hepatomegaly or acute onset of liver failure may prompt an investigation that includes liver biopsy. Liver histology ranges from normal to chronic hepatitis with or without steatosis, often prominent nuclear glycogenation, or submassive necrosis. The rhodanine copper stain identifies copper within secondary lysosomes and is widely used but not specific, because copper accumulates nonuniformly in WD and may also be detected in other conditions, particularly if cholestasis is a confounding factor. Liver ultrastructure may be helpful showing prominent mitochondrial changes such as tubular dilatation of cristae and





**Fig. 13.13** (a) Wilson disease. Chronic hepatitis with steatosis. H&E stain. (b) Wilson disease. Abnormal mitochondria with increased matrix density and dilated

cristae. Numerous normal peroxisomes and alpha granules of glycogen in the background. EM

matrix inclusions, as well as unusually complex membrane-bound insoluble lipid inclusions, dubbed lipolysosomes (Fig. 13.13a, b). The mitochondrial changes in Wilson disease overlap with those seen in primary mitochondriopathies and also with those seen in acquired conditions such as steatohepatitis where oxidative stress may be causative. Evidence from humans and animal models of genetic and acquired copper toxicity suggests that copper localizes in and promotes oxidant injury to mitochondria thereby playing an essential role in progressive liver and brain damage in Wilson disease (Sokol et al. 1994; Zischa et al. 2011).

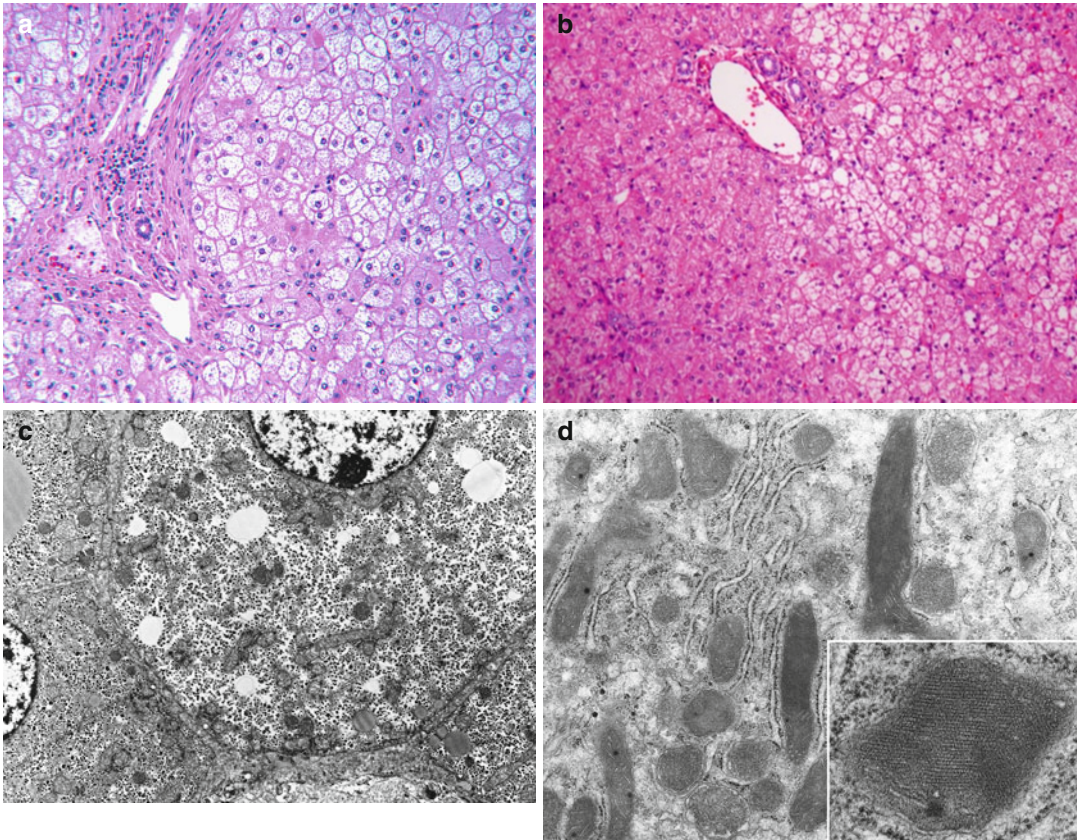
related disorders that cause hyperammonemia are lysinuric protein intolerance and citrin deficiency, the latter a disorder prevalent in ethnic East Asian adults that causes type II citrullinemia. N-acetylglutamate synthetase, carbamoyl phosphate synthetase, and ornithine transcarbamylase are located in the mitochondrial matrix; deficiency impairs urea synthesis by reducing mitochondrial citrulline production. Argininosuccinic acid synthetase and argininosuccinate acid lyase, located in the cell cytoplasm, are necessary for the final assembly of urea.

## 13.8 Urea Cycle Defects (UCD)

UCD are inherited enzyme defects that impair nitrogen elimination resulting in hyperammonemia and encephalopathy (Tuchman et al. 2008; Summar et al. 2008). The cycle includes six enzymes: ornithine transcarbamylase, argininosuccinic acid synthetase (type I citrullinemia), argininosuccinate acid lyase (“argininosuccinic aciduria”), arginase, carbamoyl phosphate synthetase, and N-acetylglutamate synthetase. Two

### 13.8.1 Clinical Manifestations

Signs and symptoms of UCD may be present at birth or delayed until later infancy, childhood, and beyond and are often episodic, varying with the defect, protein content of the diet, and stress of intercurrent illness. Recurrent vomiting and somnolence are typical. All are autosomal recessive traits with the exception of OTC deficiency, the most common, which is x-linked and produces symptomatic carriers who may have lethal acute liver failure. Hepatomegaly is common in UCD.



**Fig. 13.14** (a) Urea cycle defect (argininosuccinic lyase defect). Glycogen excess in acute liver failure. (b) Urea cycle defect (ornithine transcarbamylase (OTC) defect). Focal glycogenosis. (c) Urea cycle defect (OTC).

Glycogen excess causes dispersion of mitochondria. EM. (d) Urea cycle defect (OTC). Mitochondria vary from normal to elongated; several contain matrix paracrystalline inclusions. *Inset*: detail of matrix inclusion. EM

### 13.8.2 Pathology

The histology and ultrastructure of liver in these disorders may be normal or abnormal. Liver biopsy is not necessary as laboratory diagnosis, biochemical or genetic, usually suffices. It is likely that the described changes in liver morphology are dependent more upon the status of the patient at the time of biopsy, such as hyperammonemic crisis or nutritional state, than upon the particular defect. Light microscopy may be normal when ammonia levels are normal, or in explanted livers. More often seen are macro- and/or microvesicular steatosis, cytoplasmic glycogen excess, and variable portal fibrosis. Intralobular cholestasis and hepatocellular necrosis are inconstant features. Excess glycogen accumulation in nonfatty livers from patients

with UCD may resemble a nonlysosomal glycogen storage disease (Fig. 13.14a) and, in some instances, takes the form of quasiclonal clusters of glycogen-laden hepatocytes (Fig. 13.14b) (Badizadegan and Perez-Atayde 1997; Miles et al. 2005). Hypothetical etiologies for glycogen accumulation in UCD abound reflecting incomplete understanding of the altered biochemistry. These include gene expression altered by the lobular microenvironment, effect of hyperammonemia on glycolysis, and/or a low-protein/carbohydrate-rich diet. Fibrosis may progress to cirrhosis despite control of hyperammonemia (Mori et al. 2002).

When glycogen excess is present, electron microscopy reveals that glycogen granules usually are well admixed with subcellular organelles (Fig. 13.14c) without displacement of

organelles to the cytoplasmic periphery as tends to be true for nonlysosomal glycogenoses. One may also see admixed normal and polymorphous mitochondria with occasional megamitochondria, shortened cristae, and paracrystalline matrix inclusions (Fig. 13.14d) (Latham et al. 1984). Such changes are observed in several other conditions (alcoholism, cavernous transformation of portal vein, steatohepatitis) and are best thought of as nonspecific indicators of mitochondrial stress. Importantly, mitochondrial ultrastructural changes typical for epidemic RS, a hyperammonemic state in which only the two urea cycle enzymes located in mitochondria are selectively impaired, are absent (Brown et al. 1976).

### 13.8.3 Diagnosis

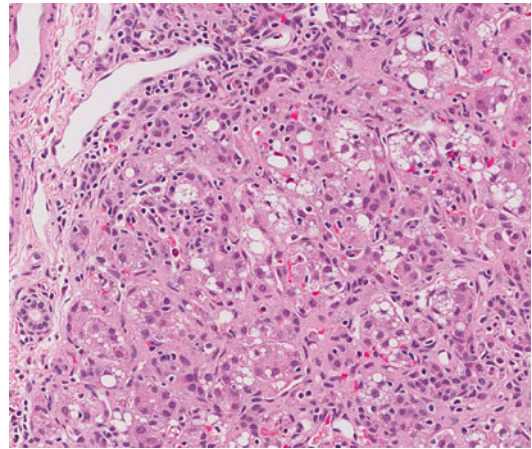
Liver tissue can be used for diagnosis by measuring enzyme activity, but diagnosis usually is made by biochemical and/or genetic tests performed on body fluids.

## 13.9 Citrin Deficiency

Citrin is a hepatic mitochondrial aspartate-glutamate carrier protein. Mutations cause type II citrullinemia in adults or cholestasis in infants. Both forms are most prevalent in ethnic East Asians. Affected infants have hyperammonemia, hypoproteinemia, and, in some cases, galactosemia. The liver histology in young infants with citrin deficiency may overlap with features of “neonatal hepatitis” but differs because of the prevalence of steatosis. The histology, reported in small numbers of cases consists of macrovesicular steatosis, lobular cholestasis, prominent acinar transformation, and progressive pericellular fibrosis (Fig. 13.15) (Yeh et al. 2006). These changes may regress with dietary management.

## 13.10 Galactosemia

Galactosemia is an autosomal recessive disorder caused by a deficiency of galactose-1-phosphate uridylyltransferase activity. Galactose cannot be

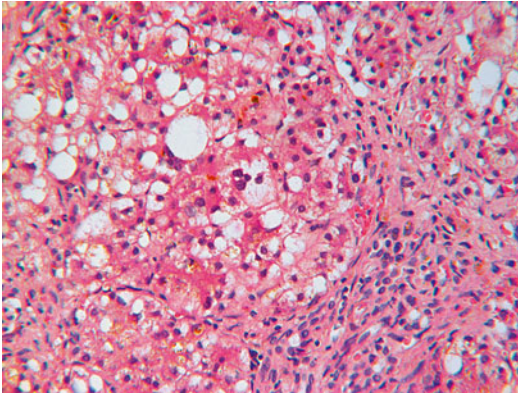


**Fig. 13.15** Citrin deficiency in infant. Combined micro- and macrovesicular steatosis, acinar transformation of liver cell plates, smoldering inflammation, and pericellular fibrosis are shown. H&E stain (Used with permission of Grace E. Kim, UCSF)

metabolized by about 1/10,000 infants, in whom it is particularly toxic to the liver and the eye (Bosch 2006). All newborn screening programs are designed to detect galactosemia; the diagnosis is confirmed by enzyme assay in erythrocytes. The most common severe form of galactosemia presents as feeding difficulties, jaundice, hepatosplenomegaly, and growth failure. Liver biopsy findings evoke the usual broad differential diagnosis of a neonatal hepatitis-like pattern without signs of a cholangiopathy. Features such as frequent acinar arrangements of hepatocytes, fibrosis, and fatty change suggest a metabolic disease but are not specific (Fig. 13.16). Restriction of dietary galactose ameliorates signs of systemic disease. Recognition and treatment may prevent cirrhosis, cataracts, and neurological deterioration.

## 13.11 Hereditary Fructose Intolerance

Fructose, like galactose, a normal nutrient for most persons, is a noxious sugar for about 1/20,000 infants. The most common and most severe form of fructosemia is due to fructose B aldolase deficiency (Ali et al. 1998). Foods containing fructose cause hypoglycemia, signs



**Fig. 13.16** Galactosemia. Steatosis, lobular cholestasis, acinar clusters, and portal fibrosis are notable features. H&E stain

of liver injury, and growth failure in affected infants. The severity of symptoms correlates with the fructose content of the diet and duration of exposure. Liver damage may be severe resulting in cirrhosis and liver failure. A neonatal hepatitis-like pattern is uncommon, probably because recognition is usually delayed. Liver biopsy findings are more likely to be limited to fatty change and fibrosis without evidence of cholangiopathy (Fig. 13.17a). At the level of ultrastructure, autophagy may be extensive (Fig. 13.17b). These changes typically reverse when dietary fructose is withdrawn (Fig. 13.17c).

### 13.12 Lysosomal Storage Disorders

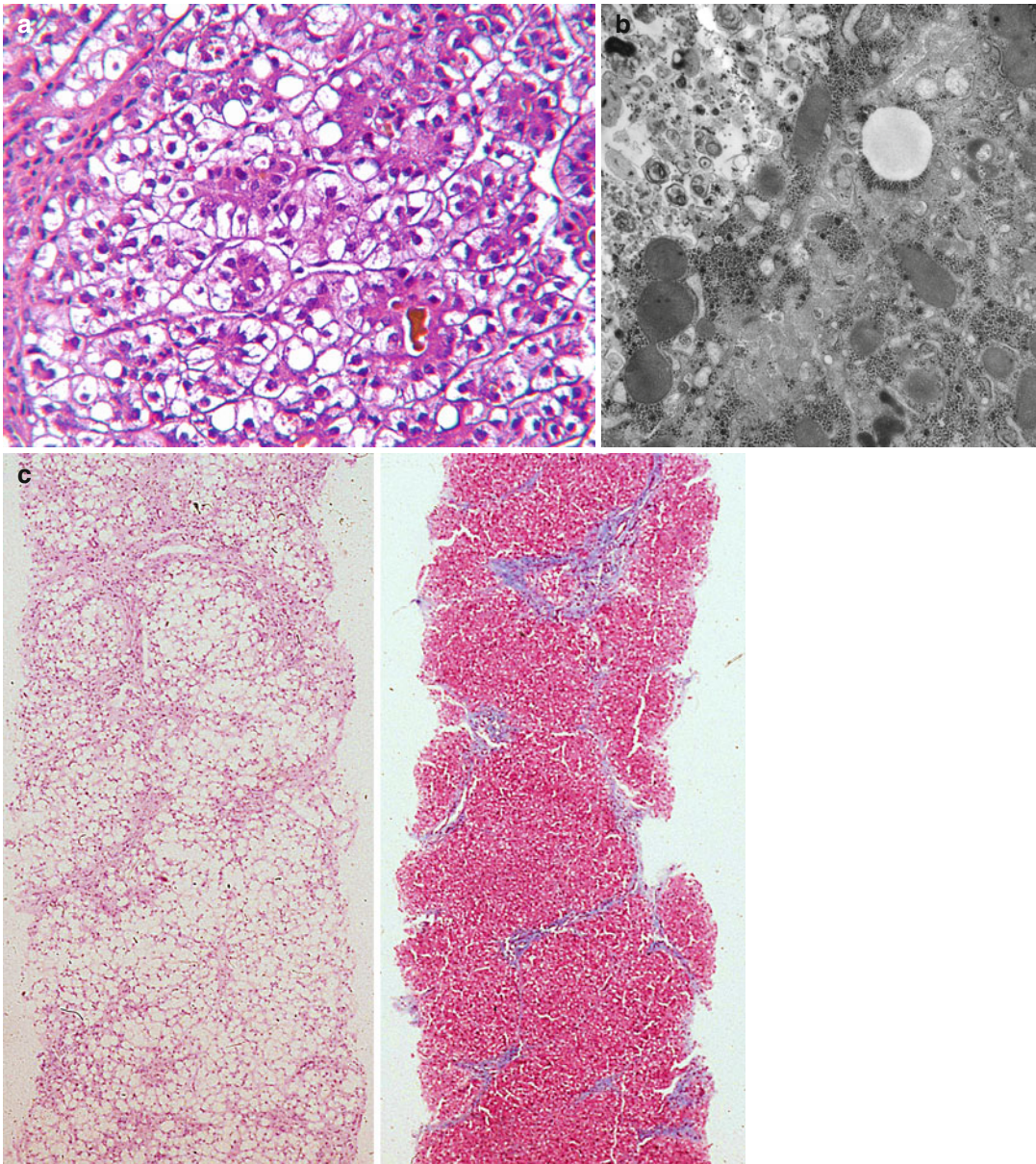
More than 50 lysosomal storage disorders (LSD) are known (Filacamo and Morrone 2011). Most result from deficient or absent activity of specific acid hydrolases. Several, such as sialidosis and cystinosis, are due to defective transport of products of lysosomal enzyme activity. As the molecular genetic bases are elaborated, the original entities are evolving into a multiplicity of genotypes and phenotypes produced by different mutations in genes encoding enzyme protein or mutations in genes that control transcription and intracellular trafficking of gene products (Koprivica et al. 2000). The resulting phenotypic variability is expressed in rate of disease

progression or in severity of clinical and tissue manifestations in a particular organ such as the liver.

Common disease-specific clinical patterns of organ involvement guide clinicians and pathologists during a diagnostic evaluation. Involvement limited to liver suggests the possibility of type I or type VI glycogenosis or cholesterol ester storage disease. Involvement limited to the liver, spleen, bone marrow, and lymph nodes suggests a reticuloendothelial storage disorder such as Gaucher disease or type B Niemann-Pick disease. Generalized lysosomal storage disorders that involve multiple organ systems including the nervous system are type II glycogenosis; mucopolysaccharidoses I, II, and III; GM1 gangliosidosis; Niemann-Pick disease types A and C; and the neuropathic form of Gaucher disease. Leukodystrophy due to lysosomal enzyme defects has major clinical manifestations limited to the central nervous system, but peripheral nerves are involved in metachromatic leukodystrophy. In mucopolysaccharidosis, hepatocyte involvement produces few clinical manifestations; in Fabry disease, the hepatocytes are not involved at all. Progressive hepatic fibrosis is unusual in lysosomal storage disorders except for Gaucher disease, Niemann-Pick disease type C, and unpredictably in both Niemann-Pick disease type B and cholesterol ester storage disease.

Gaucher disease, the most common lysosomal storage disorder, is a model for the complexity that has resulted from phenotypic and genotypic investigations in recent years. Gaucher disease has three major forms: type 1 is highly variable in severity and rate of progression, may first manifest at any age, and lacks involvement of the central nervous system; type 2 is the acute infantile neuropathic form; and type 3 is the subacute neuropathic form. All are due to a deficiency of glucocerebrosidase activity. Mutational analysis has revealed a high rate of mutation as well as more than 100 different mutations in the affected gene. Thus far, phenotype-genotype correlation is insufficient to reliably categorize patients based upon molecular analysis (Koprivica et al. 2000).

Modification of phenotype in humans, dramatic in some cases, has now been achieved by



**Fig. 13.17** (a) Fructose intolerance. Steatosis with extreme acinar transformation of hepatocyte plates and canalicular cholestasis. Creeping periportal fibrosis is shown at left of image. H&E stain. (b) Fructose intolerance. Adjacent

hepatocytes exhibit focal and extreme autophagy, respectively. Mitochondria are normal. EM. (c) Fructose intolerance. Liver biopsy cores obtained before and after removal of fructose from diet. H&E stain

effective enzyme replacement therapy for three lysosomal storage disorders: type 1 Gaucher disease (Grabowski et al. 2004), type IIa glycogen storage disease (van der Ploeg and Reuser 2008), and Fabry disease (Ries et al. 2005). More

an example such as cholesterol ester storage disease and Niemann-Pick disease type B are under study (Thurberg et al. 2012). Allogeneic bone marrow transplant has been effective in LSD that primarily affects the reticuloendothelial system.

### 13.12.1 Diagnosis

Diagnostic alternatives to liver biopsy are plentiful for the clinician who suspects a lysosomal storage disorder based upon family history, clinical presentation, pattern of organ involvement, radiological features, or examination of leukocytes in a blood smear. These include biochemical or genetic testing of cultured skin fibroblasts, amnion epithelium, or leukocytes and diverse sources of either lysosomal enzymes or nDNA or using skin, conjunctive, amnion, and skeletal muscle for electron microscopy. The liver is likely to be subjected to biopsy only when hepatomegaly is a dominant feature at the time of initial examination.

### 13.12.2 Pathology

It is important to determine which of the following cell types display features of storage: hepatocytes, Kupffer cells, endothelial cells, portal macrophages, or fibroblasts. Kupffer cells and portal macrophages are selectively involved in lysosomal disorders involving the reticuloendothelial system, because the lysosomal degradation pathway is extremely active in macrophages. Gaucher disease type I is an example of pure reticuloendothelial storage disease.

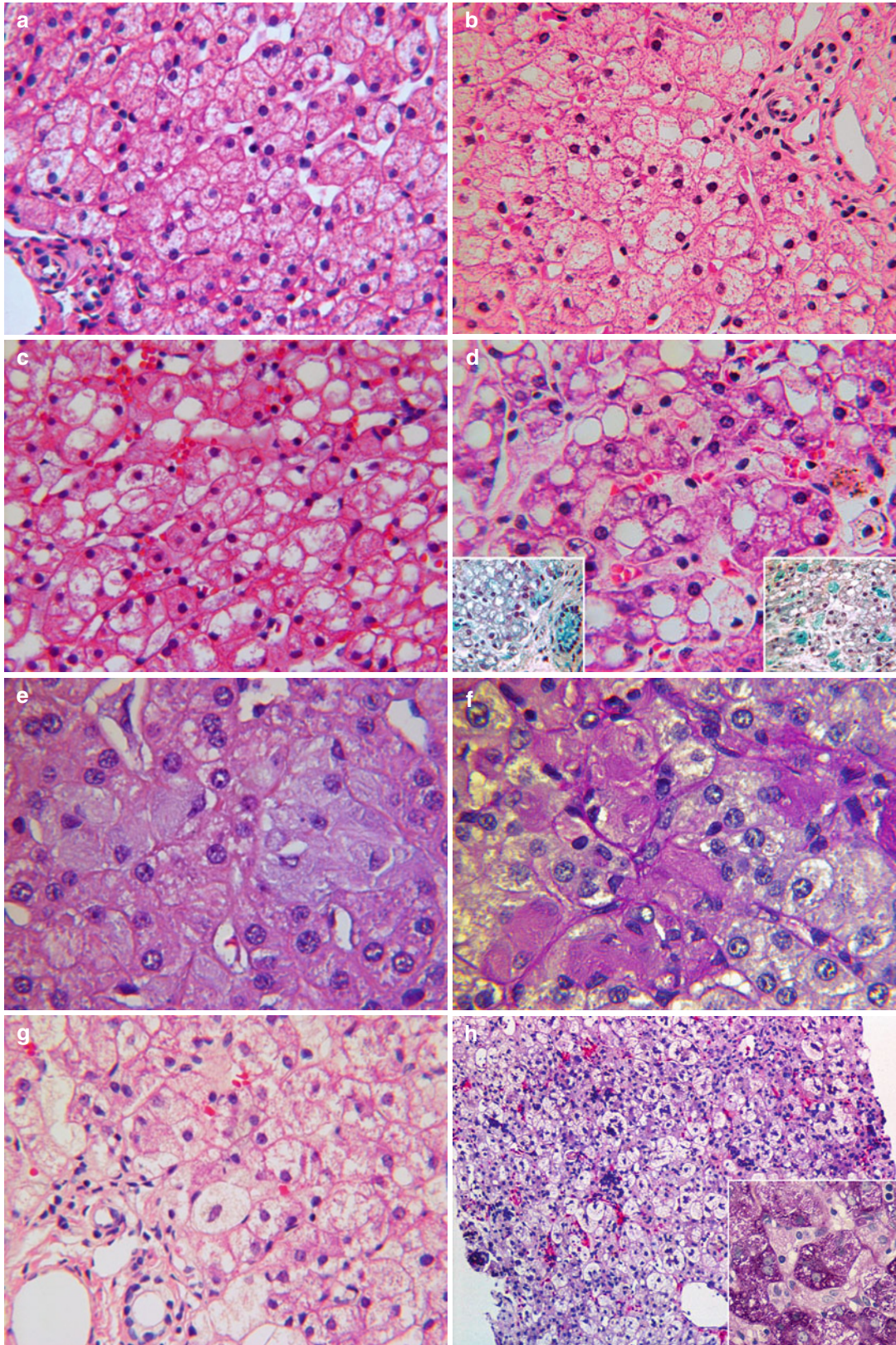
Storage lysosomes in the cytoplasm of hepatocytes create a characteristic light microscopic appearance of abnormal clarity, often with a lacy pattern created by numerous tiny clear empty-appearing vesicles that may mimic microvesicular steatosis (Fig. 13.18a–d). The possibility of lipid vesicles alone or intermixed with storage

lysosomes can be investigated by performing a neutral fat stain on a small sample of the biopsy that has been frozen fresh for histochemical and biochemical study. Vesicles may also be identified as lysosomes by demonstration of acid phosphatase activity in frozen sections or using an immunostain for LAMP1. Normal lysosomes are concentrated in the apical cytoplasm, whereas activity is observed throughout the cytoplasm in lysosomal storage disease. In many LSD, the partially degraded storage material is readily dissolved in aqueous fixatives or lost during processing; the lysosomal vesicles may appear to be empty by light microscopy and, to some extent, by electron microscopy as well. In such cases, biochemical or genetic confirmation is required. The stored material is especially apt to be lost in processing in mucopolysaccharidoses and disorders of glycoprotein degradation such as mannosidosis and fucosidosis. Conversely, stored material composed of glycogen or complex glycolipids tends to survive. An example is Gaucher disease where the storage product is insoluble and retained in paraffin sections as weakly PAS-positive material that has a distinctive “crinkled paper” appearance; Gaucher storage material in the liver is confined to the Kupffer cells and portal macrophages (Fig. 13.18e, f).

When concern for metabolic disease narrows to specific concern for LSD based on light microscopy, having prepared a small portion of the biopsy sample for electron microscopy enables the pathologist to verify that the vesicles are defined by a unit membrane and to characterize the storage product, thereby narrowing the range of diagnostic possibilities. The membrane-bound vesicles in LSD typically contain a monomorphous

**Fig. 13.18** (a) Glycogen storage disease. Type II (Pompe disease). Liver cells with slightly lacy cytoplasm are minimally expanded and sinusoids are patent as a result. Inflammation is absent. H&E stain. (b) Hurler disease (MPS type I). Liver cells and Kupffer cells are variably expanded due to the presence of vacuoles of variable size. Stainable lipid was absent. H&E stain. (c) GM1 gangliosidosis. Vacuoles of variable size are present in hepatocytes and Kupffer cells. H&E stain. (d) Sialidosis (autopsy). Small vesicles are universal in hepatocytes and Kupffer cells. H&E stain. (e) Gaucher disease. Kupffer cells are massively expanded by the presence of crystallized

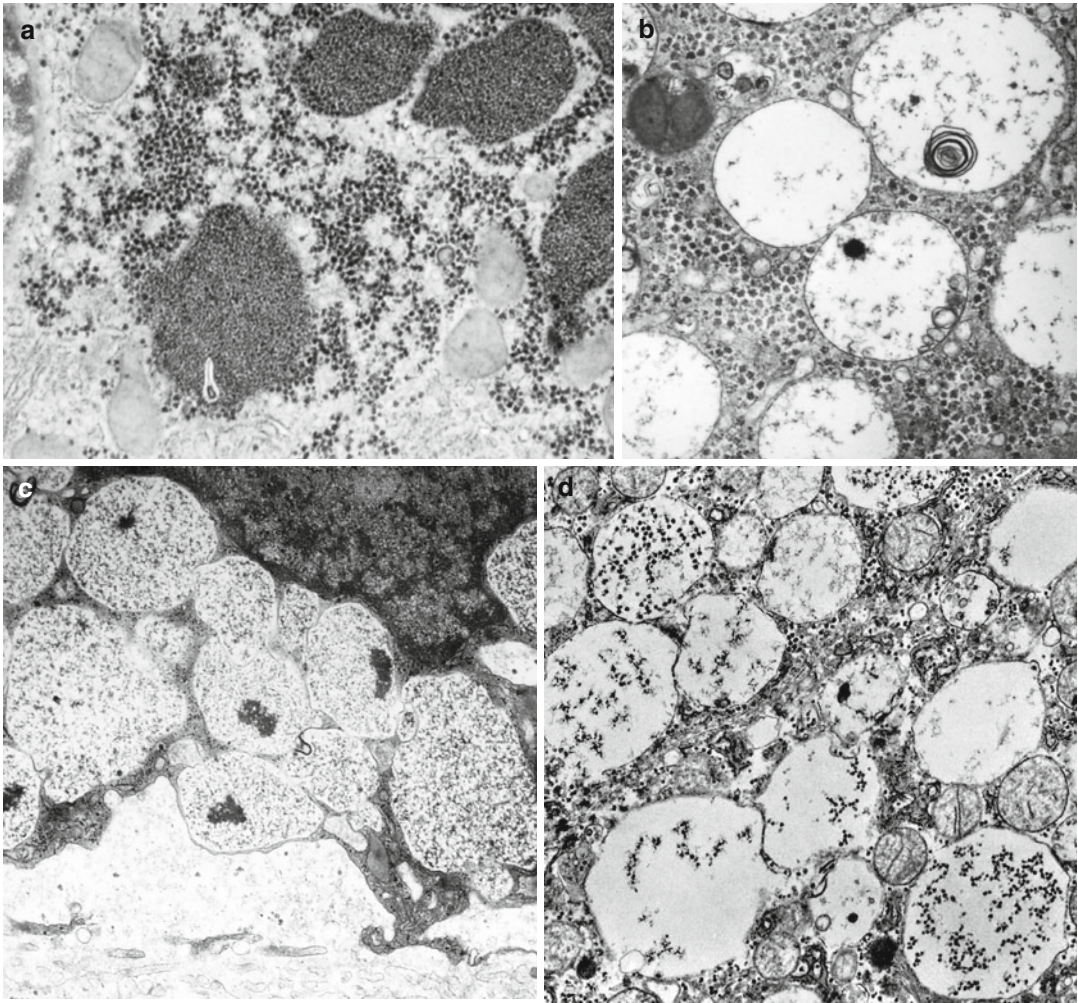
cerebroside which aggregate to produce a linear “wrinkled paper” texture. H&E stain. (f) Gaucher disease. Storage material in Kupffer cells is weakly PAS positive. PAS stain. (g) Niemann-Pick disease. Storage material in hepatocytes, Kupffer cells and portal macrophages makes then indistinguishable except for subtle differences in nuclear shape. (h) Farber disease in infant. Pale expanded vacuolated cytoplasm of hepatocytes and Kupffer cells is associated with giant cell transformation and sinusoidal erythroplasia. H&E stain. *Inset*: storage material is more abundant in Kupffer cells than in hepatocytes. PAS stain (Used with permission of Raffaella Morotti, Yale University)



storage product that is often specific for a group of lysosomal disorders and is occasionally characteristic of a particular defect (Fig. 13.19a–f).

Storage lysosomes in type II glycogenosis almost exclusively contain partially degraded glycogen (Fig. 13.19a). In mucopolysaccharidoses and in GM1 gangliosidosis, the ultrastructure

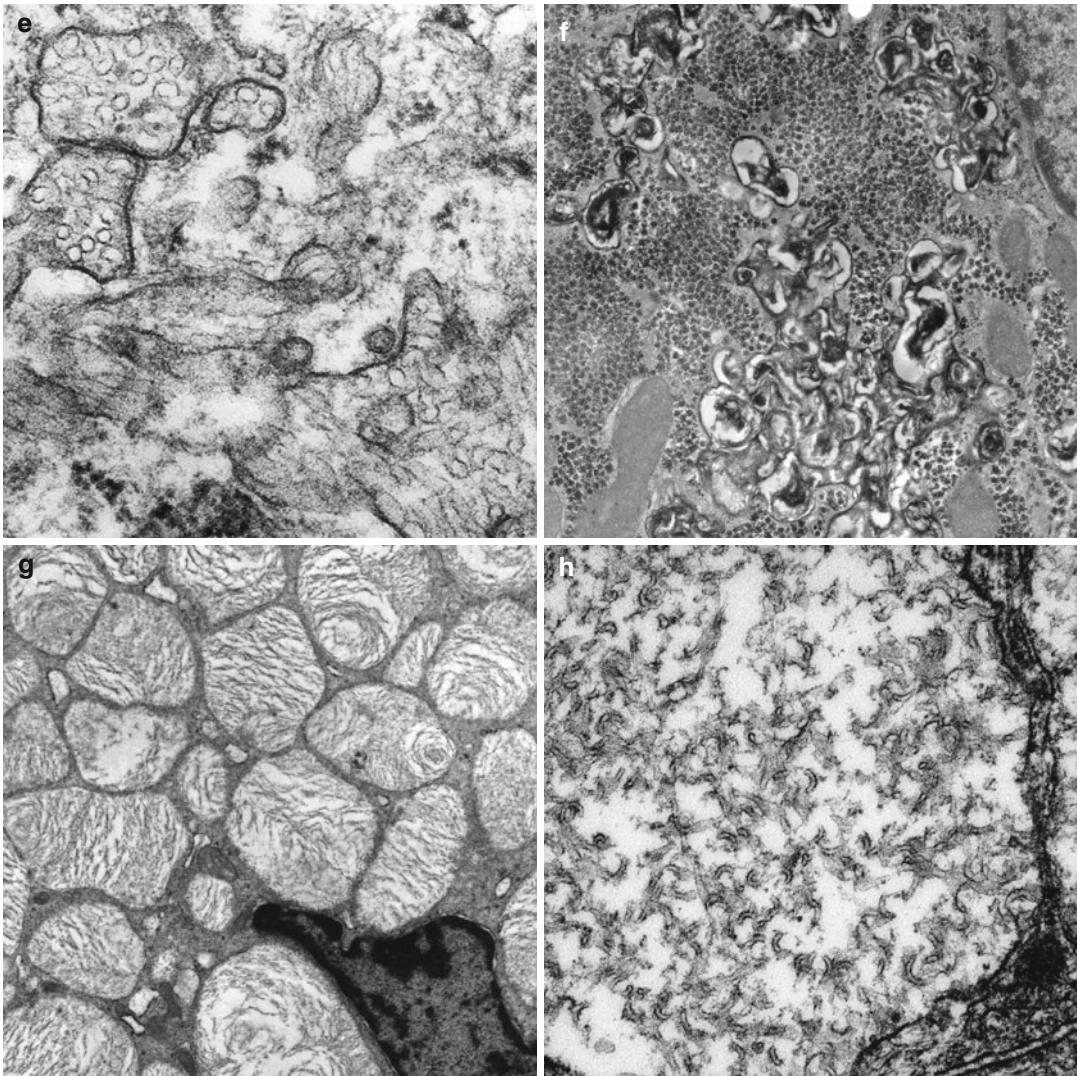
of the stored material is an amorphous lacy reticulogranular material (Fig. 13.19b–d). The Gaucher storage product has a unique ultrastructure of platelike tubules (Fig. 13.19e) and can be mobilized from the reticuloendothelial cells by enzyme replacement therapy thus ameliorating the nonneuropathic manifestations of this disease



**Fig. 13.19** (a) Glycogen storage disease type II. Partially degraded glycogen is located within lysosomal membrane. Note normal alpha glycogen particles in adjacent cytoplasm. (b) Hurler disease (type I MPS). Storage lysosomes contain amorphous almost completely degraded material that has been lost during processing. EM. (c) Sanfilippo disease (MPS type III). Amorphous granular material accumulates in storage lysosomes of a Kupffer cell. EM. (d) Sialidosis. Amorphous granular material accumulates in hepatocyte lysosomes. Mitochondria exhibit usual postmortem

artifacts. EM. (e) Gaucher disease. Membrane-bound packets of crystallized tubules pack the cytoplasm of Kupffer cells. EM. (f) Niemann-Pick disease type B. Membrane-bound inclusions of dense membranous material are dispersed among normal mitochondria and well-glycogenated cytoplasm of hepatocyte. EM. (g) Niemann-Pick disease type B. Membrane-bound inclusions of stacked membranous material fill the cytoplasm of a Kupffer cell. EM. (h) Farber lipogranulomatosis. Membrane-bound curvilinear tubular lipid crystals within the cytoplasm of a Kupffer cell. EM





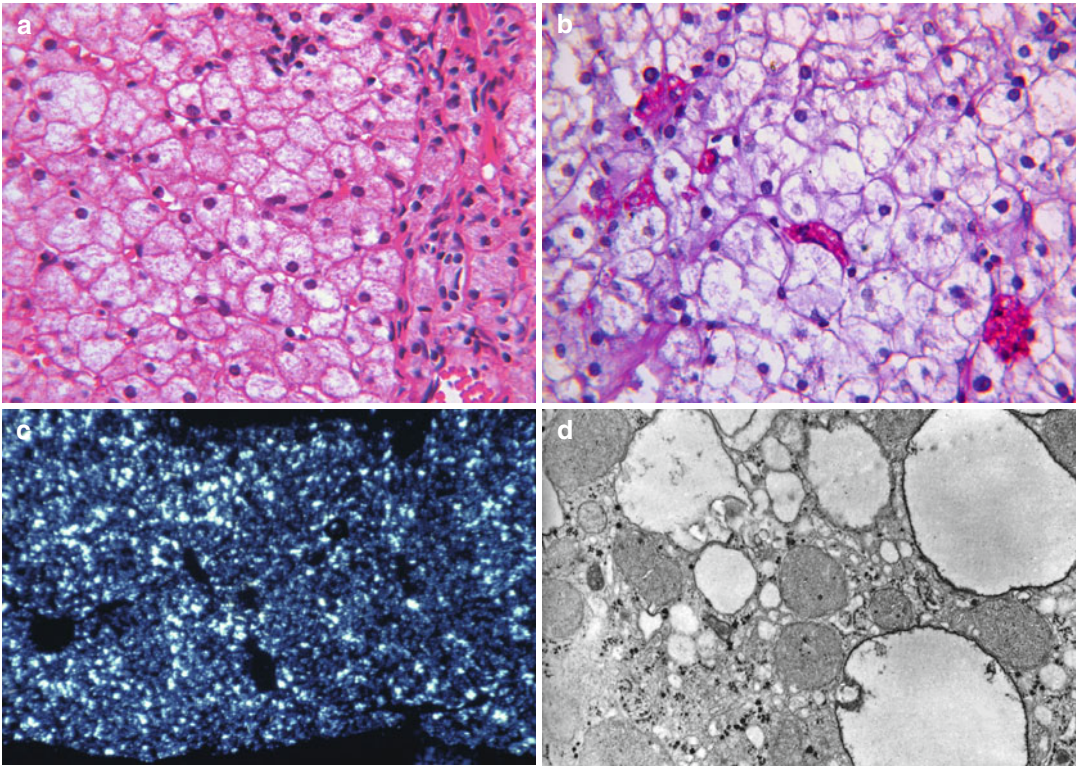
**Fig. 13.19** (continued)

in the liver, spleen, bone marrow, and lung (Willis et al. 2008). In Niemann-Pick disease types A and B due to sphingomyelinase deficiency, the Kupffer cells and, to a variable extent, hepatocytes have a characteristic foamy appearance due to lysosomal accumulation of sphingomyelin, a membranous sudanophilic phospholipid. This material has distinctive myelin-like ultrastructural qualities (Fig. 13.19f, g) that resemble, to some extent, the phospholipid profiles that result from autophagy and may be prevalent in cholestasis. Morphologically and chemically similar material accumulates in renal epithelium and

blood vessels in Fabry disease. In Farber lipogranulomatosis, due to acid ceramide deficiency, the vacuolated cytoplasm of Kupffer cells, and to a lesser extent hepatocytes (Fig. 13.19g), contains lysosomes filled with minute tubular curvilinear bodies.

### 13.12.3 Acid Lipase Deficiency

Acid lipase deficiency is responsible for both Wolman disease in young infants and cholesterol ester storage disease (CESD), a milder form



**Fig. 13.20** (a) Cholesterol ester storage disease (CESD). Hepatocytes are slightly pale and variably vacuolated. Portal area to left contains plump macrophages containing granular lacy amphophilic material. H&E stain. (b) CESD. Hepatocytes have expanded vacuolated cytoplasm. Scattered Kupffer cells contain abundant PAS-positive

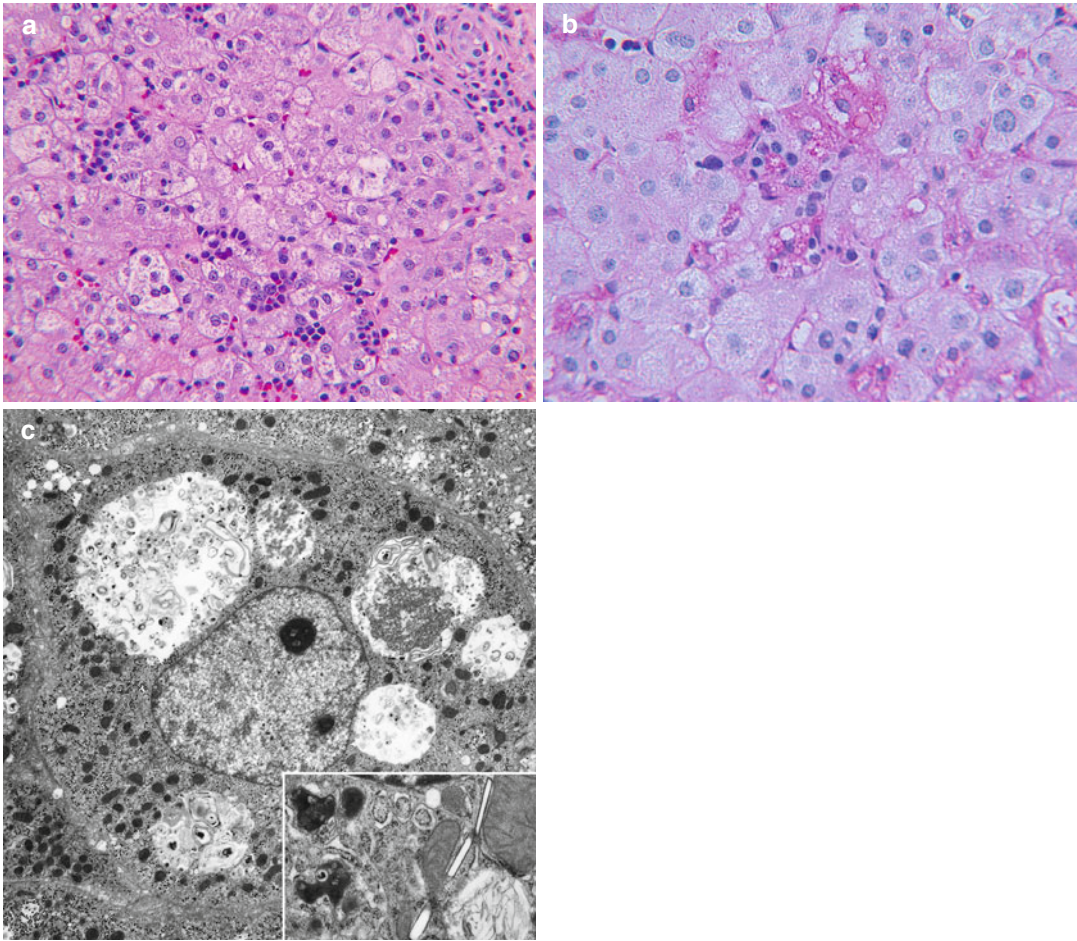
diastase-resistant residual bodies. PAS stain after diastase digestion. (c) CESD. Frozen section of liver with massive accumulation of cholesterol ester crystals. Unstained. Polarized light. (d) CESD. Hepatocyte lipid accumulates in membrane-bound vesicles (lysosomes), unlike usual hepatocyte lipid

that causes asymptomatic hepatomegaly in older infants and children. Because of the nature of the stored lipid, the liver may be orange-yellow rather than pale yellow, as in neutral lipid storage diseases. In both types of acid lipase deficiency, the stored lipid is membrane bound by electron microscopy (unlike ordinary lipid vesicles) and contains cholesterol ester crystals that are faintly detectable in H&E-stained sections, but dramatically demonstrable in unstained frozen sections using polarized light (Fig. 13.20a–d). In CESD, Kupffer cells and especially portal macrophages contain a complex of insoluble lipids in lysosomes that are weakly basophilic. This finding, though nonspecific, as it may also be seen in Niemann-Pick disease, and following hepatocyte injury unrelated to LSD, completes an unusual light microscopic constellation that strongly

suggests CESD. Delicate progressive fibrosis occurs over time without active inflammation, but the long-term natural history is uncertain.

#### 13.12.4 Niemann-Pick Disease, Type C (NPC)

Niemann-Pick disease, type C (NPC) is a rare disorder that results from mutations in proteins, NP1 (95 %) and NP2 (5 %), that transport unesterified cholesterol between lysosomes, endosomes, and other organelles (Patterson et al. 2012). The result is accumulation of glycolipids and cholesterol in lysosomes. NPC is biochemically unrelated to NPA and NPB, but clinically and morphologically overlaps with these two sphingomyelin storage disorders and with several



**Fig. 13.21** (a) Niemann-Pick type C (NPC) in an infant. INH-like appearance includes giant cell transformation and extramedullary hematopoiesis. Lipid inclusions are inconspicuous. H&E. (b) NPC. Kupffer cells may be conspicuously enlarged due to accumulation of secondary

lysosomes and lipid. (c) NPC. Hepatocytes contain large autophagosomes not unlike those that may be seen in INH. *Inset*: autophagy accompanied by cholesterol crystal profiles. EM. Used with permission of Milton Finegold, Texas Children's Hospital

other unrelated neurovisceral lipidoses. A morphological hallmark, especially in the spleen and bone marrow, is accumulation of foamy reticulo-endothelial cells. Cholestatic liver disease, which is neonatal hepatitis like, may begin in infancy and slowly progress to cirrhosis (Kelly et al. 1993). Isolated liver cell necrosis, lobular inflammation, and pericellular fibrosis may be early findings (Fig. 13.21a). In most reported cases, liver disease is overshadowed by neurological deterioration in later life that is due to progressive neuronal loss.

In NPC disease, enlarged vacuolated Kupffer cells in infants and young children may exceed that found in other neonatal cholestatic disorders. The expanded cytoplasm of Kupffer cells contains bulky residual bodies that are PAS positive and diastase resistant (Fig. 13.21b) and may contain free cholesterol demonstrable in frozen sections with an immunostain for filipin. Electron microscopy demonstrates complex storage material that seems to result from excessive autophagy that yields abundant phospholipid-rich membranous material (Fig. 13.21c) overlapping with

features seen in NPA and NPB. Rarely, lipid crystals may be found in the cytoplasm, but lack specificity. Disproportionate splenomegaly should prompt examination of bone marrow for storage histiocytes. Fibroblast culture may be used to detect accumulated nonesterified cholesterol. Because mutations in NP1 or NP2 are found in most cases, genetic analysis has become the test of choice (Park et al. 2003).

### 13.13 Endoplasmic Reticulum Storage Disorders

Abnormal accumulation of protein in the endoplasmic reticulum of hepatocytes at the point of synthesis is characteristic of three rare disorders, alpha-1-antitrypsin (A1AT) storage disease, fibrinogen storage disease, and chymotrypsin storage disease.

#### 13.13.1 Alpha-1-Antitrypsin Storage Disease

The most common of endoplasmic reticulum storage disorders is alpha-1-antitrypsin storage disease, most often associated with the Z phenotype, an abnormally folded protein that accumulates in the endoplasmic reticulum of hepatocytes (Perlmutter 2004). Misfolded A1AT cannot be secreted resulting in reduced levels of A1AT activity in the blood. For reasons that are unclear, accumulation of abnormally folded AAT protein in hepatocytes is highly variable in this genetic disease, resulting in substantially different clinical presentations ranging from transient neonatal cholestasis (Fig. 13.22a) to progressive liver disease in a small minority of affected children to absence of clinical liver disease. The common association with chronic lung disease occurs exclusively in adults. The paradox that most children and adults with the ZZ phenotype do not develop clinical liver disease remains unexplained. To explain this discrepancy, hypotheses have invoked the modifying influence of other genes that influence protein processing or adverse influence of intercurrent illness.

The abnormal protein is recognizable in liver tissue samples as PAS + diastase-resistant globules

surrounded by a clear halo most obvious in zone 1 hepatocytes (Fig. 13.22b). These globules are often not apparent in the first few months of life when A1AT deficiency mimics idiopathic neonatal hepatitis. Immunostains for normal or mutated A-1-antitrypsin are helpful in atypical cases. The stored material in the endoplasmic reticulum usually has a distinctive ultrastructural appearance (Fig. 13.22c). Low blood activity of alpha-1-antitrypsin, an acute phase reactant, is a useful screening test. Definitive diagnosis of abnormal protein phenotypes requires protein electrophoresis with isoelectric focusing or mutation analysis.

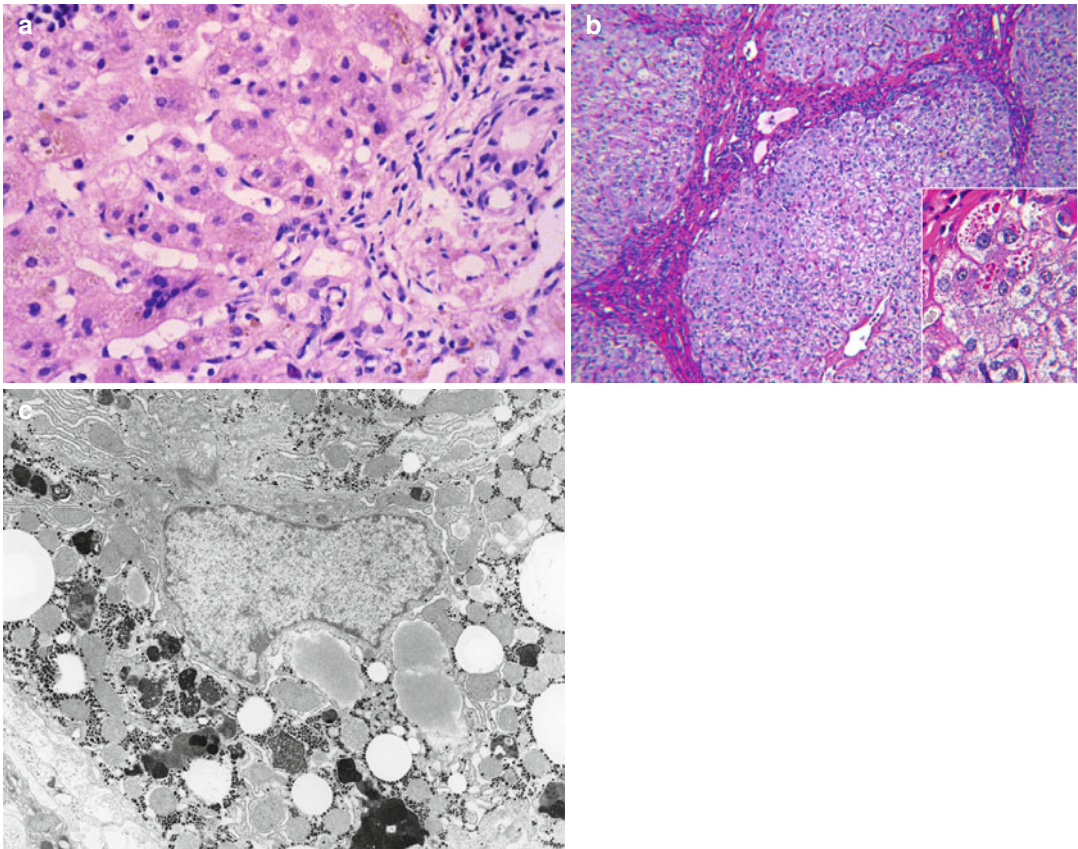
A similar clinical phenotype of chronic liver and lung disease is described in adults with the much rarer alpha-1 antichymotrypsin mutation that results in low serum levels of this serine protease due to retention in hepatocyte endoplasmic reticulum. There are no reported examples in children.

#### 13.13.2 Congenital Afibrinogenemia and Dysfibrinogenemia

Congenital afibrinogenemia and dysfibrinogenemia are rare genetic disorders due to mutations in one of three polypeptide chains that constitute fibrinogen, usually leading to bleeding or thrombosis. Very rarely, mutations in fibrinogen result in hypofibrinogenemia caused by retention of defective fibrinogen in the endoplasmic reticulum (Fig. 13.23a, b) leading to chronic liver disease of variable severity. To date, only four of the many known fibrinogen mutants are associated with fibrinogen storage and progressive liver disease (Brennan et al. 2000, 2010).

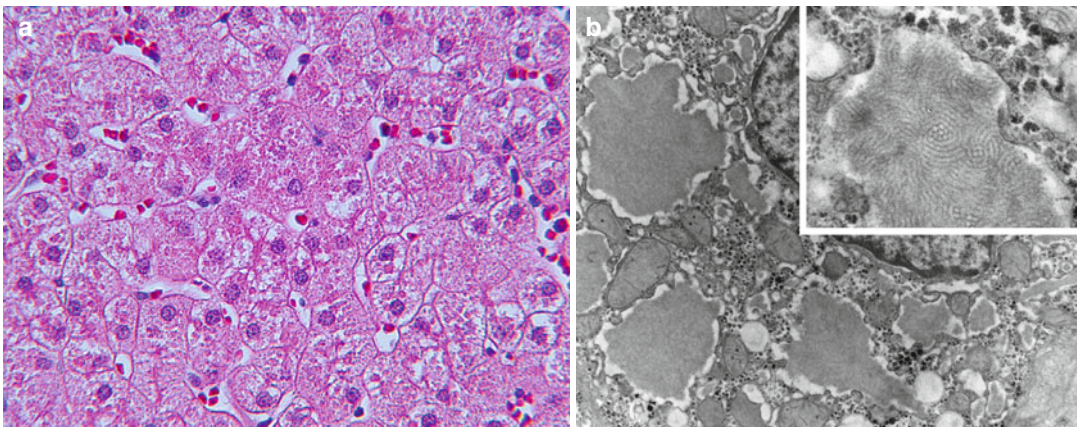
#### 13.13.3 Cystinosis

Cystinosis is a rare autosomal recessive trait that causes cystine to accumulate in lysosomes due presumably to lack of a membrane transport carrier, resulting in progressive renal tubular and glomerular disease in infants and children. Cystine crystal deposits have been identified in many other organs in patients with cystinosis and may be responsible for local tissue injury at many sites, including the liver, where nodular



**Fig. 13.22** (a) A1AT deficiency, infant at 2 months of age. Lobular cholestasis with occasional giant cell transformation and mild bile duct injury resembles INH. PAS-positive globules were absent. H&E. (b) A1AT deficiency in older child with established portal cirrhosis. Clusters of

periportal hepatocytes contain pale eosinophilic protein globules enhanced by PAS stain. d-Pas with *inset*. (c) A1AT deficiency. Dilated cisterns of endoplasmic reticulum adjacent to nucleus contain unsecreted protein. EM



**Fig. 13.23** (a) Fibrinogen storage disease. Hepatocyte cytoplasm contains slightly coarse granular deposits of proteinaceous material. There is no other abnormality.

H&E stain. (b) Fibrinogen accumulates in dilated cisterns of endoplasmic reticulum throughout the liver. *Inset*: mutant fibrinogen crystallizes in situ. EM

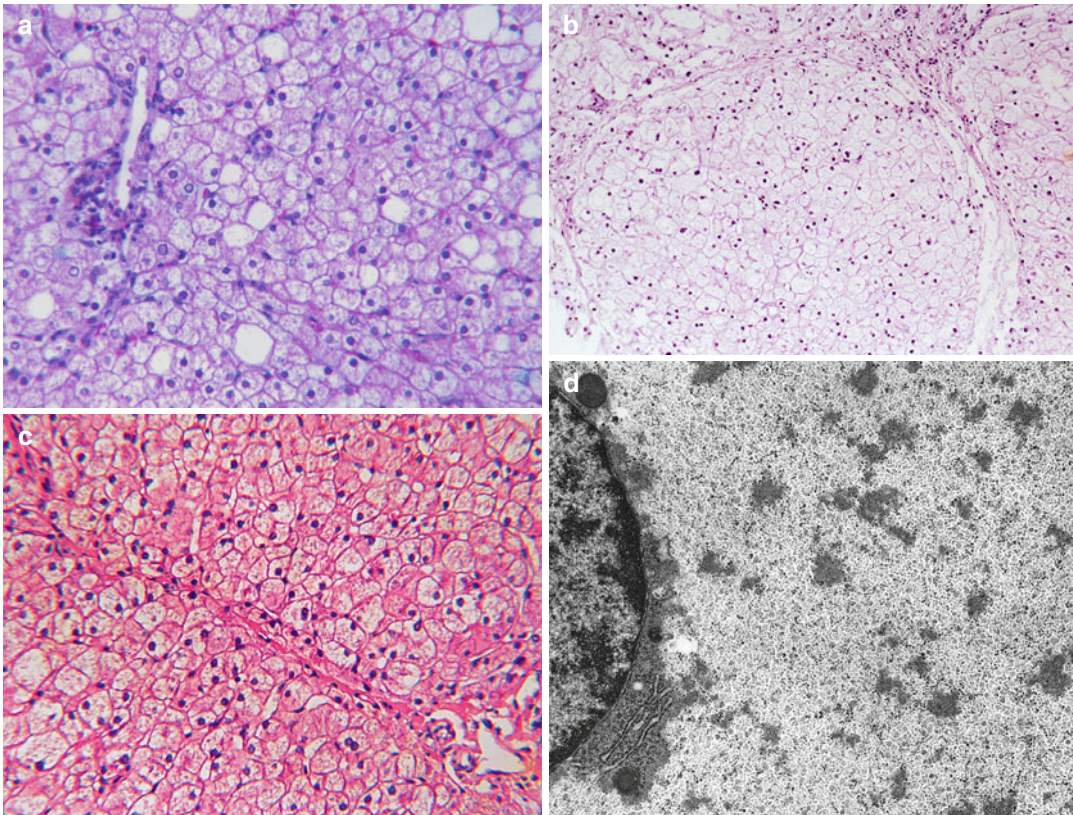
regenerative hyperplasia and noncirrhotic portal hypertension are described as late complications (DiDomenico et al. 2004). In the liver, the cystine crystals are mainly localized in Kupffer cells and portal area macrophages. Clinically significant liver disease in nephropathic cystinosis is rare.

### 13.14 Nonlysosomal Glycogenosis and Polyglucosan Storage Disorders

#### 13.14.1 Pathology

Glycogen storage diseases exhibit substantial phenotypic, biochemical, and clinical

heterogeneity (Shin 2006). Nonetheless, so far as is known, the light and electron microscopic appearance of most nonlysosomal glycogenoses is similar (McAdams et al. 1974). Hepatocytes in glycogenoses other than types II and IV are dramatically expanded due to glycogen accumulation that produces diffuse cytoplasmic clarity and a mosaic pattern due to compression of sinusoids (Fig. 13.24a–c). Often the hepatocyte membrane appears thick compared to the clarity of the cytoplasm. Electron microscopy usually demonstrates that pools of excess glycogen have displaced hepatocyte organelles to the perinuclear region and to the periphery adjacent to the cell membrane (Fig. 13.24d).



**Fig. 13.24** (a) Glycogen storage disease type I. Hepatocytes with expanded cytoplasm of abnormal clarity also contain scattered large lipid droplets and compress sinusoids. Occasional zone I nuclei contain glycogen. H&E stain. (b) Glycogen storage disease type III. The abnormal features resemble those in Fig. 13.25a. However, delicate fibrous septation indicates evolution towards cir-

rhosis. H&E stain. (c) Glycogen storage disease type VI. The abnormal features resemble types I and III except for lack of glycogenated nuclei. (d) All nonlysosomal glycogenoses. The accumulated glycogen is usually partially degraded with no alpha particles and displaces organelles both to the perinuclear region and to the periphery beneath the cell membrane. EM

The PAS stain demonstrates liver glycogen, but has notable limitations in the recognition of glycogen storage disease. This nonquantitative stain helps to identify the material responsible for hepatocyte cytoplasmic expansion, but has no value for determining when hepatocyte glycogen content exceeds the upper limit of normal, which is high – approximately 6 % of wet weight.

Several minor differences among the subtypes of nonlysosomal glycogenosis are consistent enough to permit suggesting a particular diagnosis based on the appearance in paraffin sections stained with hematoxylin and eosin. Two features, macrovesicular lipid and glycogenation of hepatocyte nuclei in zone 1, are common and often very prominent in types I and III glycogenoses. Microvesicular lipid can also be observed in the hepatocytes of type Ia glycogenosis, possibly related to mitochondrial stress or severe hypertriglyceridemia. Cytoplasmic lipid and nuclear glycogenation tend to be minor features in types VI and VIII and IX (deficiencies of liver phosphorylase, phosphorylase kinase activator, and phosphorylase kinase, respectively). Cytoplasmic lipid and nuclear glycogen are not usual characteristics of type II glycogenosis, a lysosomal glycogenosis.

Fibrosis is usually absent in type I glycogenosis, but may occur in long-term survivors of dietary management. Fibrosis in type III glycogenosis tends to be mild but may progress. Fibrosis is typically mild or absent in subtypes of glycogenosis involving defects in liver phosphorylase. Progressive liver fibrosis is typical in type IV glycogenosis. Liver adenoma and hepatocellular carcinoma occur with increased frequency in types I, IV, and VI glycogenoses.

Type IV glycogenosis is a notable exception to other glycogen storage diseases, both in terms of microscopic and ultrastructural features, and its varied presentations (a lethal neonatal form and a later onset form with predominant involvement of the liver,  $\pm$  skeletal muscle, or heart) (Moses and Parvari 2002). Isolated liver involvement with progression to cirrhosis is the most common presentation. The stored material is not glycogen but amylopectin, an unbranched polysaccharide that chemically resembles starch. In frozen

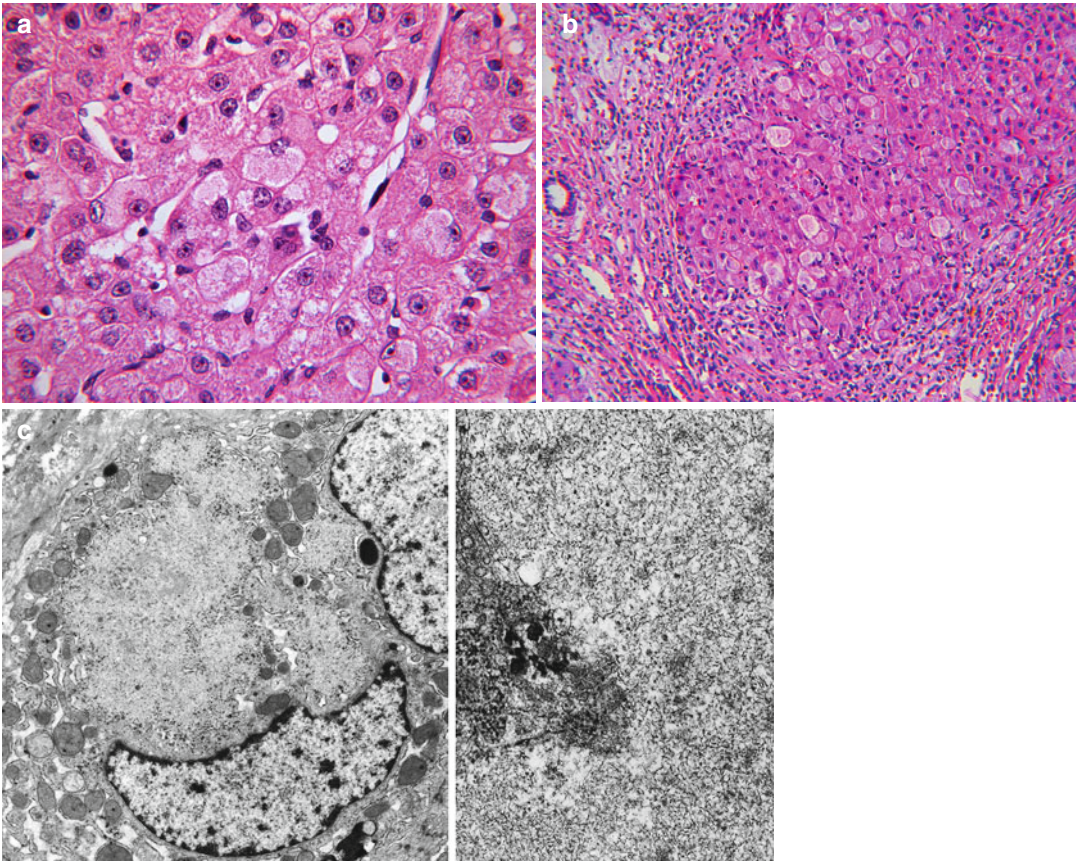
sections, this material is metachromatic, staining blue-brown with Lugol's iodine. The stored material is PAS positive but diastase resistant and gradually accumulates as discrete pale inclusions within the hepatocyte cytoplasm in a distinctive localized pattern (Fig. 13.25a, b). Electron microscopy demonstrates amylopectin arranged in bulky non-membrane-bound granulofibrillar inclusions (Fig. 13.25c, d).

### 13.14.2 Diagnosis

The most efficient approach to making a correct diagnosis of glycogenosis requires clinical suspicion and then selection from available screening methods. If a liver biopsy is performed initially, and clinical suspicion for a storage disorder is high, at least two cores of liver tissue or an open biopsy specimen should suffice. One core of liver tissue must be frozen in anticipation of a need for enzyme analysis. It is helpful to first perform electron microscopy to identify the stored material and its location in lysosomes or free in the cytoplasm. When excess nonlysosomal glycogen displaces other organelles to the periphery in most hepatocytes, the glycogen concentration should be measured and a homogenate screened for glycolytic enzyme defects.

Polyglucosan accumulation in hepatocytes that resembles type IV glycogenosis may be observed in Lafora disease, a very rare genetic-based progressive type of myoclonic epilepsy. The stored polysaccharide results in cytoplasmic inclusions that are reactive with PAS and diastase sensitive, as well as weakly reactive with colloidal iron, indicating a weakly acidic polysaccharide. The inclusions accumulate in hepatocyte cytoplasm as demarcated pale pools of material that is granular to fibrillar by electron microscopy and displaces hepatocyte organelles in a manner that resembles type IV glycogenosis. However, assays of branching enzyme activity are normal. Lafora bodies are an insoluble product of disordered glycogen synthesis (Turnbull et al. 2010).

Hepatocytes with a similar but more diffuse ground glass cytoplasmic texture may also be observed in chronic hepatitis B and as a result

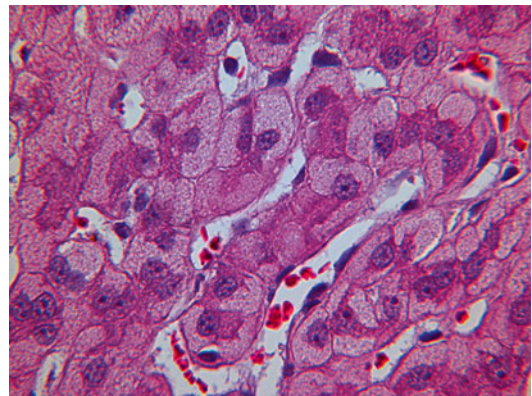


**Fig. 13.25** (a) GSD, type IV. Early change consists of accumulation of pale storage material in scattered hepatocytes. Kupffer cells are not usually involved. H&E stain. (b) GSD, type IV. Later changes include smoldering portal inflammation with progressive periportal fibrosis and cirrhosis.

Individual hepatocytes contain storage material that is often surrounded by a halo. H&E stain. (c) GSD, type IV. Low and high magnification of storage material that displaces hepatocyte organelles but is not defined by a unit membrane. The amylopectin has a granulo-fibrillar morphology. EM

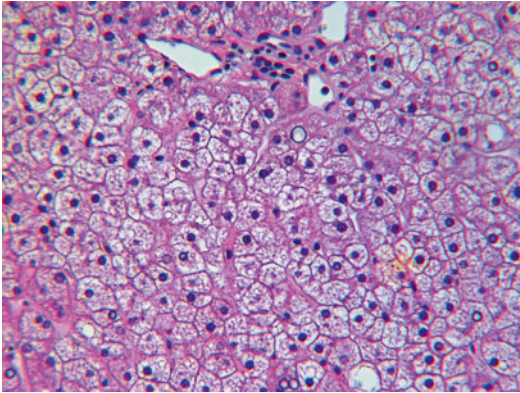
of drug-induced proliferation of smooth endoplasmic reticulum. Recently, finely granular inclusions of particulate glycogen sequestered in basal (perisinusoidal) hepatocyte cytoplasm, and occasionally intermixed with other organelles such as mitochondria and endoplasmic reticulum (Fig. 13.26), have been reported following liver and bone marrow transplantation (Lefkowitz et al. 2006). These inclusions are common in liver allografts, seem to have no clinical significance, and may slowly resolve. Multidrug effect (polypharmacy) is a suggested etiology, but pathobiology is unknown.

Experience shows that extremely well-glycogenated liver may mimic the histological appearance of glycogen storage disease



**Fig. 13.26** Polypharmacy inclusions resemble those of type IV glycogenosis but have a distinct parasinusoidal localization. Ultrastructure demonstrates inclusions are composed mainly of sequestered glycogen H&E stain





**Fig. 13.27** Glycogenopathy of DM (Mauriac syndrome). Resemblance to a genetic glycogenosis is striking. H&E stain

and remain within the upper limit of normal glycogen content. Specific disorders, where this caveat applies, include UCD (Fig. 13.14) and poorly controlled type 1 diabetes mellitus of Mauriac syndrome (Fig. 13.27). Unusually well-glycogenated livers may also be seen in the context of nonalcoholic fatty liver disease.

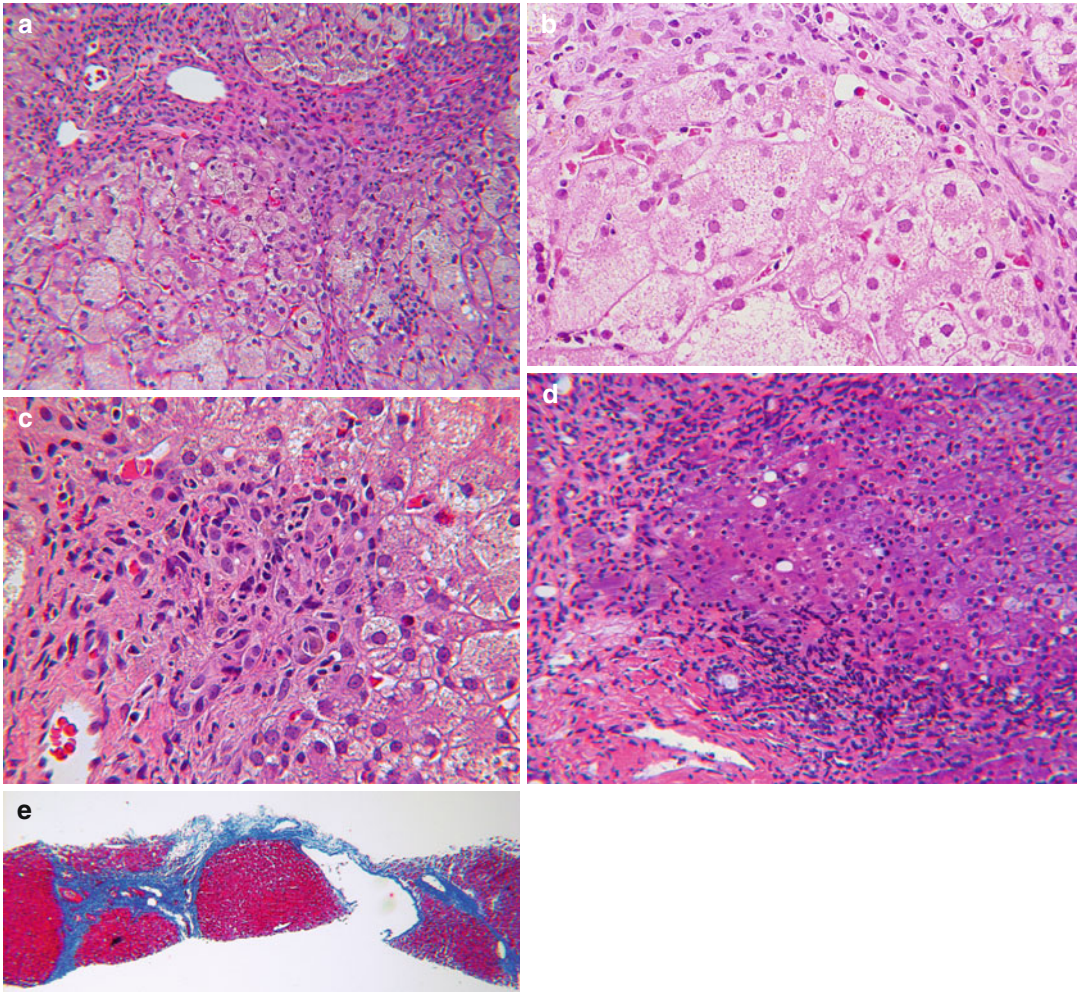
### 13.15 Bile Acid Synthetic Defects

Bile acid synthetic defects (BASD) are a relatively new category of liver disease with a wide spectrum of outcomes. Nine defects in the synthetic pathway have been identified using combined mass spectroscopy and gas chromatography to identify abnormal bile acids in blood, urine, or bile (Bove et al. 2004). The defects result in absence or low levels of normal bile acids. Direct hyperbilirubinemia and aminotransferasemia are common in affected infants who typically present with a neonatal hepatitis-like syndrome. Serum levels of gamma glutamyl transpeptidase, an enzyme produced in hepatocytes, secreted in bile, and reabsorbed by damaged bile duct epithelium, are normal, as in progressive familial cholestasis types 1 and 2 (PFIC1 and PFIC2). Liver injury in BASD is due to two factors: the absence of the normal choleric influence of the two normal major bile acids, cholic, and deoxycholic acids and the accumulation in hepatocytes of metabolic

intermediaries, hydrophobic monohydroxy bile acids proximal in the synthetic pathway to the missing enzyme. Hydrophobic bile acids are relatively toxic to hepatocytes and probably to the cholangiocytes of the smallest ductules as well. In infants with the two most common defects, 3-beta OH steroid dehydrogenase deficiency (Fig. 13.28a–d) and 5-beta reductase deficiency (Fig. 13.29a–e), the histology overlaps with ordinary neonatal hepatitis with giant cell transformation with the following exceptions. Necrosis of giant cells may be more common than in usual neonatal hepatitis. Interlobular bile ducts are normal, but there may be a cytotoxic interface hepatitis accompanied by swollen cholangiocytes or frank necrotizing cholangiolitis affecting the smallest ductules (Fig. 13.28b, c). Extreme architectural distortion of canaliculi has been described in 5-beta reductase deficiency (Fig. 13.29d) (Daugherty et al. 1993). The cholestatic hepatopathy in all BASD responds to therapy with normal bile acids, although fibrosis may persist if initial recognition and therapy are delayed (Fig. 13.29d). Rapid evolution to liver failure in early infancy occurs in the very rare oxysterol-7-alpha hydroxylase defect.

Important exceptions exist to the pattern of presentation of BASD in early infancy as “neonatal hepatitis.” Signs and symptoms due to 3-beta OH steroid dehydrogenase deficiency uncommonly present in older infants and children, and rarely in adults. In these patients, an indolent course is typical. Signs include persistent direct hyperbilirubinemia, elevated serum aminotransferases, fat-soluble vitamin deficiency, itching, and poor growth. Fibrosis tends to be very slowly progressive (Fig. 13.28d), possibly depending on the degree of interface inflammatory activity. Thus, there is significant overlap with PFIC1 and PFIC2.

The newest recognized class of BASD are disorders of bile acid conjugation, of which two have been identified. These patients produce bile acids that are not normally amidated and, as a result, are ineffective promoters of fat absorption. Cardinal signs are poor growth and fat-soluble vitamin deficiency. In patients who lack conjugated bile acids, transient neonatal cholestatic



**Fig. 13.28** (a) Bile acid defect. 3-beta OH steroid dehydrogenase deficiency in young infant. Extreme lobular cholestasis with giant cell transformation is associated with portal and lobular inflammation and early portal fibrosis. H&E stain. (b) Bile acid defect. 3-beta OH steroid dehydrogenase deficiency in young infant. Marked lobular cholestasis with giant cell transformation and subtle interface hepatitis. H&E stain. (c) Bile acid defect.

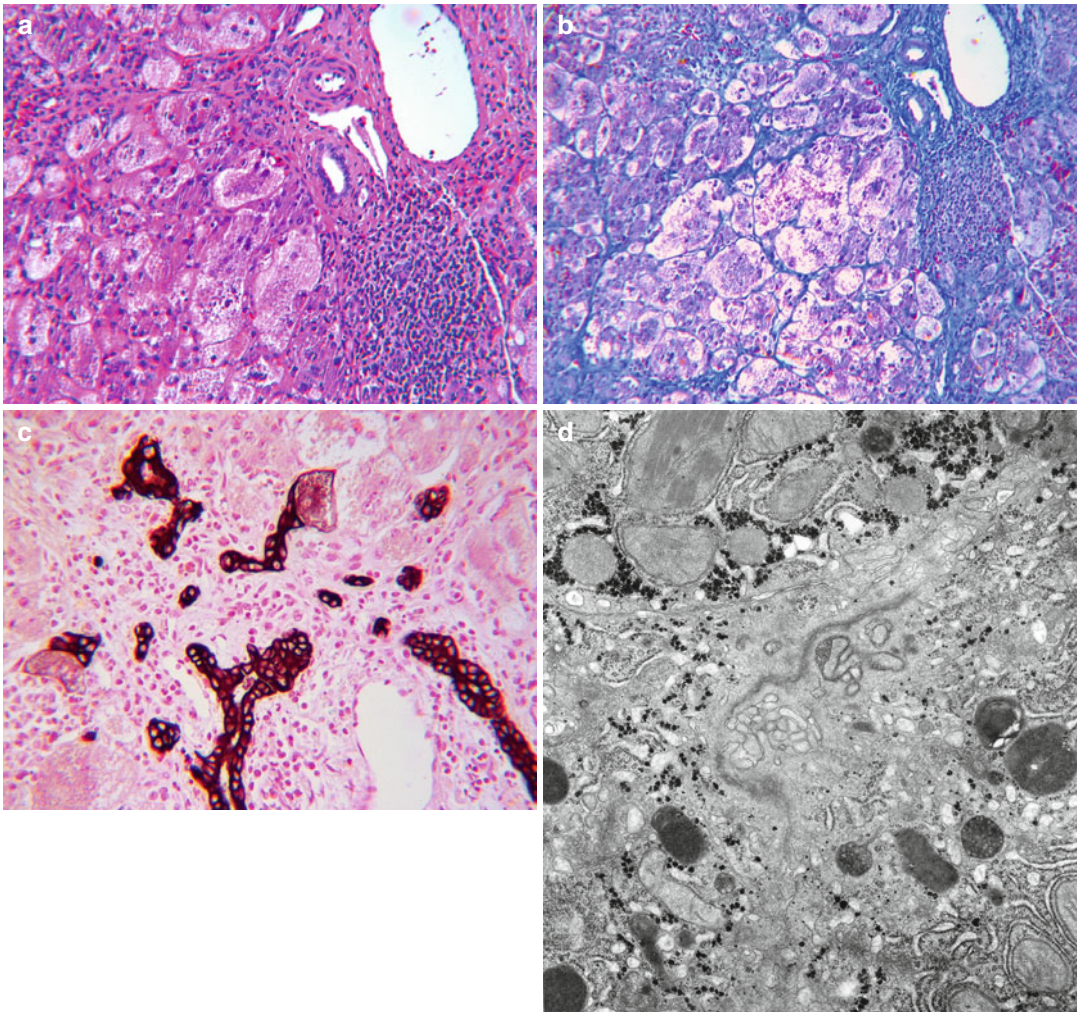
3-beta OH steroid dehydrogenase deficiency in young infant. Focal florid ductulitis. H&E stain. (d) Bile acid defect. 3-beta OH steroid dehydrogenase deficiency presenting with chronic fibrosing interface hepatitis in an older infant. H&E stain. (e) Bile acid defect. 5-beta reductase deficiency. Adult treated with colic acid since infancy. Residual periportal fibrosis but no cholestasis or active hepatitis. Trichrome stain

hepatitis with cholangiopathic features has been observed (Setchell et al. 2013).

Timely institution of normal bile acid replacement therapy appears to be beneficial for preventing chronic liver disease in the two most common defects, 3-beta OH steroid dehydrogenase deficiency and 5-beta reductase deficiency, but has not been effective in the much rarer oxysterol-7-alpha hydroxylase deficiency that is lethal in infancy.

### 13.16 Peroxisomal Diseases

Peroxisomes are subcellular organelles essential for beta-oxidation of very long-chain fatty acids, and synthesis of plasmalogens and bile acids. Peroxisomes are more abundant in hepatocytes than any other cell type. Metabolic disorders caused by peroxisome dysfunction are divided into two biologic groups that have overlapping clinical and morphological phenotypes.

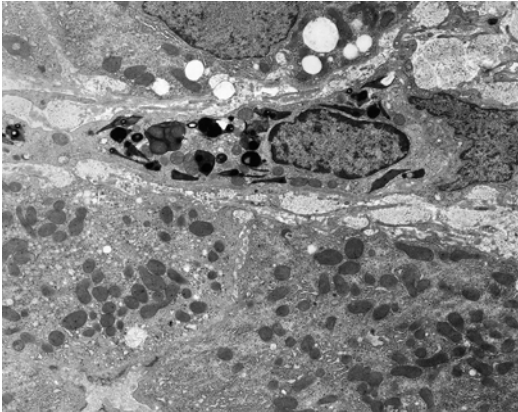


**Fig. 13.29** (a) Bile acid defect. 5-beta reductase deficiency in a young infant. Lobular cholestasis with marked giant cell transformation, interface inflammation, and periportal fibrosis. H&E stain. (b) Bile acid defect. 5-beta reductase deficiency in a young infant. Marked periportal and pericellular fibrosis indicate a rapidly progressive disease. H&E

stain. (c) Bile acid defect. 5-beta reductase deficiency in a young infant. Brisk ductular reaction is a response to injury of small ducts. Cytokeratin stain. (d) Bile acid defect. 5-beta reductase deficiency. Such extreme tortuosity of canaliculi with architectural rearrangement of microvilli is unusual in cholestatic disorders of early infancy. EM

Peroxisomal biogenesis disorders are multisystem recessively inherited traits characterized by abnormalities of peroxisome assembly, which result in marked deficiency or absence of peroxisomes. Principally affected are the central nervous system, liver, adrenal, and skeleton. Mutations in the *PEX* family of genes that are involved in peroxisome membrane assembly are the major cause of defective peroxisome biosynthesis (Ebberink et al. 2011). Mutations in *ABCD1*, a peroxisomal membrane transporter for very long-chain fatty acids, are related to

x-linked adrenal leukodystrophy. Approximately 80% of peroxisomal biogenesis disorder patients are classified in the Zellweger syndrome spectrum (ZSS) (Yik et al. 2009). Three single peroxisomal enzyme deficiency disorders have also been recognized: straight-chain acyl-CoA oxidase deficiency results in accumulation of very long-chain fatty acids in the serum; D-bifunctional protein deficiency causes familial encephalopathy with seizures; and alpha methylacyl-CoA racemase deficiency results in accumulation of abnormal bile acids and pristanic



**Fig. 13.30** X-linked adrenoleukodystrophy. A Kupffer cell contains massive secondary lysosomes of polymorphous type unusual for the component of lipid crystals implied by angulated morphology. EM

acid in serum and malabsorption of fat-soluble vitamins.

### 13.16.1 Clinical Manifestations

Zellweger syndrome, usually lethal in infants, was the first peroxisomal disease to be recognized as such. The complete phenotype includes cholestasis, evolving paucity of bile ducts, progressive hepatic fibrosis, neuronal migration abnormalities, small renal cysts, and unusual craniofacial appearance.

### 13.16.2 Pathology

Among peroxisomal biogenesis disorders and single enzyme peroxisomal defects, progressive liver disease occurs mainly in classical Zellweger syndrome, which represents the severe end of the ZSS. Reticuloendothelial cells may exhibit distinctive angulated secondary lysosomes (Fig. 13.30) in other peroxisomal biogenesis disorders such as x-linked adrenoleukodystrophy, but liver disease is typically absent in infantile Refsum disease and single peroxisomal enzyme defects. Progressive liver disease in Zellweger disease is related, at least in part to the toxicity of accumulated abnormal bile salts, as well as to progressive destruction

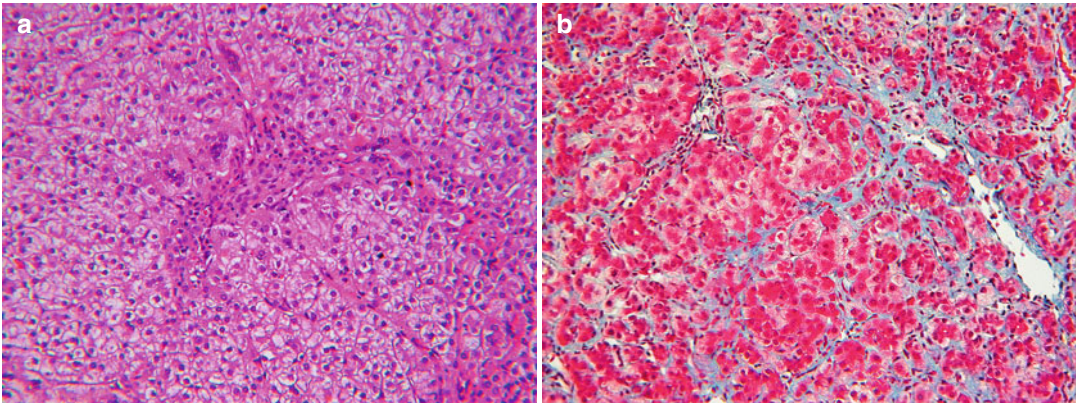
of small bile ducts leading to paucity, lobular cholestasis, and both periportal and pericellular fibrosis (Figs. 13.4b and 13.31a, b). Although the absence of peroxisomes is a defining feature, clinical severity is now known to vary greatly. Some patients in ZSS have small numbers of peroxisomes that may have a puny appearance with deficient matrix with partial clearing. Unlike the situation in single enzyme defects in bile acid synthesis, administration of normal bile acids to patients with classical Zellweger disease or to mice with an animal knockout model of ZSS does not prevent progressive liver disease.

### 13.16.3 Diagnosis

Diagnosis of peroxisomal disorders is based upon clinical findings, laboratory data such as elevated serum levels of very long-chain fatty acids, identification of unusual metabolites in serum such as phytanic acid and pipercolic acid, complementation assays in fibroblast culture, and, most recently, genetic testing for abnormalities in the PEX family of genes. Liver ultrastructure is helpful if peroxisomes, which are normally present in considerable number, cannot be found, or are exceedingly rare on careful search. However, secondary peroxisome alteration or depletion may pose problems in interpretation (Ribeiro et al. 2012). It appears that defects in biogenesis of this organelle may result in absence, marked reduction in numbers, or appearance of a few morphologically abnormal remnant peroxisomes, thus making reliance on liver ultrastructure in the absence of supportive laboratory studies unsuitable as a criterion for diagnosis.

## 13.17 Aminoacidopathies

Aminoacidopathies are recessively inherited single enzyme defects in catabolism of amino acids that result in accumulation of single amino acids or their metabolites in body fluids. At abnormally high concentration, these moieties impair development resulting in mental retardation, protein intolerance, metabolic crises, and significant



**Fig. 13.31** (a) Zellweger disease. Lobular cholestasis with acinar rearrangements of liver cell plates, zone 1 giant cells, and evolving bile duct paucity. H&E stain. (b)

Zellweger disease. Periportal and especially prominent pericellular fibrosis. Trichrome stain

mortality. Examples, often with genetic subtypes, variable time of onset, and variable severity, are tyrosinemia, phenylketonuria, isovaleric acidemia, propionic acidemia, methylmalonic aciduria, and the branched chain aminoacidopathy known as maple syrup urine disease. Of these, only tyrosinemia type I is associated with progressive liver disease.

Liver biopsy is not a diagnostic tool for these disorders but may show fatty change if performed at the time of a metabolic crisis.

### 13.17.1 Hereditary Tyrosinemia

#### 13.17.1.1 Clinical Manifestations

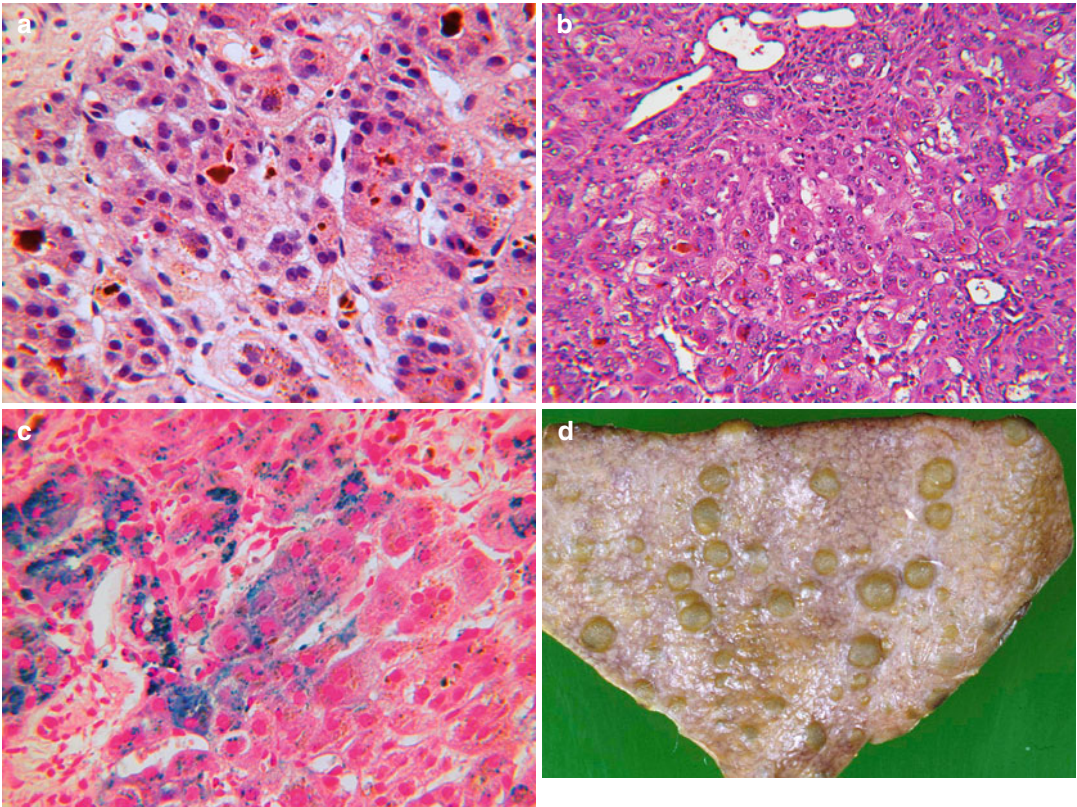
Type I tyrosinemia is caused by deficiency of fumarylacetoacetase, the last enzyme in the pathway of tyrosine degradation. Accumulation of toxic intermediate metabolites causes either acute liver failure associated with neonatal hepatitis or slowly progressive liver disease in children and young adults. Renal tubular dysfunction causes hypophosphatemic rickets in infants. Either phenotype may result in cirrhosis with high risk for hepatocellular carcinoma at a young age. Liver transplantation is effective but has been supplanted by long-term administration of an enzyme inhibitor of tyrosine degradation, 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), coupled with dietary management.

This places emphasis on early diagnosis. NTBC reverses the production of toxic intermediary metabolites and ameliorates the liver disease (Masurel-Paulet et al. 2008). However, persistent elevation of serum alpha-fetoprotein, a tendency to develop dysplastic liver nodules, and the risk for hepatocellular carcinoma are not eliminated.

#### 13.17.1.2 Pathology

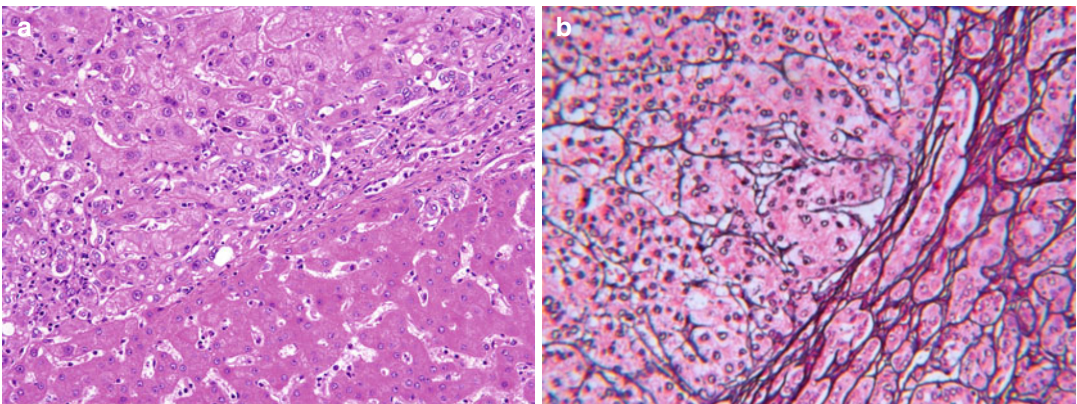
The histology of the liver in infants with hereditary tyrosinemia (Fig. 13.32a) shows lobular cholestasis, pseudoacini, giant cell transformation, focal fatty changes, necrosis, early pericellular fibrosis, and excessive inflammation (Fig. 13.32b) compared to other metabolic disorders that cause non-obstructive cholestasis in infants (Dehner et al. 1989). Hemosiderin deposits in zone 1 hepatocytes may be heavy; pseudoacini with canalicular bile plugs are often extremely prevalent (Fig. 13.32b, c). Fibrosis may be established very early, even before birth. A fascinating phenomenon is the appearance of nodules composed of normal appearing hepatocytes that contain immunoreactive fumarylacetoacetase, an apparent spontaneous reversion to a normal genotype (Demers et al. 2003).

Cirrhosis and dysplastic nodules develop in late infancy or early childhood. The dysplastic liver nodules (Fig. 13.33a, b) are difficult to distinguish from hepatocellular carcinoma. Tyrosinemia is an important model for the study of early histological and genetic changes during



**Fig. 13.32** (a) Tyrosinemia, 1-month infant. Extreme lobular cholestasis with giant cells and focal acinar rearrangements of liver Plates. H&E stain. (b) Tyrosinemia, 4-month infant. Acinar rearrangements are universal and canalicular cholestasis is severe. There is significant

portal and lobular inflammation and spotty hepatocyte necrosis. H&E stain. (c) Tyrosinemia, prominent hemosiderin deposits in zone 1 hepatocytes. Iron stain. (d) Tyrosinemia. Explanted liver with multiple dysplastic nodules



**Fig. 13.33** (a) Tyrosinemia. Interface of dysplastic nodule and normal liver. H&E stain. (b) Tyrosinemia. Interface of dysplastic nodule and normal liver. Reticulin

stain. Used with permission of Pierre Russo, Children's Hospital of Philadelphia

hepatic carcinogenesis and also for the study of genetic events that spontaneously correct the mutations in fumarylacetoacetase in liver nodules that have reverted to normal.

### 13.17.1.3 Diagnosis

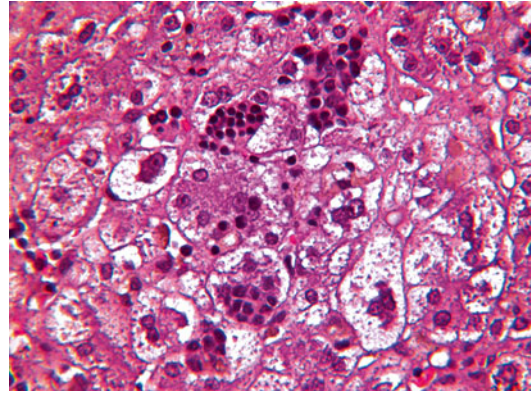
Accumulation of succinylacetone, a by-product of tyrosine degradation, in urine and serum is the basis for a specific diagnostic test.

## 13.18 Disorders of Glycosylation

Congenital disorders of glycosylation are an expanding group of rare systemic diseases with multiorgan dysfunction in which glycoproteins are under-endowed with sugar moieties, such as mannose, thereby interfering with protein transport and function (Jaeken 2010). To diagnose these diseases and to further specify the molecular defect, electrophoresis with isoelectric focusing of serum transferrin has been supplemented by mass spectroscopy. Affected children usually have neurological impairment and neuropathological features may be distinct (Agarwal et al. 2007). Liver morphological changes are absent or mild and nonspecific; thus, liver biopsy is neither helpful nor necessary. Reported findings include steatosis, dilatation of endoplasmic reticulum, membranous bodies, and rarely siderosis and micronodular cirrhosis (Iancu et al. 2007).

## 13.19 Genetic Hemolytic Disorders

A host of uncommon genetically determined hemolytic disorders are capable of causing liver injury, as a consequence of either anemia, heart failure, or overloading the immature liver with the products of rapid hemoglobin degradation, resulting in the inspissated bile syndrome. In the prenatal period, rapid hemolysis due to hemoglobinopathy or to Rh incompatibility may cause hydrops fetalis. Liver lesions include abnormal persistence and often left-shifted sinusoidal erythropoiesis, siderosis involving the reticuloendothelial cells of sinusoids and portal spaces, and



**Fig. 13.34** Erythropoietic porphyria. Lobular cholestasis, giant cell transformation, and left-shifted erythropoiesis. H&E stain

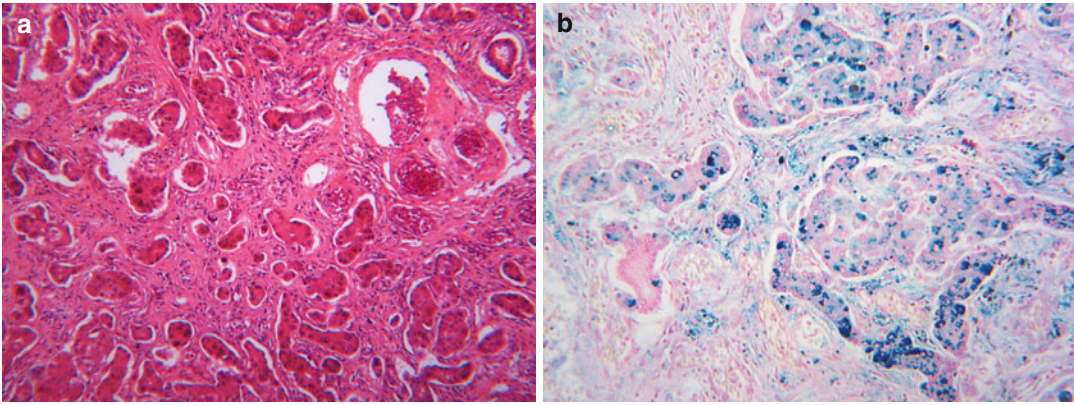
lobular cholestasis accompanied by giant cell transformation. If perfusion or oxygen-carrying capacity of the blood is impaired, liver necrosis may be superimposed.

Congenital erythropoietic porphyria is a recessive trait that impairs heme biosynthesis and shortens red cell survival (Desnick et al. 1998). The broad phenotype includes mild and severe forms, and the genotype shows extreme variation with poor genotype-phenotype correlation. Clinical presentation may occur at any age and includes rare reports of hydrops fetalis and/or liver failure during infancy. The liver lesion in affected infants is neonatal hepatitis like and presumably is related to excess hemolysis and liver immaturity, as with other neonatal hemolytic disorders. Brisk left-shifted erythropoiesis without myelopoiesis may provide a histological clue (Fig. 13.34).

## 13.20 Perinatal Hemochromatosis (PH)

### 13.20.1 Clinical Manifestations

PH is a poorly understood disorder characterized by acute liver failure beginning before, at, or shortly after birth caused by severe liver disease accompanied by excessive iron deposition in the parenchymal cells of the liver, pancreas, endocrine organs, glandular epithelia,



**Fig. 13.35** (a) Perinatal hemochromatosis at autopsy. The end-stage lesion is characterized by pervasive lobular fibrosis of nonspecific character. H&E stain. (b) Perinatal hemochromatosis. Systemic distribution of parenchymal

iron deposits that spares the reticuloendothelial system may include deposits of hemosiderin in bile duct epithelium as well as surviving hepatocytes. Iron stain

heart, and other sites (Silver et al. 1993). Death in early infancy is typical. The broad pattern of tissue siderosis in PH simulates hereditary hemochromatosis in adults, but no genetic relationship exists between the two conditions. Notable, but not understood, is the fact that reticuloendothelial cells in PH typically lack hemosiderin. Unique characteristics of PH are high recurrence rate in siblings, predilection for the perinatal period, the established absence of a connection to hereditary hemochromatosis in both affected infants and their mothers, and the diversity of the occasionally associated conditions ranging from infections to specific metabolic diseases. The associated stressors that have been identified in a minority of cases of PH are in utero infections, metabolic disorders (hereditary tyrosinemia, 3-oxosteroid 5-beta reductase deficiency, deoxyguanine kinase deficiency), in utero hemolytic disorders, exogenous iron overload, and severe hypoperfusion due to congenital heart disease or perinatal hypoxic-ischemic liver injury. Because such associations are uncommon, the high recurrence rate in siblings remains enigmatic. Whittington has proposed alloimmunity as a common cofactor and reports success in prevention of recurrence by treating mothers of an affected child with hyperimmune globulin prior to subsequent pregnancies. Perinatal hemochromatosis is now often referred to as gestational alloimmune hep-

atitis (Whittington and Hibbard 2004; Pan et al. 2010). This theoretical explanation for PH and the suggested therapeutic approach both require more validation and may not pertain in all cases.

### 13.20.2 Pathology and Diagnosis

Because protein synthetic failure limits use of liver biopsy for diagnosis of PH, knowledge of liver histology depends upon autopsy reports and study of explants, a successful rescue procedure in some cases. Liver changes include florid giant cell transformation, extensive parenchymal necrosis and collapse, and, in some cases, extensive fibrosis or established cirrhosis with regenerative nodules (Fig. 13.35a). Inflammation is usually inconspicuous. Occasionally, a pattern of sinusoidal/veno-occlusive disease is seen. Excessive iron deposition in hepatocytes is a regular feature but may not be particularly severe. Hemosiderin deposits in bile duct epithelium (Fig. 13.35b) suggest the possibility of more widespread tissue siderosis, a suspicion that may be explored further in life with a biopsy of oral submucosal glands or after death with a survey for hemosiderin deposits in parenchymal cells of other viscera. Deposition of antihuman C5b-9, the membrane attack complex of the complement system, has been reported in the cytoplasm of surviving hepatocytes (Pan et al. 2010).



It should be remembered that periportal hepatocytes normally contain hemosiderin early in infancy only to gradually lose it within several months of birth indicating that perinatal iron handling pathways may differ significantly during development from those in adults. Thus, the finding of a dusting of hemosiderin pigment in periportal hepatocytes of newborn infants is a normal feature and should not be taken as evidence for pathological storage of iron.

## References

- Agarwal B, Ahmed A, Rushing EJ, Bloom M, Kadom N, Vezina G, Krasnewich D, Santi M (2007) Congenital disorder of glycosylation-X: clinico-pathological study of an autopsy case with distinct neuropathologic features. *Hum Pathol* 38:1714–1719
- Ali M, Rellos P, Cox TM (1998) Hereditary fructose intolerance. *J Med Genet* 35:353–365
- Badizadegan K, Perez-Atayde AR (1997) Focal glycogenesis of the liver in disorders of ureagenesis: its occurrence and diagnostic significance. *Hepatology* 26:365–373
- Belay ED, Bresee JS, Holman RC et al (1999) Reye's syndrome in the United States from 1981 through 1997. *N Engl J Med* 340:1377–1382
- Bernier FP, Boneh A, Dennett X et al (2002) Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* 59(9):1406–1411
- Bosch AM (2006) Classical galactosaemia revisited. *J Inherit Metab Dis* 29:516–525
- Bove KE (2011) Chapter 10: Histologic patterns of metabolic disease. In: Saxena R (ed) *Practical hepatic pathology, a diagnostic approach*. Saunders, Philadelphia
- Bove KE, McAdams AJ, Partin JC et al (1975) The hepatic lesion in Reye's syndrome. *Gastroenterology* 69:685–697
- Bove KE, Heubi JE, Balistreri WF et al (2004) Bile acid synthetic defects and liver disease: a comprehensive review. *Pediatr Dev Pathol* 7:315–334
- Brennan SO, Wyatt J, Medicina D, Callea F, George PM (2000) Fibrinogen Breacia: hepatic endoplasmic reticulum storage and hypofibrinogenemia because of a gamma284 Gly—Arg mutation. *Am J Pathol* 157: 189–196
- Brennan SO, Davis RL, Conrad K, Savo A, Furuya KN (2010) Novel fibrinogen mutation gamma314Thr—Pro (fibrinogen AI DuPont) associated with hepatic fibrinogen storage disease and hypofibrinogenemia. *Liver Int* 30:1541–1547
- Brown T, Hug G, Lansky L et al (1976) Transiently reduced activity of carbamoyl phosphate synthetase and ornithine transcarbamylase in liver of children with Reye's syndrome. *N Engl J Med* 294:861–867
- Brunt EM et al (2011) Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology* 53(3):810–820
- Chalmers RA, Stanley CA, English N et al (1997) Mitochondrial carnitine-acylcarnitine translocase deficiency presenting as sudden neonatal death. *J Pediatr* 131:220–225
- Clayton PT (2002) Inborn errors presenting with liver dysfunction. *Semin Neonatol* 7:49–63
- Clayton PT (2003) Diagnosis of inherited disorders of liver metabolism. *J Inherit Metab Dis* 26:135–146
- Daugherty CC, Setchell KD, Heubi J, Balistreri WF (1993) Resolution of liver biopsy alterations in three siblings with bile acid treatment of an inborn error of metabolism (delta 4-3 oxosteroid 5 beta reductase deficiency). *Hepatology* 18:1096–1101
- Dehner LP, Snover DC, Sharp HL et al (1989) Hereditary tyrosinemia type I (chronic form): pathologic findings in the liver. *Hum Pathol* 20:149–158
- Demers SI, Russo P, Lettre F, Tanguay RM (2003) Frequent mutation reversion inversely correlates with the clinical severity in a genetic liver disease, hereditary tyrosinemia. *Hum Pathol* 34:1313–1320
- Desnick RJ, Glass IA, Xu W et al (1998) Molecular genetics of congenital erythropoietic porphyria. *Semin Liver Dis* 18:77–84
- DiDomenico P, Berry G, Bass D et al (2004) Noncirrhotic portal hypertension in association with juvenile nephropathic cystinosis: case presentation and review of the literature. *J Inherit Metab Dis* 27:693–699
- Dimauro S (2011) A history of mitochondrial diseases. *J Inherit Metab Dis* 34:261–276
- Ebberink MS, Mooijer PA, Gootjes J, Koster J, Wanders RJ, Waterham HR (2011) Genetic classification and mutational spectrum of more than 600 patients with a Zellweger spectrum disorder. *Hum Mutat* 32:59–69
- Fellman V, Kotarsky H (2011) Mitochondrial hepatopathies in the newborn period. *Semin Fetal Neonatal Med* 16:222–228
- Filacamo M, Morrone A (2011) Lysosomal storage disorders: molecular basis and laboratory testing. *Hum Genomics* 5:156–169
- Finsterer J (2004) Mitochondriopathies. *Eur J Neurol* 11:163–186
- Garone C, Tadesse S, Hirano M (2011) Clinical and genetic spectrum of mitochondrial neurogastrointestinal encephalomyopathy. *Brain* 134:3326–3332
- Gilbert-Barness E, Barness LA (eds) (2000) *Metabolic diseases: foundations of clinical management, genetics, and pathology*. Eaton Pub, Natick
- Gilbert-Barness E, Kapur RP, Oligny LL et al (eds) (2007) *Potter's pathology of the fetus, infant and child*. Mosby/Elsevier, Philadelphia
- Gordon N (2006) Alpers syndrome: progressive neuronal degeneration of children with liver disease. *Dev Med Child Neurol* 48:1001–1003
- Grabowski GA, Andria G, Baldellou A et al (2004) Pediatric non-neuronopathic Gaucher disease: presentation, diagnosis and assessment. Consensus statements. *Eur J Pediatr* 163:58–66

- Holve S, Hu D, Shub M et al (1999) Liver disease in Navajo neuropathy. *J Pediatr* 135:482–493
- Horvath GA, Davidson GA, Stockler-Ipsiroglu SG, Lillquist YP, Waters PJ, Olpin S, Andresen BS, Palaty J, Nelson J, Vallence H (2008) Newborn screening for MCAD deficiency: experience of the first three years in British Columbia. *Can. J Public Health* 99: 276–280
- Iancu M, Hajna M, Manov I, Cherug S, Knopf C, Mandel H (2007) The liver in congenital disorders of glycosylation: ultrastructural features. *Ultrastruct Pathol* 31: 189–197
- Ibdah JA, Bennett MJ, Rinaldo P et al (1999) A fetal fatty-acid oxidation disorder as a cause of liver disease in pregnant women. *N Engl J Med* 340:1723–1731
- Jaeken J (2010) Congenital disorders of glycosylation. *Ann N Y Acad Sci* 1214:190–198
- Kahn E, Daum F, Markowitz J et al (1986) Nonsyndromic paucity of interlobular bile ducts: light and electron microscopic evaluation of sequential liver biopsies in early childhood. *Hepatology* 6:890–901
- Karadimas CL, Vu TH, Holve SA et al (2006) Navajo neurohepatopathy is caused by a mutation in the MPV17 gene. *Am J Hum Genet* 79:544–548
- Kelly DA, Portmann B, Mowat AP et al (1993) Niemann-Pick disease type C: diagnosis and outcome in children, with particular reference to liver disease. *J Pediatr* 123:242–247
- Kleiner DE et al (2005) Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 41(6):1313–1321
- Koprivica V, Stone DL, Park JK et al (2000) Analysis and classification of 304 mutant alleles in patients with type 1 and type 3 Gaucher disease. *Am J Hum Genet* 66:1777–1786
- Labarthe F, Dobbelaere D, Devisme L et al (2005) Clinical, biochemical and morphological features of hepatocerebral syndrome with mitochondrial DNA depletion due to deoxyguanosine kinase deficiency. *J Hepatol* 43:333–341
- Latham PS, LaBrecque DR, McReynolds JW et al (1984) Liver ultrastructure in mitochondrial urea cycle enzyme deficiencies and comparison with Reye's syndrome. *Hepatology* 4:404–407
- Lefkowitz JH, Lobritto SJ, Brown RS Jr et al (2006) Ground-glass, polyglucosan-like hepatocellular inclusions: a "new" diagnostic entity. *Gastroenterology* 131:713–718
- Lindner M, Hoffmann GF, Matern D (2010) Newborn screening for disorders of fatty acid oxidation: experience and recommendations from an expert meeting. *J Inherit Metab Dis* 33:521–526
- Mandel H, Hartman C, Berkowitz D et al (2001) The hepatic mitochondrial DNA depletion syndrome: ultrastructural changes in liver biopsies. *Hepatology* 34:776–784
- Masurel-Paulet A, Poggi-Bach J, Rolland MO et al (2008) NTBC treatment in tyrosinaemia type I: long term outcome in French patients. *J Inherit Metab Dis* 31: 81–87
- McAdams AJ, Hug G, Bove KE (1974) Glycogen storage disease, types I to X: criteria for morphologic diagnosis. *Hum Pathol* 5:463–487
- Miles L, Heubi JE, Bove KE (2005) Hepatocyte glycogen accumulation in patients undergoing dietary management of urea cycle defects mimics storage disease. *J Pediatr Gastroenterol Nutr* 40:471–476
- Mori T, Nagai K, Mori M et al (2002) Progressive liver fibrosis in late-onset argininosuccinate lyase deficiency. *Pediatr Dev Pathol* 5:597–601
- Moses SW, Parvari R (2002) The variable presentations of glycogen storage disease type IV: a review of clinical, enzymatic and molecular studies. *Curr Mol Med* 2:177–188
- Nicastro E, Rannucci G, Vajro P, Vegnente A, Iorio R (2010) Re-evaluation of the diagnostic criteria for Wilson disease in children with mild liver disease. *Hepatology* 52:1948–1956
- Olpin SE, Evans M-J (2004) Investigations of inherited metabolic disease after death. In: Ruddy GN (ed) *Essentials of autopsy practice: recent advances, topics and developments*. Springer, London, pp 17–44
- Pan X, Kelly S, Melin-Aldana H et al (2010) Novel mechanism of fetal hepatocyte injury in congenital alloimmune hepatitis involves the terminal complement cascade. *Hepatology* 51:2061–2068
- Park WD, O'Brien JF, Lundquist PA, Kraft DL, Karnes PS, Patterson MC, Snow K (2003) Identification of 58 novel mutations in Niemann-Pick disease type C: correlation with biochemical phenotypes and importance of PTC-1-like domains in NPC1. *Hum Mutat* 22:313–325
- Partin JC, Schubert WK, Partin JS (1971) Mitochondrial ultrastructure in Reye's syndrome (encephalopathy and fatty degeneration of the viscera). *N Engl J Med* 285:1339–1343
- Patterson MC, Hendriksz CJ, Walterfang M, Sedel F, Vanier MT, Wijburg F (2012) Recommendations for the diagnosis and management of Niemann-Pick disease, type C: an update. *Mol Genet Metab* 106:330–344
- Perlmutter DH (2004) Alpha-1-antitrypsin deficiency: diagnosis and treatment. *Clin Liv Dis* 8:839–859
- Popescu I, Dima SO (2012) Domino liver transplantation: how far can we push the paradigm? *Liver Transpl* 18:22–28
- Ribeiro D, Castro I, Fahimi HD, Schrader M (2012) Peroxisome morphology in pathology. *Histol Histopathol* 27:661–676
- Ries M, Gupta S, Moore DF et al (2005) Pediatric Fabry disease. *Pediatrics* 115:e344–e355
- Sampietro M, Iolascon A (1999) Molecular pathology of Crigler-Najjar type I and II, and Gilbert's syndromes. *Haematologica* 84:150–157
- Sarzi E, Bourdon A, Chretien D et al (2007) Mitochondrial DNA depletion is a prevalent cause of multiple respiratory chain deficiency in childhood. *J Pediatr* 150: 531–534
- Saudubray JM, Martin D, de Lonlay P et al (1999) Recognition and management of fatty acid oxidation

- defects: a series of 107 patients. *J Inher Metab Dis* 22:488–502
- Schwimmer JB et al (2005) Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 42(3):641–649
- Setchell KD, Heubi JE, Lavine J, Shah S, Suskind D et al (2013) Genetic defects in bile acid conjugation cause fat-soluble vitamin deficiency. *Gastroenterol* 144:945–955
- Shin YS (2006) Glycogen storage disease: clinical, biochemical, and molecular heterogeneity. *Semin Pediatr Neurol* 13:115–120
- Silva MF, Aires CC, Luis PB et al (2008) Valproic acid metabolism and its effects on mitochondrial fatty acid oxidation: a review. *J Inher Metab Dis* 31:205–216
- Silver MM, Valberg LS, Cutz E et al (1993) Hepatic morphology and iron quantitation in perinatal hemochromatosis. Comparison with a large perinatal control population, including cases with chronic liver disease. *Am J Pathol* 143:1312–1325
- Sinha J, Magid MS, VanHuse C et al (2007) Bile duct paucity in infancy. *Semin Liver Dis* 27:319–323
- Sokol RJ, Twedt D, McKim J, Devereaux MW, Karrer FM, Kam I, von Steigman G, Narkewicz MR, Bacon BR, Britton RS et al (1994) Oxidant injury to hepatic mitochondria in patients with Wilson disease and Bedlington terriers with copper toxicosis. *Gastroenterology* 107:1788–1798
- Stocker JT, Dehner LP, Husain AN (eds) (2011) *Stocker & Dehner's pediatric pathology*, 3rd edn. Lippincott Williams & Wilkins, Philadelphia
- Summar ML, Dobbelaere D, Brusilow S et al (2008) Diagnosis, symptoms, frequency and mortality of 260 patients with urea cycle disorders from a 21-year, multicentre study of acute hyperammonaemic episodes. *Acta Paediatr* 97:1420–1425
- Thurberg BL, Wasserstein MP, Schians T, O'Brien F, Richards S, Cox GF, McGovern MM (2012) Liver and skin histopathology in adults with acid sphingomyelinase deficiency (Niemann-Pick disease type B). *Am J Surg Pathol* 36:1234–1246
- Treem WR, Witzleben CA, Piccoli DA et al (1986) Medium-chain and long-chain acyl-CoA dehydrogenase deficiency: clinical, pathologic and ultrastructural differentiation from Reye's syndrome. *Hepatology* 6:1270–1278
- Tuchman M, Lee B, Lichter-Konecki U et al (2008) Cross-sectional multicenter study of patients with urea cycle disorders in the United States. *Mol Genet Metab* 94:397–402
- Turnbull J, Wang P, Girard JM, Ruggieri A, Wang TJ et al (2010) Glycogen hyperphosphorylation underlies lafora body formation. *Ann Neurol* 68:925–933
- van der Ploeg AT, Reuser AJ (2008) Pompe's disease. *Lancet* 372:1342–1353
- Vockley J, Whiteman DA (2002) Defects of mitochondrial beta-oxidation: a growing group of disorders. *Neuromuscul Disord* 12:235–246
- Whittington PF, Hibbard JU (2004) High-dose immunoglobulin during pregnancy for recurrent neonatal haemochromatosis. *Lancet* 364:1690–1698
- Williams TB, Daniels M, Puthenveetil G, Chang R, Wang RY, Abdenur JE (2012) Pearson syndrome: unique endocrine manifestations including neonatal diabetes and adrenal insufficiency. *Mol Genet Metab* 106:104–107
- Willis A, Vanhuse C, Newton KP et al (2008) Farber's disease type IV presenting with cholestasis and neonatal liver failure: report of two cases. *Pediatr Dev Pathol* 11:305–308
- Wong LJ, Brunetti-Pierri N, Zhang Q et al (2007) Mutations in the MPV17 gene are responsible for rapidly progressive liver failure in infancy. *Hepatology* 46:1218–1227
- Yang Z, Yamada J, Zhao Y et al (2002) Prospective screening for pediatric mitochondrial trifunctional protein defects in pregnancies complicated by liver disease. *JAMA* 288:2163–2166
- Yeh JN, Jeng YM, Chen HL et al (2006) Hepatic steatosis and neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) in Taiwanese infants. *J Pediatr* 148:642–646
- Yik WY, Steinberg SJ, Moser AB et al (2009) Identification of novel mutations and sequence variation in the Zellweger syndrome spectrum of peroxisome biogenesis disorders. *Hum Mutat* 30:E467–E480
- Zischa H, Lichtmanegger J, Schmitt S, Jagemann N, Schulz S, Wartini D, Jennen L, Rust C, Larochette N, Galluzzi L, Chajes V, Bandow N, Gilles VS, DiSpirito AA, Esposito J, Goertlicher M, Summer KH, Kroemer G (2011) Liver mitochondrial membrane crosslinking and destruction in a rat model of Wilson disease. *J Clin Invest* 121:1508–1518