Clinical Aspects of Liver Diseases

31 Metabolic disorders and storage diseases

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1 Definition

Primary metabolic disorders and storage diseases are caused by endogenous factors, usually a gene mutation. Since the congenital defect is predominantly or exclusively located in the liver, the resulting diseases also become manifest in this organ.

Secondary metabolic disorders and storage diseases are present in almost all liver diseases and occur with more or less pronounced intensity. • They are, however, also caused by faulty nutrition as well as by many exogenous factors or noxae – just as latent metabolic disorders may generally become manifest due to such factors.

Thesaurismoses are storage diseases caused by the accumulation of metabolic products or substances in body fluids, organs or cells as a result of metabolic disorders.

2 Pathogenesis

► The pathogenic processes leading to the development of **primary** metabolic disorders or storage diseases are essentially caused by *two mechanisms*: (*1.*) formation of atypical macromolecules and (*2.*) extensive or complete blockage of a metabolic pathway. • These pathologically formed macromolecules cannot be broken down or secreted, with the result that they accumulate in the cells, including hepatocytes, or in the extracellular spaces. • Blockage of a metabolic pathway leads to (*1.*) insufficient production of certain metabolites, (*2.*) metabolite accumulation at the point of blockage, or (*3.*) formation of abnormal metabolites. This gives rise to the respective primary metabolic disorder or storage disease. It is, however, largely unclear what kind of mechanisms are actually responsible for liver cell damage.

► The pathogenesis of **secondary** metabolic disorders or storage diseases depends on the specific metabolic influence resulting from the character of the existing liver disease as well as other individual factors (e. g. malnutrition, alcohol abuse, chemicals, toxins). • Either a severe deficiency or an excessive supply of nutrients can cause persistent disorders in the hepatic metabolism and possibly result in morphological changes. That may also trigger a latent metabolic disorder which leads to further hepatocellular damage. This is especially true when the hepatic metabolism is compromised by a combination of stress factors, such as are present with diabetes mellitus, obesity, alcoholism or hyperlipidaemia.

► In primary or secondary metabolic disorders or storage diseases, almost all **metabolic functions** of the liver may be affected.

Bilirubin metabolism (= jaundice) **Bile acid metabolism (= cholestasis)**	
Amino acid metabolism	Lipid metabolism
Carbohydrate metabolism	Lipoprotein metabolism
Copper metabolism	Mucopolysaccharide metabolism
Glycolipid metabolism	Porphyrin metabolism
Iron metabolism	Protein metabolism

Genetically induced disorders of **bilirubin metabolism** affect (*1.*) bilirubin conjugation or (*2.*) bilirubin excretion through the canalicular membrane. This results in *functional hyperbilirubinaemia*. (s. tabs. 12.1, 12.4) (*see chapter 12*)

Genetically induced disorders of **bile acid metabolism** cause non-obstructive intrahepatic *cholestasis*. Cholestasis due to primary storage diseases also belongs to this group of disorders. (s. tab. 13.4) (*see chapter 13*)

3 Non-alcoholic fatty liver disease

3.1 Physiological aspects

Usually, the liver contains 0.8–1.5% of its wet weight in the form of extractable, finely dispersed structural fats, which cannot be detected by normal histological techniques. Under the light microscope, the liver fat, which is mainly made up of small droplets of triglycerides, only becomes visible when an increase up to 2–3% occurs. Above this value, hepatocytes "register" this event as a pathological process per se.

Triglycerides: Triglycerides are formed by the esterification of fatty acids, with glycerophosphate being produced by glycolysis. • Short- and medium-chain fatty acids from foodstuffs as well as fatty acids derived from lipolysis within adipose tissue are bound to albumin and transported to the liver cells through the portal vein. Long-chain fatty acids from foodstuffs become inserted as triglycerides in the chylomicrons within the mucosal cells of the small intestine. After having been broken down by endothelial lipoprotein lipase, the chylomicrons reach the hepatocytes in the form of remnants together with fatty acids. There, the fatty acids are beta-oxidized (to acetate and ketone bodies) in order to release energy or to synthesize phospholipids, cholesterol ester and triglycerides. The liver cell is also capable of de-novo synthesis of fatty acids from acetyl coenzyme A. Triglycerides are synthesized rapidly, particularly

when there is a sufficient supply of α-glycerophosphate and acetyl coenzyme A. This also explains the influence of glucose and insulin on the regulation of the hepatocellular metabolism. High carbohydrate (and alcohol) levels increase the esterification of fatty acids to form glycerides. Partial conjugation of lipids and apoproteins takes place at the contact surfaces (i.e. membranes) of the smooth and rough endoplasmic reticulum, while the carbohydrate component is subsequently added within the Golgi complex, so that a complete VLDL particle is formed. It is only in this form, and together with cellular membrane lipids, that triglycerides can be actively exported from the liver cell by way of exocytosis. Otherwise, both retention and thus storage occur in the liver cell. • VLDL particles secreted from the hepatocyte are once more broken down in the capillaries by lipoprotein lipase to form LDL and fatty acids. In this way, the fatty acids can be reused as an energy source, stored in fat depots or remetabolized in the hepatocytes. • The lipids stored in fat depots are likewise broken down by triglyceride lipase into fatty acids (and glycerin) and reach the liver cells again via the blood stream, where the same possibilities of metabolization exist. • The liver cell is capable of either synthesizing or metabolizing lipid substances separately within itself, both in terms of time and place. *It should be noted that these metabolic processes can occur parallel to and independently of each other.* (s. p. 47) (s. fig. 3.8)

3.2 Definition

Liver steatosis is defined as a condition when there are small or medium-sized fat droplets in singular, disseminated liver cells and when the fat content is 3–10% of liver wet weight. • **Fatty liver** is defined as a condition when the fat storage is >10% of liver wet weight, when >50% of the hepatocytes contain fat droplets in different sizes (small to large) and when fat deposition shows a diffuse pattern in the parenchyma. • *Storage of fat in the liver cell is the most common form of morphological hepatocellular damage.*

▶ **Symptom:** Low-grade fat storage in liver cells, ranging from the deposition of tiny to medium-sized droplets, is deemed to be a mere symptom (or "metabolic siding") and therefore a harmless phenomenon. • Such a finding can be seen as a "dynamic" (short-term) liver steatosis after a high-fat meal, particularly together with alcohol.

▶ **Disease:** A diffuse fatty liver showing medium and large-sized droplets is regarded as a disease when the severity increases to a point where >50% of hepatocytes are involved (= *"from symptom to disease"*). Occasionally, excessive fat storage occurs (up to 100% of hepatocytes involved) with a high mortality rate, e.g. in acute alcohol intoxication (s. fig. 28.6) and acute poisoning due to chemicals or toxins. A maximum fat storage rate of 24% of liver wet weight has been observed.

3.3 Pathogenesis

When the hepatocytes are continuously inundated by fatty acids, from either the intestines or fat depots, their oxidative degradation or synthesis capacity may be reduced and triglyceride binding to lipoproteins can be depleted. This is most likely to happen whenever apoprotein synthesis or lipoprotein formation is compromised by noxae (e.g. alcohol). • Fat storage in hepatocytes is a *question of equilibrium* since accelerated or increased formation of triglycerides in the liver cell is *not* compensated by sufficient synthesis of lipoproteins and adequate secretion of VLDL from the liver cell. • Therefore, both exogenous and endogenous pathogenic factors may be responsible for the development of liver steatosis. In this process, additive as well as potentiating metabolic disorders sometimes occur, resulting in a biochemical/morphological *vicious circle.* (s. tab. 31.1)

Exogenous
1. **Increase in lipid uptake from the intestine**
2. **Enhanced supply of glyceride precursors** (glucose, fructose, galactose)

Endogenous
1. **Increase in peripheral lipid mobilization**
   - Lipolysis ↑ (= triglyceride lipase activity ↑) (by ACTH, cortisol, catecholamines, prostaglandins, caffeine, alcohol, nicotine)
2. **Inhibition of lipid utilization in hepatocytes**
   - β-oxidation ↓
   - Fatty acid-binding protein ↓
3. **Increase in lipid synthesis in hepatocytes**
   - Formation of fatty acids ↑
   - Formation of triglycerides ↑
4. **Reduction in lipid export**
   - Secretion of VLDL ↓
   - Synthesis of apoproteins B, C_1–C_3, E ↓
   - Disturbance in gluconeogenesis

Tab. 31.1: Pathogenesis of liver steatosis and fatty liver

3.4 Morphology

▶ The earliest histological criteria of **fatty liver** were described by W. Bowman in 1842. • As early as 1861, F.T. Frerichs demonstrated the development of hepatic steatosis and its reversibility in dogs. He gave a detailed description of minute fat droplets in liver cells and their continuous growth. Even at that time, he differentiated between **fatty infiltration** and **fatty degeneration.** Indeed, he considered fatty degeneration to be more "pernicious" than fatty infiltration. (1861, volume I, pp 285–324; figs. 41, 42) • (see below: W.S. Hartroft et al., 1968)

3.4.1 Fatty infiltration

Fat storage in liver cells starts with tiny droplets being deposited inside the endoplasmic reticulum and cytoplasm. This fatty deposition in the form of **small droplets** without a surrounding membrane is also called *fatty infiltration* (type A) (W.S. Hartroft et al., 1968). (14) Ini-

Formation of fat droplets

1. Deposits of tiny fat droplets
   Formation of small fat droplets
   = **small-droplet liver steatosis**

↓

2. Increase in size of fat droplets
   = **medium-droplet liver steatosis**

↓

3. Further increase in size of fat droplets
   Confluence of fat droplets
   Impairment of nuclear functions
   = **large-droplet liver steatosis**

↓

= mixed-droplet liver steatosis
= **formation of fat cysts**

Localization of liver steatosis

1. **peripheral**
2. **centroacinar**
3. **zonal**
4. **diffuse**

Liver steatosis

1. *Low-grade liver steatosis*
   = ca. 5% of WW
   = 10–15% of hepatocytes
2. *Medium-grade liver steatosis*
   = 6–8% of WW
   = 15–30% of hepatocytes
3. *Pronounced liver steatosis*
   = 8–9% of WW
   = 30–50% of hepatocytes

Fatty liver

4. ***Severe fatty changes in liver cells***
   = 10–12% of WW
   = > 50% of hepatocytes
   (medium-sized and large droplets, diffuse)
5. ***High-grade fatty changes in liver cells***
   = 12–17% of WW (300–500 mg/g)
   = > 70% of hepatocytes

Tab. 31.2: Formation, localization and various courses of liver steatosis resulting in the development of fatty liver (WW = wet weight of liver)

tially, only a few hepatocytes are affected by small-droplet deposits, whereas later on whole clusters are grouped together, mainly at the periphery of the lobules or in the centroacinar area. • Slowly but steadily, the droplets become larger, resulting in **medium-droplet steatosis.** The stored fatty droplets increase further in size and eventually fill the hepatocytes completely (= *macrovesicular steatosis*). (19, 23, 39, 48) (s. tab. 31.2)

As a result of **large-droplet steatosis,** the hepatocytes become enlarged, the fine cytoplasmatic structures are destroyed and the nucleus is pushed towards the cellular membrane. The functions of the nucleus are impaired or even halted. This also results in a strong tendency of the fatty cells to develop necrosis even under mildly toxic conditions. At the same time, the hepatocyte loses more and more glycogen. **Glycogen vacuolations of the nuclei** form when nuclear glycogen is removed. (s. fig. 31.1) They are observed in various diseases (e. g. diabetes mellitus), mostly in the area of the portal fields. • So-called **fat cysts** of various sizes are the result of large-droplet steatosis due to the cellular membrane being torn apart. (s. fig. 31.2) This fat-storing process results ultimately in a diffuse **mixed-droplet** fatty liver. (s. fig. 31.3) • **Peripheral** fat deposits (zone 1) are mainly caused by infectious or toxic damage directly affecting the

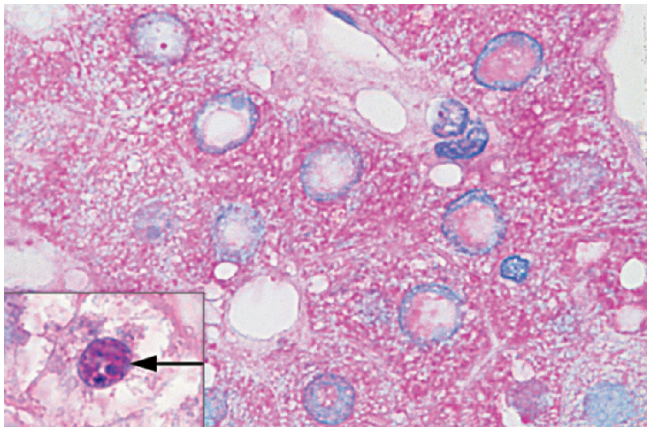

Fig. 31.1: Glycogenated nuclei (so-called glycogen vacuolations of the nuclei) in diabetes mellitus. *Insert:* nucleus strongly laden with glycogen (↑) (PAS) (s. pp 402, 540, 605, 613, 630, 639)

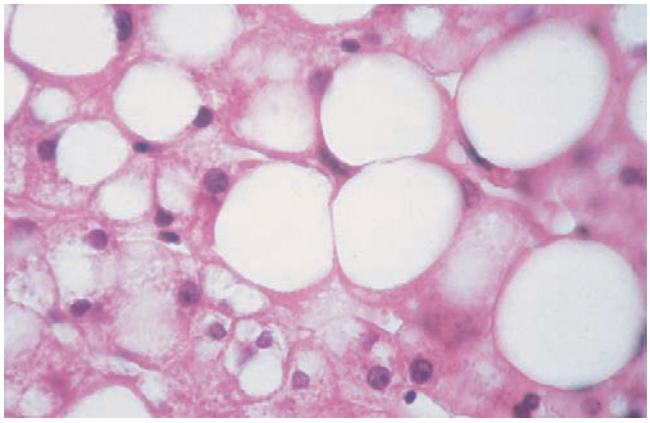

Fig. 31.2: Large-droplet (coarse-vacuolar) fatty liver in diabetes mellitus (preliminary stage of fat cyst formation) (HE)

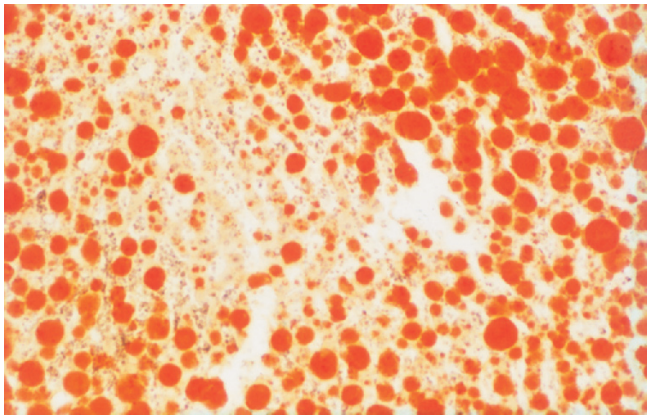

Fig. 31.3: Pronounced mixed-droplet fatty liver (Sudan red)

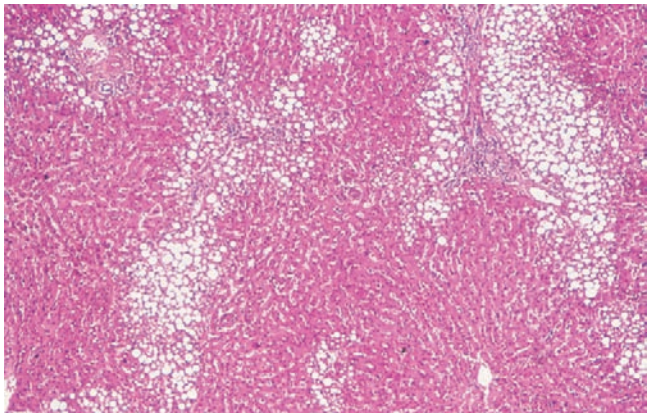

Fig. 31.4: Steatosis in periportal liver parenchyma. Clinical diagnosis: chronic phosporus poisoning (HE)

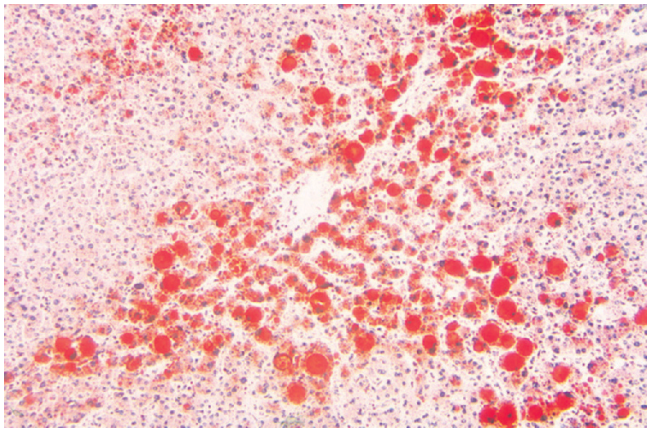

Fig. 31.5: Centroacinar steatosis. Clinical diagnosis: chronic alcohol abuse (Sudan red)

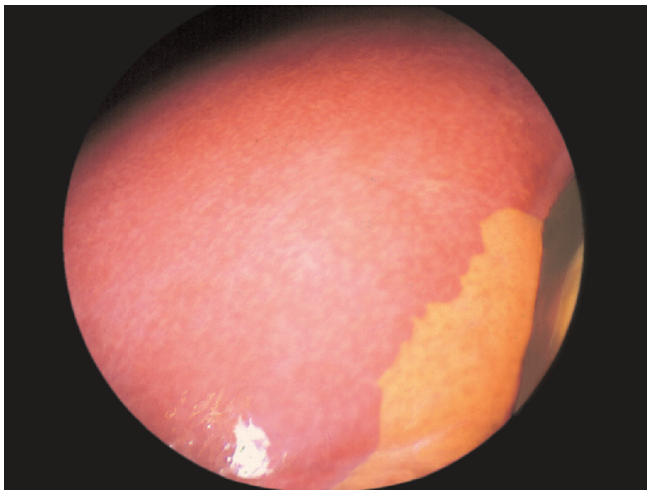

Fig. 31.6: Fatty liver with pronounced, thumb-sized fatty changes at the periphery of the left lobe (so-called "yellow spot")

periphery, where the liver cells are more involved in lipid metabolism via the blood stream. (s. fig. 31.4) • **Centroacinar** steatosis (zone 3) is often found in O_2 deficiency, nutrition-related damage, diabetes mellitus and alcohol abuse. It is seen as the more severe form of damage. (s. fig. 31.5) • **Zonal** steatosis occurs when only certain zones or areas of a lobule are affected. • This eventually results in **diffuse** steatosis of the whole lobule. (s. fig. 31.3)

In general, the left lobe shows less homogeneous steatosis than the right lobe. Regionally more pronounced fatty changes – which were formerly known laparoscopically as "yellow spots" (s. fig. 31.6), can be clearly detected as **focal** (s. fig. 8.3) or **segmental** fatty infiltration (s. fig. 8.4) by means of CT. (8, 12, 17, 25, 39)

3.4.2 Fatty degeneration

This form of microvesicular fat storage in hepatocytes, which is also termed type B (W.S. Hartroft et al., 1968) (14), is a rare but prognostically serious condition. The cytoplasm is filled with small non-confluent fat particles which are surrounded by a delicate membrane. This may give the hepatocytes a foamy appearance. The nucleus remains largely unmodified in the centre of the cell.

Such *microvesicular degeneration* can be found in various thesaurismoses, different liver conditions and numerous drug-induced diseases. (15) (s. tab. 31.3)

1. **Acute fatty liver of pregnancy**
2. **Alcoholic foamy fat syndrome**
3. **Jamaican vomiting sickness** (33)
4. **Kwashiorkor** (29, 37)
5. **Reye's syndrome**
6. **Thesaurismoses**
   - cholesterol ester storage disease
   - disturbance of the urea cycle
   - HDV hepatitis in northern South America
   - Wolman's disease
7. **Drugs**
   - acetylsalicylic acid
   - amineptin
   - fialuridine
   - ibuprofen
   - ketoprofen
   - pirprofen
   - tetracycline
   - valproic acid
   - warfarin
   - *etc.*

Tab. 31.3: Causes of microvesicular fatty degeneration (with some references)

3.4.3 Phospholipidosis

Enhanced lysosomal storage of phospholipids due to the inhibition of phospholipases is another special form of hepatic steatosis. The hepatocytes are enlarged and exhibit a distinctive foamy lucency of the cytoplasm. Crystalline inclusions and an agglomeration of myelin structures are visible in the lysosomes using electron microscopy. Mallory-Denk bodies may also be found. This idiosyncratic metabolic liver damage can even develop into cirrhosis. (s. tabs. 29.2; 31.4)

1. Amiodarone	**4. Chlorpromazine**
2. Amitriptyline	**5. Imipramine**
3. Chloroquine	**6. Perhexiline maleate**

Tab. 31.4: Some drugs causing phospholipidosis

3.5 Causes

Causes of fatty liver are manifold, and combinations of causes quite common. **Acquired causes** are by far the most frequent, but there are also rare causes, e. g. coeliac disease (9, 24), parenteral nutrition. (27, 28) • **Congenital metabolic disorders** can also lead to the development of a fatty liver, as in the case of a rare thesaurismosis. • It is of considerable therapeutic and prognostic importance to differentiate between an alcoholic fatty liver (AFL) and alcoholic steatohepatitis (ASH) (s. pp 529, 531) as well as between non-alcoholic fatty liver (NAFLD) and non-alcoholic steatohepatitis (NASH). (2, 19, 23, 34) (s. tabs. 31.5–31.7)

The frequency of NAFLD in the general population is given as 3–58%, whereby the great variability is due to socio-economic factors (average value is 20–23%). The development of NAFLD is more closely correlated with obesity than with alcohol abuse. In combined obesity and alcohol abuse, the frequency of fatty liver is estimated to be 50–60%. About one third of overweight patients suffer from type II diabetes, which in turn is responsible for NAFLD. Obesity can also lead to the formation of ASH and NASH. Consequently, if several causal factors of NAFLD coincide, the frequency and severity of fatty liver increases.

1. **Nutritional causes**
   Gastric bypass
   Hyperalimentation/obesity (1, 4, 5, 18, 32, 49, 73)
   Jejunoileostomy
   Malnutrition (11)
   – malabsorption, starvation, kwashiorkor (29, 37)
   Parenteral feeding (27, 28)
2. **Metabolic disorders**
   Diabetes mellitus (21, 32, 38)
   Gout
   Hyperlipidaemia (54)
   Thesaurismoses (s. tab. 31.6)
3. **Alcohol**
4. **Drugs** (s. tabs. 29.11; 31.3, 31.4, 31.7)
5. **Chemical substances** (47)
6. **Phytotoxins, mycotoxins**
7. **Infections**
   Bronchiectasis
   Chronic osteomyelitis
   Chronic tuberculosis
   Hepatitis C
   HIV infection
   Sprue
   Ulcerative colitis/Crohn's disease (9, 24)
   Yellow fever
8. **Oxygen deficiency**
   – anaemic – respiratory – cardiac
9. **Endocrinopathies**
   Acromegaly
   Cushing's syndrome
   Myxoedema (56)
10. **Liver surgery**
    Liver resection, jejuno-ileal bypass
    Primary dysfunction of a transplanted liver (44, 59)
11. **Cryptogenic fatty liver** (10)

Tab. 31.5: Acquired causes of liver steatosis or fatty liver (including some references)

Abetalipoproteinaemia	Mucoviscidosis
Cholesterol ester storage	Niemann-Pick disease
Fructose intolerance	Refsum's disease
Galactosaemia	Sphingolipidosis
Glycogenoses	Tyrosinaemia
Homocystinuria	Weber-Christian disease
Hypoalphalipoproteinaemia	Wilson's disease
Mauriac syndrome	Wolman's disease
Mucolipidosis	*etc.*

Tab. 31.6: Congenital causes of liver steatosis or fatty liver (so-called thesaurismoses)

1. **Medication**
   Flurazepam
   Glucocorticosteroids
   Hydrazine
   Mercaptopurine
   Methotrexate
   Naproxen
   Nifedipine
   Phenylbutazone
   Probenecid
   Rifampicin
   STH
   Tamoxifen, *etc.*
2. **Chemical substances**
   Antimony
   Arsenic
   Chloronaphthalene
   Carbon tetrachloride
   Chloroform
   Chromium
   DDT
   Dinitrobenzene
   Dioxins
   Hexachlorocyclohexane
   Lead
   Pentachloroethane
   Phosphorus
   Tetrachloroethane
   Toluilendiamine, *etc.*
3. **Phytotoxins and mycotoxins**
   Aflatoxins
   Amanitins
   Gyromitrin, *etc.*

Tab. 31.7: Liver steatosis or fatty liver due to medication, chemical substances or toxins (s. tab. 29.11!)

3.6 Non-alcoholic steatohepatitis

► In 1980 the term non-alcoholic steatohepatitis (NASH) was introduced by J. Ludwig et al. to denote chronic liver disease with increased enzymatic activity and the histological picture of alcohol-induced hepatitis. (57) • *The histological feature itself was described by* H. Thaler *as early as 1962 in his paper "Fatty liver and its relationship to cirrhosis".* (72) • Over the following years, transition from a diabetic fatty liver into cirrhosis (S. Itoh et al., 1979) and from an obesity-induced fatty liver into cirrhosis (M. Adler et al., 1979) were reported.

3.6.1 Definition

Histologically, non-alcoholic steatohepatitis shows moderate to high-grade, mainly macrovesicular fatty degeneration of the liver cells with inflammatory infiltrates and formation of fibrosis. Cirrhosis often develops. • Despite the morphological similarity to alcohol-induced fatty liver hepatitis, no (noteworthy)

alcohol consumption is involved in NASH. Viral or autoimmune hepatitis are not detectable either. • There are no or only moderate subjective complaints. The transaminases are normal or slightly elevated. NASH is mostly associated with obesity and/or type II diabetes. • *Thus NASH is regarded as the hepatic manifestation of a metabolic syndrome.*

3.6.2 Epidemiology

Once alcohol consumption, viral hepatitis or autoimmune hepatitis have been ruled out, NASH is deemed the most common cause of a long-term increase in the transaminases. • The information available on prevalence and general frequency is not yet sufficient. In 2−6% of the US population, however, an increase in GPT (ALT) was detected without chronic liver disease being diagnosed. (53) Based on liver biopsies, a prevalence of 1.2−9.0% was determined. NASH is frequently associated with obesity (ca. 40%), non-insulin-dependent diabetes mellitus (ca. 20%) and hyperlipidaemia (ca. 20%). Women (particularly in middle age) are more often affected than men. (49, 53, 73, 74) • NASH may indeed also be found in children. (58, 64, 69). In this context, it was associated with obesity in 83% and with a disorder of the lipid metabolism in 50% of cases, while diabetes mellitus could only be detected in 5% of patients (but with increasing frequency during the further course of life, as was shown in the follow-up). (64)

3.6.3 Pathogenesis

The development of **liver steatosis** is attributable to various exogenous and endogenous mechanisms, which may combine with and/or potentiate each other. Numerous causal factors must be considered in the pathogenesis of fatty liver. (s. tab. 31.1) • With the increasing storage of fat, the liver cells also become more and more vulnerable to **noxae.** Thus oxidative stress may cause augmented oxidation of free fatty acids in the peroxisomes as well as enhanced activity of cytochrome P450 2E1. The biotoxometabolites (e. g. malonyldialdehyde, 4-hydroxynonenal) arising during this process provoke inflammatory infiltrations and fibrosis.

Second-hit hypothesis: The *first hit* is considered to be the development of *fatty liver*, particularly due to hyperalimentation and obesity resulting in insulin resistance. *However, the presence of a fatty liver is no prerequisite for the development of NASH!* • As *second hit* follows the mobilization of free fatty acids from fat depots, especially in cases of central obesity, and their transport to the liver cells. This leads to a massive increase of *free radicals* due to *oxidative stress* with *lipid peroxidation* and induction of *cytokines* (TNFα, TGFβ, IL8, IL1). (s. pp 71−73, 408) As a result, there is a reactive formation of uncoupling protein (UCP2) with a subsequent decrease in hepatocyte ATP and a disturbance of macrophage function with higher sensitivity to endotoxin. This leads to an inflammatory reaction, cell death and the formation of fibrosis. (43, 62)

A *genetic basis* seems to be necessary as a predisposition of NASH; however, such mechanisms are still unknown. It is discussed that genes can influence the degree of oxidative stress, the severity of steatosis, the regulation of immune reactions and apoptosis.

The pathogenesis of NASH is multifactorial, i.e. in the presence of steatosis, it is attributed to the additional (even combined) influence of different noxae (e. g. oxygen deficiency, endotoxins, medicaments, chemicals, iron, biotoxometabolites). • There should be no laboratory findings pointing to alcohol abuse; chronic hepatitis B and C as well as autoimmune hepatitis (71) must also be ruled out.

► From present knowledge, it can be assumed that numerous cases of cryptogenic chronic hepatitis or cryptogenic cirrhosis are attributable to the presence of NASH in terms of aetiopathogenesis.

3.6.4 Morphology

The diagnosis of NASH and the assessment of its prognosis are most reliably derived from liver histology. • In the lobule, *steatosis* is distributed mainly in a macrovesicular and diffuse manner, but sometimes it is concentrated microvesicularly and perivenously. Glycogen vacuolation of nuclei is common. • The *inflammatory reaction* consists of granulocytic and lymphocytic infiltrates, which are more frequently found in the portal and perivenous area than intralobular, with or without focal necrosis. Cell ballooning, Mallory's hyaline and ubiquitin (60) are generally present, while megamitochondria are also found occasionally. Steatosis and mild lobular chronic inflammation alone are insufficient for diagnosing NASH. The histological alterations are mainly localized in zone 3. • First of all, *fibrosis* appears within the perisinusoidal area, then additionally within the portal field (41); subsequently, it develops as bridging fibrosis giving rise to architectural remodelling, and eventually results in *cirrhosis* with portal hypertension. The histological picture of NASH can be mistaken for chronic hepatitis C, steroid-treated AIH, drug-induced hepatitis or the early stage of Wilson's disease. It is even more difficult for the pathologist to differentiate between a cirrhosis as a result of NASH, chronic hepatitis C and AIH. Often a diagnosis of cryptogenic cirrhosis has to be made. (10, 39, 42, 43, 50, 52, 58, 60, 63, 67, 69) (s. fig. 31.7)

► There are no morphological differences between alcoholic steatohepatitis and NASH. • In some 30(−50)% of patients with NASH, predominantly micronodular cirrhosis develops within five years.

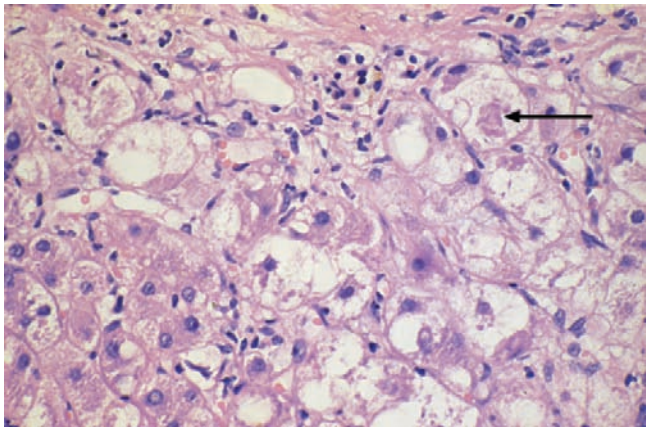

Fig. 31.7: Non-alcoholic steatohepatitis: Hydropic degenerated hepatocytes with Mallory's hyaline (←); lymphocytic and granulocytic infiltration as well as activated Kupffer cells (HE)

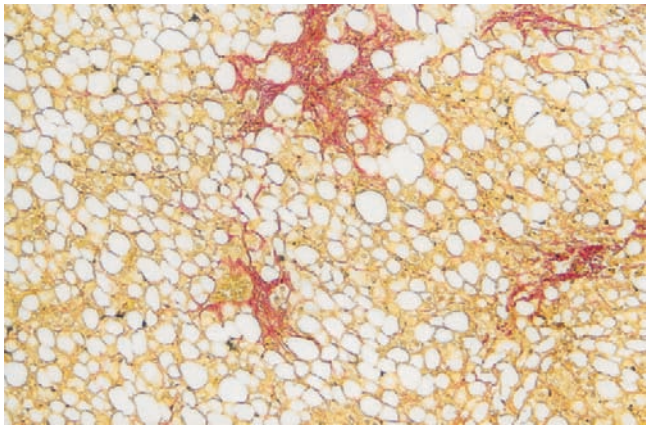

Fig. 31.8: Non-alcoholic steatohepatitis with massive steatosis and fibrosis. A 10-year-old boy with diabetes mellitus type 2

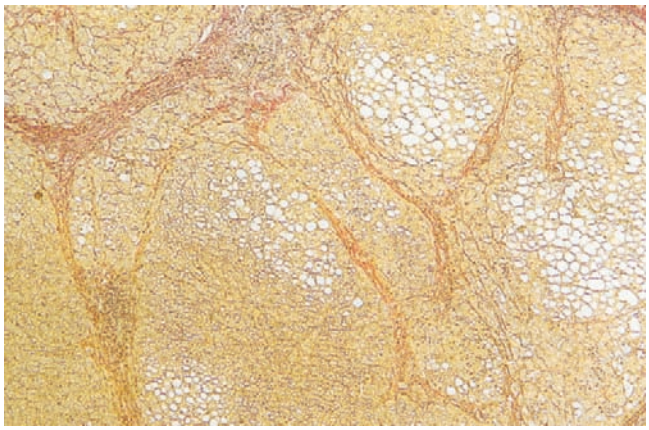

Fig. 31.9: Liver cirrhosis in non-alcoholic steatohepatitis. A 24-year-old adipose woman with insufficiently treated diabetes mellitus type 2 (Sirius red)

3.7 Diagnosis

Clinical findings: Steatosis does not generally cause any subjective complaints or clinical symptoms; it remains undetected or is discovered incidentally when diagnostic screening is being carried out for another disease. The presence of *risk factors* (overweight, diabetes, gout, hyperlipidaemia, medication, malnutrition, etc.) is of considerable diagnostic importance. • *Subjective complaints* include fatigue, repletion, malaise or loss of appetite. In hepatomegaly, right upper quadrant abdominal discomfort may occur, especially when stooping or lying on the right side. In obesity, which is present in most cases, hepatomegaly is often not palpable, so that the actual size of the liver can only be demonstrated by sonography (or CT). *Hepatomegaly* correlates with the severity of the fatty liver, allowing determination of the course. *Splenomegaly* is found in 20−25% of patients. (36) • In advanced cases, *skin stigmata* of liver disease and signs pointing to portal hypertension might be in evidence. Left ventricular diastolic dysfunction can be shown in ECG. (13) (s. tab. 31.8)

Laboratory diagnostics: With increasing severity of a fatty liver or NASH, some laboratory parameters are pathological in 80−90% of cases, i. e. 10−20% of patients show normal values. There is an initial rise in *γ-GT*, and subsequently in *GPT* and *GOT*. As a rule, the mildly to moderately increased values are detected purely by chance during a medical check-up. The DeRitis quotient is < 1 (cf. alcoholic hepatitis with > 1). While this constellation in itself is a useful hint of liver cell damage induced by toxins or metabolic processes, an increase in *cholinesterase* actually suggests the presence of disturbed lipid metabolism or a fatty liver. Decreased functioning of the liver, detectable at an early stage, can be determined by measuring the *galactose elimination capacity* (s. p. 114) or by applying the *indocyanine green test* (s. p. 114); these tests may also be used in long-term follow-up. • Other important laboratory parameters include alkaline phosphatase, bilirubin, electrophoresis, ferritin, leptin (46, 65), thioredoxin (70) and parameters connected with lipid metabolism. Progressive and severe courses correlate closely with laboratory values well outside the normal range. Sonographic signs of liver steatosis indicate screening for *diabetes mellitus*, if necessary with the help of the oral glucose tolerance test. (6, 34, 50, 52, 53, 68, 70) (s. tab. 31.8)

Sonography: Hepatomegaly can be confirmed by sonography (vertical diameter liver enlargement in MCL > 11−12 cm), with the liver mostly showing a plump form. Echogenicity is increased due to the high number of water/fat boundary layers, and the single reflexes are coarsened (unlike the renal parenchyma). The hepatic veins are difficult to visualize. In pronounced fatty liver, sonographic signs of portal hypertension may appear (= sinusoidal block). There is a positive correlation between the degree of fatty changes and the rise in echogenicity (= *large, white liver*). Fatty changes below 10−20% cannot be reliably detected by US. (s. p. 138) • Often, it is difficult to differentiate *focal fatty changes* (circumscribed density of the echo pattern) from malignant foci, so that further clarification using scintigraphy or CT is required. *Fibrosis* shows an inhomogeneous parenchymal structure in relation to the degree of severity. (7, 8, 12, 22) (s. tab. 31.8)

CT: In a fatty liver, CT scanning shows diffuse density reduction with a corresponding decrease in Hounsfield units (HU). (26) A fatty liver is markedly darker than the spleen (= *large, grey liver*). The vessels and bile ducts are hyperdense as compared to the parenchyma. There is a linear relationship between the extent of fat deposition and the reduction in density. The diagnostic reliability of a fatty liver determined by CT is 85–95%. *Focal fatty changes* or segmental fatty changes are easy to differentiate since, in contrast to metastases, they do not have any vascular branching. A 10% increase in the relative fat content results in a density reduction of 17–20 HU; i.e. in fatty changes of 80%, a density decrease to about −50 HU occurs. (s. p. 180) • Investigations have also been carried out to determine whether it is possible to quantify the fat content of the liver by **MRT.** (17, 20, 26) (s. tab. 31.8)

1. **Determination of potential risk factors** (obesity, alcohol, diabetes, gout, malnutrition, chemicals, hyperlipidaemia, medication, *etc.*)
2. **Hepatomegaly** **Splenomegaly**
3. **Laboratory parameters** • γ-GT ↑, GPT ↑, GOT ↑ • cholinesterase ↑, ferritin ↑ – galactose elimination capacity + – indocyanine green test + – alkaline phosphatase ↑ – triglycerides ↑, cholesterol ↑, glucose ↑ – lipid electrophoresis +, leptin ↑, thioredoxin ↑
4. **Sonography** **CT**
5. **Morphology** • percutaneous biopsy • laparoscopy + biopsy

Tab. 31.8: Diagnosis of fatty liver or NASH, involving determination of the degree of severity, differential diagnosis and course

Liver biopsy: Only morphology provides a definitive diagnosis of fatty changes in the liver cells or a fatty liver. Apart from revealing *fatty changes*, it is even more important histologically to detect *inflammatory reactions* and to determine whether there is any sign of *progressive fibrosis* (e.g. due to iron overload). (6) The result of grading and staging (42) is decisive for the prognosis. (s. tabs. 34.2, 34.3) • Laparoscopic evaluation of the liver surface is of considerable diagnostic importance, as is the targeted biopsy of hepatic areas which are suspected of being pathological.

3.8 Prognosis

A fatty liver has a good prognosis, since complete reversibility can be achieved if the causes are fully eliminated. However, existing fibrotic processes generally remain. • A fatty liver should in no way be underestimated, since it involves many **dangers:** (*1.*) the manifold liver cell functions may be distinctly compromised, causing unfavourable effects on the liver or the organism as a whole; (*2.*) fatty changes in the liver cells make them susceptible to noxae or toxins, so that there is an increased tendency towards steatonecrosis and an impaired regeneration capacity; (*3.*) a fatty liver responds strongly to inflammatory processes with mesenchymal reactions; (*4.*) a severe fatty liver sometimes results in a narrowing of the intrahepatic vessels with ensuing impairment of biliary flow and haemodynamics, or it may even lead to the development of portal hypertension. Prognosis is essentially dependent on whether the causes of fatty liver as well as any additional risk factors can be eliminated. NASH develops into fibrosis or cirrhosis within five to ten years in 10–40% of cases. Some 10–15% of these patients die within 10 years as a result of complications associated with cirrhosis. • Risk factors showing that NASH is progressing include: age > 50 years, body mass index > 30, type II diabetes, hyperlipidaemia, DeRitis quotient > 1, thrombopenia, and the presence of Mallory-Denk bodies. (16, 34, 52, 63, 67) • *Metastases* originating from a colorectal carcinoma are rarely found in fatty liver.

3.9 Complications

The occurrence of complications points to the fact that fatty liver should not generally be regarded as a harmless disorder. A fatty liver and the various risk factors may give rise to complications which occasionally manifest as a separate disease. (19, 34, 63) (s. tab. 31.9)

1. Development of fatty liver hepatitis progressing to fatty fibrosis (50%) or cirrhosis (15%) (e.g. via *non-alcoholic steatohepatitis*)
2. Formation of intrahepatic cholestasis with or without jaundice, possibly even similar to obstructive jaundice (3)
3. Fat embolism (R. Virchow, 1886) (30)
4. Compression and narrowing of sinusoids (31) with potentially reversible portal hypertension – but also with formation of collaterals and ascites
5. Intrahepatic narrowing of the inferior vena cava, with occurrence of leg oedema
6. Hepatic insufficiency (3%)

Tab. 31.9: Possible complications of fatty liver

Such complications must be reckoned with in fatty liver if (*1.*) the cause or any additional risk factors have not been eliminated and continue to have an effect, (*2.*) the underlying cause has not been identified despite all efforts (= *cryptogenic fatty liver*) and thus no real starting point

for therapeutic measures is given, and (*3*.) specific causes of fatty liver, and the occurrence of NASH in particular, already have a tendency towards progression. • In such patients, there is evidence of *liver cell necrosis* and inflammatory *infiltration*, both of which are reversible. However, in some cases, particularly with simultaneous *lipid peroxidation*, a vicious circle can develop with subsequent mesenchymal reactions. The outcome is increasing *fibrosis* or, in some cases, even *cirrhosis*.

3.10 Therapy

The search continues for a medicament that can normalize the disturbed hepatocellular lipid metabolism or bring about the release of the fat stored in liver cells.

► Fatty liver has no specific therapy. Exclusion of the *cause* and elimination of additional *risk factors* − in as far as these two basic therapeutic requirements can be accomplished − usually result in complete regression of steatosis. • Should these measures fail to bring about a regression within a period of three to six months, it is assumed that *either* exclusion of the cause and elimination of risk factors have not been successful *or* the real underlying cause was not identified, so that no effective treatment measures were actually applied. • A lack of regression thus necessitates (*1*.) renewed investigation of the cause and risk factors as well as (*2*.) consistent implementation of the therapy that results from this!

Strict *alcohol abstinence* is called for − even in cases where alcohol is not the cause of fatty liver. The same applies to the adjustment of the *blood-sugar levels* in diabetes mellitus or the *uric acid values* in gout. *Hyperlipidaemia* may likewise necessitate a specific drug therapy depending on the respective type and course. • In *obesity* due to overeating, it is imperative to lose weight (gradually!) by means of reduced caloric intake, especially a low-carbohydrate ketogenic diet (35), based on the principles of the physiology of nutrition. Proteins and water-soluble vitamins should be administered at a higher daily dosage than is usually required. The effect of dietary measures can generally be supported by *orlistat*. Regression of a fatty liver is best achieved by constant weight reduction (200−250 g/day). Given a normal body weight, reducing the intake of fat in the diet (below the level that is usually needed) does not influence the regression of hepatic steatosis. (11, 26)

Although the therapeutical principles described above are generally accepted knowledge, pharmacological possibilities for facilitating the release of fat from the liver cells by administering **specific medication** have been repeatedly sought in the past. This process is ongoing, but first results have been relatively promising.

Adjuvant therapy: In view of the fact that it has hitherto proved difficult to restore a fatty liver by means of medication, attention has focused on the possibility of averting the pathomorphological forms of damage described above (and thus the development of a complicative progression) with the help of medicaments as an adjuvant therapy.

Prevention of fibrosis is of great importance. Three substances have already proved their worth in the **inhibition of fibrosis:** *essential phospholipids* (EPL or PPC), *silymarin* and *UDCA*. • The antifibrogenic effect of EPL has been demonstrated repeatedly in experiments; it is mainly based on the stimulation of collagenase. (s. fig. 40.3) • The antifibrogenic efficacy of silymarin is attributed to the proven inhibition and transformation of Ito cells as well as the reduction in gene expression of ECM and TGF-β. (s. tab. 40.13) • An antifibrogenic effect of UDCA in alcoholic liver disease has also been reported.

The occurrence of **lipid peroxidation** normally results in inflammatory tissue reactions and progression of the morphological process. Under experimental conditions, elimination of the free radicals responsible for lipid peroxidation has proved useful in therapy. The administration of antioxidants may thus be advisable as adjuvant therapy. Effective active agents include *essential phospholipids* (s. fig. 40.3), *silymarin* (s. tab. 40.13) and *N-acetylcysteine*. In this connection, it was possible to observe stimulation of superoxide dismutase, inhibition of lipoxygenase, reduction in malonyl dialdehyde, decreased consumption of glutathione, etc. Administration of *vitamin C* and *vitamin E* as antioxidants led to the regression of fibrosis. (51) Likewise, *probucol*, an agent with antioxidant properties, caused a significant decrease in GPT and GOT. The use of *pentoxifylline* (1600 mg/day) led to a decrease in liver enzymes.

Lipotropic substances show a particular affinity to fats and have counteracted hepatic steatosis in animal experiments. • In a 12-month course of therapy involving patients with a fatty liver, *EPL* led to a significant and lasting improvement and even to normalization of the increased transaminases and γ-GT within four weeks. (61) • *Choline* is substantially involved in the mobilization of triglycerides from the liver cell, because it uses these neutral fats to form transportable phospholipids. • However, choline needs *betaine* for demethylation. Thus betaine has an important function in transmethylation processes of lipid metabolism. Resynthesis of methionine likewise requires the assistance of betaine (increase of SAMe concentration in the liver). A dosage of 20 g/day (over 1 year) led to a reduction of the transaminases, liver steatosis and inflammatory activity. (40) • *Atorvastatin* (10 mg/day) was effective in patients with hyperlipidaemia. *Clofibrat* proved ineffective in patients with NASH. *Gemfibrozil* (600 mg/day), which reduces the mobilization of free fatty acids from the fat depots,

brought about a decrease in the transaminases. • By contrast, the use of *pioglitazone* or *rosiglitazone*, a peroxisome proliferator-activated receptor (PPAR), led to an improvement in the histological and biochemical findings. This could imply that insulin resistance is significant in the pathogenesis of NASH. (60) *Metformin*, an insulin-sensitizing drug, led to positive changes in the histological pattern.

The use of *ursodeoxycholic* acid is advisable in cases involving a cholestatic course of NAFLD or NASH. It has a cytoprotective and anti-apoptotic effect. With a dosage of 13–15 mg/kg BW/day, it was possible to achieve an improvement in the transaminase values and the fat content of the liver. An increase in dosage to 20–25 mg/kg BW/day might be advisable. (55) A combination of UDCA and vitamin E (over two years) showed good results. • In animal experiments, *taurine* led to a restorative effect of NASH (inhibition of lipid peroxidation, improvement in lipid and glucose metabolism, decreased synthesis of TFNα and TFNβ, enhanced synthesis of adiponectin). (45)

A *phlebotomy* reduces transaminase levels and increases iron parameters in patients with NASH. This is understandable, since intrahepatic iron storage correlates with the severity of fibrosis.

Liver transplantation may be indicated in patients with considerable cirrhosis-related complications. However, NASH can also reoccur in the transplanted liver. (44, 60) This observation points to a systemic disorder of the lipid metabolism.

4 Faulty nutrition

A **healthy liver** is capable over a longer time of tolerating considerable changes in the pattern of food intake (irregular meals) or with respect to the quantity and quality of the food itself. • However, even a healthy liver reacts with functional disturbances or morphological changes to long-term **malnutrition.** During a state of hunger or a period of low protein intake, for example, almost all toxic substances have more severe effects, while the ability of the immune system to fight infections is compromised. • General **hyperalimentation** results in surplus calorie supply. The excess in carbohydrates and fat usually goes together with simultaneous protein deficiency. In this context, both the kind of fat or carbohydrate and any imbalance between lipogenic and lipotropic substances are important.

Animal experiments have shown that **faulty nutrition,** i.e. > 90% fat, < 10% protein and < 2 mg choline per day, leads to pronounced fatty liver and even fatty cirrhosis within a few weeks. The same changes could be observed when the protein intake remained more or less normal, while extremely little methionine and choline was offered. • With a **partial surplus** of certain foodstuffs, the special nature of the excessive nutritional components is also of considerable importance. • The term **partial malnutrition** may, for example, be associated with a pronounced protein deficiency (and thus possibly inadequate production of lipoproteins) or a lack of lipotropic substances (such as methionine, choline, cystine, glycocollbetaine, pyridoxine, casein and various N- or S-methylated substances). *Protein deficiency has particularly severe consequences when toxic substances are absorbed at the same time or when the organism has to fight bacterial or parasitic infections.* • A **diseased liver** reacts to both a serious deficiency in and an excessive supply of different nutrients (e.g. proteins, certain kinds of amino acids, various lipids, trace elements) with unfavourable or even complicative developments during the course of disease.

4.1 Malnutrition

Functional disturbances and morphological cell damage of the liver can be observed after prolonged general malnutrition. They are fully reversible using nutritional therapy. In *anorexia nervosa*, there may also be an increase in the transaminases or a pathological tendency shown in liver function tests.

Lipofuscin: The presence of lipofuscin is without pathological relevance and can be observed when medication is taken (e.g. phenacetin, chlorpromazine) (s. fig. 21.3), with advancing age and during prolonged malnutrition. The yellowish-brown pigment granules, some 1 µ in size, are PAS-positive, orcein-negative and acid-proof. They are produced from cell-own material and stored in the centroacinar hepatocytes, i.e. between the nucleus and bile canaliculi (= *centroaxial pigment pathways*).

Ceroid: The presence of ceroid is occasionally seen in both malnutrition and in acute viral hepatitis. It is PAS-positive, orcein-positive and acid-proof. This orange-brown granular pigment is mainly stored centroacinarly. (s. fig. 21.6)

Brown atrophy: In malnutrition, the liver shows a decrease in cell and nucleus size, glycogen depletion, pigment deposits, occasional siderosis and proliferation of Kupffer cells. The liver as a whole becomes smaller (by as much as two thirds of its normal weight). Due to its pigment deposits, particularly siderin, the liver takes on a brown colour. These changes have been subsumed under the term brown atrophy (H. Popper, 1948).

4.2 Kwashiorkor

Kwashiorkor (meaning in the Ghanaian language "a disease which develops in a baby when it is replaced by a new baby and is weaned from the breast onto starch paps") was first described by C. Williams in 1933. It

is caused by a diet which is poor in protein, particularly animal protein, whereas the supply of carbohydrates and fat calories is too high. Two new hypotheses of aetiology emerged suggesting that the disease stems from (*1.*) the action of excess free radicals (M.N.H. Golden et al., 1987) or (*2.*) the action of dietary toxins, e.g. cyanogens, aflatoxins (R.G. Hendrickse, 1991). Manifestation is attributable to intercurrent intestinal infections. In both children and adults, the symptoms include dermatosis, muscle atrophy, oedema due to hypoalbuminaemia, diarrhoea and growth retardation. The hair shows typical depigmentation (red hair), at the same time becoming straight, thin and soft. There is severe fatty liver (mainly in zone 1), often with enormous hepatomegaly. Striking dark red skin patches appear, mostly in the periumbilical and/or inguinal areas as well as in the nuchal region. This skin discoloration together with the red hair gave the illness the name "red boy". When animal protein is supplied, e.g. skimmed milk, complete restitution can be achieved. • Kwashiorkor as such does not result in liver fibrosis or cirrhosis. However, lymphocytic infiltration of the portal fields and progressive periportal/perilobular fibrosis (as well as pancreatic fibrosis and endocardial sclerosis) are often observed; there is also evidence of necrosis and collapse areas as well as signs of cirrhosis. Such changes are due to the high susceptibility of kwashiorkor patients to infections and toxic effects (e.g. aflatoxin). (29, 37) • A remarkable histological effect of EPL was seen in African children with a fatty liver due to nutritional imbalance as well as protein and vitamin deficiency. (s. p. 894)

4.3 Tropical juvenile cirrhosis

This clinical picture is also caused by animal protein deficiency. However, it was common practice in the affected regions to use beaver oil as a laxative, and this is known to be extremely toxic to the liver. Fatty liver, hepatomegaly and jaundice were observed; ultimately, cirrhosis of a more biliary type developed.

4.4 Infantile sclerosis

This disease has mainly been encountered in Jamaica. Severe fatty liver with hepatomegaly developed together with early ascites (but no jaundice) and an increasing deterioration in liver synthesis performance. Death occurs in liver coma. Morphologically, there was evidence of fibrosis as well as veno-occlusive disease, features which suggested a combination of protein-deficient nutrition and phytotoxins.

4.5 Obesity

In 30–50% of cases of obesity, *fatty liver* is detected. A statistical evaluation of available data (1996) revealed that 15–17% of the German population displayed a **body mass index** of >30; BMI = *body weight (in kg): body size (in m^2)*. Another 40% are overweight, with a BMI of 25–30. (4) Thus the German population occupies a leading position in the world with regard to the percentage of persons who are considerably overweight. The degree of obesity correlates with the severity of fatty liver. Adipose people suffering from android distribution of fat (i.e. bulk of the fat in the abdominal area) also develop more pronounced fatty liver. (18) Steatosis is predominantly macrovesicular and localized in zone 3. The increased release of fatty acids is responsible for diminished glucose utilization, whereby blood sugar rises and insulin secretion is stimulated, with simultaneous insulin resistance. Apart from that, any excessive carbohydrates are used for liponeogenesis. The outcome is a *metabolic disorder similar to diabetes* with increasing fatty changes in the liver; this condition is further aggravated by an enhanced production of endogenous cortisol due to adiposity, with the result that a vicious circle is established. (1, 2, 5, 32, 49, 58, 73, 74)

Leptin, an anti-obesity hormone, can prevent "lipotoxicity" from damaging hepatocytes by limiting triglyceride accumulation. A deficiency of leptin could be a risk factor for NASH. (65) Hyperleptinaemia correlates with the severity of fatty liver, but not with inflammation or fibrosis. • *Thioredoxin* is a stress-inducible, thiol-containing protein. It is elevated in NASH compared to patients with simple fatty liver. There is a correlation with increasing iron accumulation in the liver cells. Therefore, the pathogenesis of NASH may be associated with iron-related oxidative stress.

4.5.1 Diabetes mellitus

In type II diabetes, *fatty liver* is detectable with a frequency of 30–40%, principally due to adiposity and insulin resistance. The course is generally more aggressive with rapid progression to cirrhosis; mortality is also higher. (39) In diabetics, fatty acid oxidation in the liver cell remains undisturbed as far as coenzyme A acetate; glucose deficiency then stops any further oxidation within the citric-acid cycle. The additional energy required has to be supplied through increased degradation of fatty acids in the fat depots (= lipolysis) or reduced storage (= lipogenesis). This inevitably results in *hyperlipidaemia*, fatty liver and ketosis. Often, so-called *glycogen vacuolization of the nuclei* is found, mainly in the vicinity of the portal fields. Ketoacidosis increases lipolysis, causing steatosis to progress. • In a type I diabetes, however, fatty liver is less common and is indeed only to be expected in a ketotic metabolic situation, e.g. if the diabetes is not well-regulated. The course is more favourable; the mortality rate is not influenced. (2, 21, 32, 38, 61, 74) (s. fig. 31.1)

4.5.2 Hyperlipidaemia

Disorders of lipid metabolism, particularly type IV (endogenous hypertriglyceridaemia), cause fatty liver to

develop. Hyperlipidaemia is present in approximately 50% of patients with sonographically determined fatty liver. (2, 54) • In a similar way, patients suffering from **gout** are very often found to have a fatty liver. Both these metabolic disorders frequently appear in combination with obesity.

5 Reye's syndrome

► This syndrome was described by W.R. Brain et al. in 1929. (77) In 1963 it was differentiated as a clinical pathological entity by R.D.K. Reye et al. and defined as a feverish disease of unclear aetiology in infancy. Almost half the children who were found to be suffering from this syndrome died of various cerebral manifestations, including brain oedema. (87)

5.1 Causes

Causes, which are generally linked to a genetic *disposition*, include *viral infection* (e.g. influenza, varicella) and the administration of *salicylates* (involved in some 95% of cases). (86) • In the USA, as many as 2,900 cases were observed in 1973 alone, with more than 800 fatalities; a further 1,207 cases were reported between 1980 and 1997. (76) About 98% of these patients were younger than 20 years of age. The frequency peak was 1978 to 1980; the disease appeared predominantly in December to April, i.e. during the influenza peak. It only occurs rarely nowadays: from 1994 to 1997 only two cases were observed. It should be mentioned that during administration of *Azadirachza indica* (= Margosa oil) toxic liver damage of the Reye type was reported. (75, 78, 80, 81, 83) (s. p. 279)

5.2 Clinical picture

The onset of the disease is an influenza-like, feverish syndrome. Uncontrollable vomiting results in severe hypoglycaemia. Progressive CNS disturbances develop along with convulsions, clouding of consciousness and brain oedema. Laboratory values show a pronounced increase in the transaminases, distinct hyperammoniaemia and moderate cholestasis (without jaundice). Striking features are elevated serum levels of alanine, glutamine and lysine. There are severe blood coagulation disorders. • The tumour-necrosis factor is increased. Hepatomegaly and microvesicular fatty changes in the liver cells predominate. The hepatocytes are swollen and depleted of glycogen. Cell necrosis is found at the periphery of the lobules. The smooth endoplasmic reticulum and peroxisomes are clearly increased, while the mitochondria are swollen and deformed; no signs of inflammation are present. (s. fig. 31.10) Development of chronic liver disease has not been observed as yet. The mortality rate is 30–50%. (78, 79, 82, 85, 88)

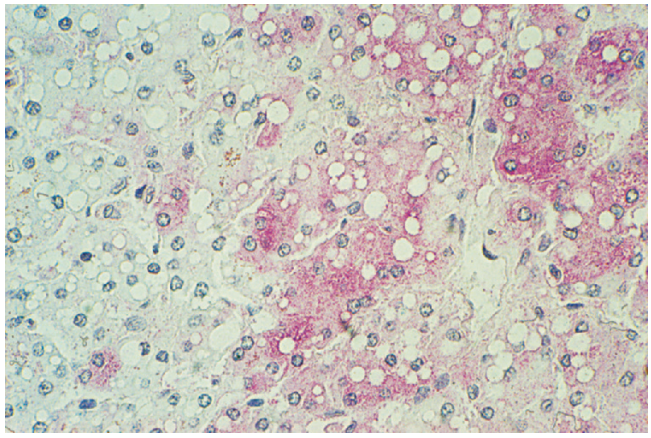

Fig. 31.10: Reye's syndrome (newborn): fine-droplet fatty changes of hepatocytes as well as glycogen depletion; no signs of inflammation (PAS)

6 Acute fatty liver of pregnancy

► The first descriptions of acute fatty liver of pregnancy were given by H.J. Stander et al. (1934) (102); H.L. Sheehan (1940) later defined this disease as a separate syndrome. (100)

6.1 Clinical picture

This severe clinical picture occurs more often than has previously been assumed; nowadays, however, the survival rate is higher. Incidence is about 1 in 13,000 births. (91, 98, 99, 107) • The cause is still unknown. Up to now, a correlation with high-dosage intravenous tetracycline has been assumed. (92) An analogy to Reye's syndrome is also under discussion, as this clinical picture is considered to be part of the spectrum of pre-eclampsia. Primigravidas and multigravidas are equally affected. There is evidence of an already existing genetic enzyme defect (3-hydroxyacyl-CoA dehydrogenase?). (96, 104) Acute fatty liver of pregnancy occurs in the third trimester, particularly in the 34^{th}–36^{th} week. The disease begins in a state of complete well-being and a hitherto uncomplicated pregnancy. It comprises nausea and vomiting (often coffee-ground-like), polydipsia, right-sided abdominal pain, headaches and jaundice. Pruritus is rare. Sometimes, gastrointestinal bleeding, oedema and ascites occur. Encephalopathy develops with neurological symptoms similar to eclampsia. Hypertension, tachycardia, proteinuria and oliguria as well as signs of acute pancreatitis are frequently observed. The whole clinical picture is characterized by multiple organ insufficiency with haemorrhagic diathesis and fulminant hepatic failure. However, relatively asymptomatic and moderate courses with jaundice are also known.

Laboratory values show progressive hyperbilirubinaemia up to 15 mg/dl, an increase in alkaline phosphatase (3 to 5 times the normal range) and triglycerides as well as a rise in the transaminases up to 500 U/l. Usually, γ-GT is normal. Uric acid, which is mostly elevated, is seen as an

early laboratory marker. Severe hypoglycaemias are evident. A striking feature is pronounced leucocytosis (> 50,000/µl), with neutrophils predominating. The differential blood count shows giant thrombocytes, target cells and normoblasts. There is a tendency towards bleeding due to thrombopenia as well as to a decrease in Quick's value, fibrinogen and AT III; disseminated intravascular coagulopathy is usually present. (93, 99)

Computer tomography provides evidence of massive fatty deposits in the liver. (94, 105) • *Sonography*, however, does not generally demonstrate such steatosis. There is pronounced hepatomegaly. (90)

Liver biopsy may be required for diagnosis – unless there are any contraindications. (s. tab. 7.4) In order to detect the characteristic microvesicular (mainly centroacinar) fatty changes, fixation without alcohol and examination of the biopsy material in the form of a frozen section are necessary. (101) The hepatocytes are swollen and resemble so-called foam cells. The nucleus is pyknotic. The mitochondria are deformed. (s. fig. 31.11)

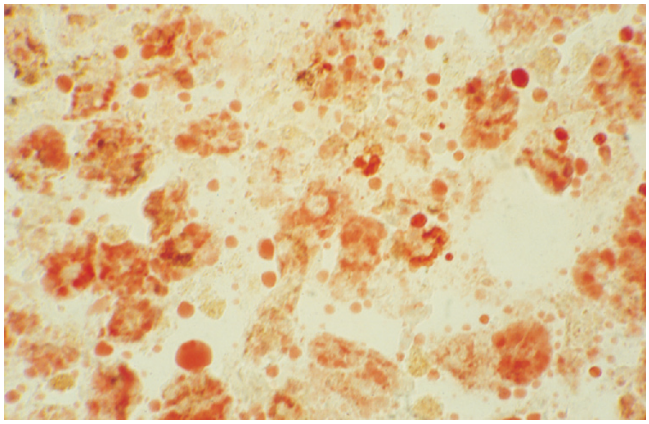

Fig. 31.11: Microvesicular fatty changes of liver cells in acute fatty liver of pregnancy (Sudan red)

Complications: Additional complications were observed during the course of disease, including pronounced cholestasis (106), portal hypertension and liver rupture (95).

6.2 Prognosis

The prognosis depends on early diagnosis and subsequent early (even premature) delivery. When a pregnant woman in the third trimester suddenly suffers from complaints and/or when the laboratory liver values are pathological, acute fatty liver of pregnancy should be suspected and other hepatobiliary diseases excluded as soon as possible. • The previous mortality rates for mother (ca. 90%) and child (ca. 70%) have been reduced to 10–35% and 7–50% respectively. The duration of the disease up to the death of the mother was on average 11 days (3 days up to 6 weeks), and in cases of survival from 2 to 8 weeks. (91, 93, 98, 103) • After acute fatty liver of pregnancy has been overcome, the liver values normalize within a few days. There is no danger of chronic liver damage. In subsequent pregnancies, there is no tendency to relapse, and there are no contraindications against further pregnancies. (89, 107) However, a certain risk remains, so that the women affected should be given the relevant information. Usually, the patient prefers not to risk a further pregnancy.

6.3 Treatment

Immediate delivery (per section or vaginally) is the most important treatment. Further therapy consists of intensive medical care. (106) In individual cases, liver transplantation may be indicated. (97)

7 Mauriac's syndrome

This clinical picture was described by P. Mauriac in 1930. Pathogenesis is attributed to pluriglandular dysregulation. In combination with difficult-to-control infantile or juvenile diabetes mellitus, secondary glycogenosis may develop due to large deposits of glycogen and fat in the hepatocytes, resulting in *hepatomegaly.* • In addition to considerable fluctuations between hyperglycaemia and hypoglycaemia, there is evidence of hypercholesterolaemia, hyperlipidaemia and acetonuria. The clinical picture is characterized by repeated abdominal colic, meteorism, venectasias of the abdominal wall, buffalo obesity, moon face, retarded growth and osteoporosis. Careful monitoring and stabilization of diabetes mellitus are the most essential aspects of the treatment. (108)

8 Protein storage diseases

Numerous proteins are produced in the liver. *Genetically induced disorders* of protein metabolism may cause proteins to be stored in the hepatocytes or even deposited in the intestinal tract. Primary storage diseases in this context include: (*1.*) α_1-antitrypsin deficiency, (*2.*) amyloidosis, and (*3.*) α_1-antichymotrypsin deficiency.

8.1 α_1-antitrypsin deficiency

► The association between lung emphysema and α_1-antitrypsin (α_1AT) deficiency was recognized by C. B. Laurell et al. in 1963. The development of liver cirrhosis due to α_1AT deficiency was first observed in children by E. Freier et al. in 1968 and in adults by N. O. Berg et al. in 1972.

8.1.1 Pathogenesis

This disease is biochemically more correctly termed **α_1-protease inhibition deficiency** since the protein acts against trypsin and numerous serine proteases, in particular chymotrypsin and leucocyte elastase. In the pulmonary alveoli, for example, elastase inhibits the break-

down of elastin. • Serum α_1AT is a glycoprotein with 394 amino acids and 3 carbohydrate chains. It is generally synthesized in the Golgi apparatus of the hepatocytes, although small amounts are also formed in the gastrointestinal tract and in macrophages. Due to gene mutation, the transport of α_1AT from the endoplasmic reticulum (high-mannose type) to the Golgi apparatus is inhibited, so that the "secretion-competent complex" cannot be produced (= *α_1AT deficiency*). (118) Thus α_1AT is not transferred to the blood by way of exocytosis, but remains stored in the endoplasmic reticulum in the form of **globular deposits** and is subsequently broken down. • The α_1AT gene is localized on chromosome 14 q 32 and possesses about 100 allelic variants, which are named according to the **Pi nomenclature** (= *protease inhibitor*). In homozygosity, *normal allele PiM* regulates the formation of the normal phenotype PiMM, which is present in > 90% of the population. • Alpha$_1$-antitrypsin deficiency is caused by an autosomal recessive mutation of two alleles. • **Homozygous defective alleles** are: PiZZ, PiSS, PiZnull, PiPP, PiWW, Pinull, PiMmalton. They show plasma concentrations between 0% and 15% of the normal α_1-AT value. *These homozygous combinations of defective alleles are accompanied by a high risk of pulmonary emphysema, newborn cholestasis, infantile hepatitis, cirrhosis and hepatocellular carcinoma*. • PiMS, PiMZ, PiSZ, PiFZ and PiMnull are deemed to be **heterozygous defective alleles.** In Europe, the allele type PiMZ is found in 3%, PiMS in 7%, and PiSZ as well as PiZZ in 1% of the population. Plasma values range between 42% and 60% of the normal α_1AT value. Heterozygosity thus causes only moderate and/or intermediary α_1-AT deficiency, but is nevertheless seen as a *predisposing factor of liver disease.* (110, 111, 113, 119, 124, 126)

8.1.2 Clinical picture

The great variability of this clinical picture depends upon allele type, age at initial manifestation and individual progression. • About 0.02 – 0.06% (1 : 1,500 – 3,500) of all newborns are **homozygous** carriers of defective alleles. In 10 – 12% of these, conjugated hyperbilirubinaemia with intrahepatic cholestasis and pruritus develops within the first months of life. A lethal course is possible; in most cases, the cholestasis present in the newborn disappears by the age of six or seven years. Hepatosplenomegaly generally persists. In 10 – 15% of the patients, cirrhosis is most likely to develop. Additional genetic or exogenous factors, especially indomethacine (123), are held responsible for manifestation and progression. An α_1-antitrypsin deficiency of the PiZZ type caused *cirrhosis* in 45 – 50% (as shown after autopsy) and *liver carcinoma* in 25 – 30% of adults. In some 50% of homozygous patients, nothing more than an increase in the transaminases was observed. Uninhibited neutrophilic elastase results in a loss of elasticity in the lung, so that emphysema and obstructive pulmonary disease develop. However, a combined hepatopulmonary disease is rarely found. Extrahepatic manifestations include pancreas fibrosis, panniculitis, glomerulonephritis and arterial aneurysm. (s. fig. 31.12) • **Heterozygous** α_1-antitrypsin deficiency does not cause any manifest hepatic or pulmonary diseases, but it is considered to be a predisposing factor. Heterozygotes of type PiZ have a higher risk of HCC, both in a non-cirrhotic liver and in the absence of liver disease. Heterozygous carriers are in any case overrepresented in patients suffering from cryptogenic or viral chronic hepatitis and cirrhosis. A higher prevalence of PiMZ was found in idiopathic haemochromatosis; greater frequency was likewise observed in pregnancies and twin births. There was a higher prevalence of PiZ in polyarthritis rheumatica. (109, 113, 115, 117, 119, 120, 125 – 127)

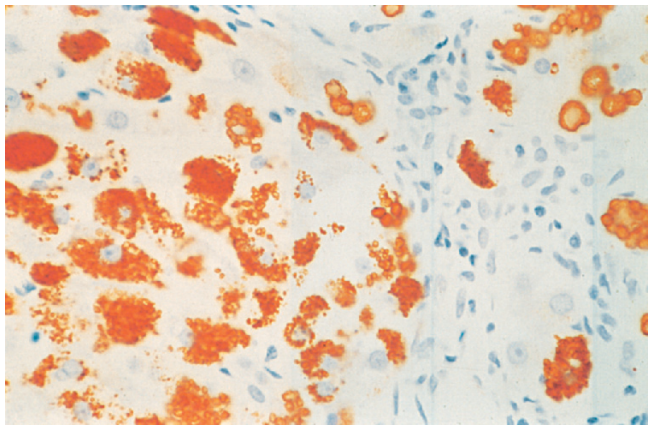

Fig. 31.12: Serologically confirmed α_1-antitrypsin deficiency type PiZ in a juvenile patient (homozygosity). Globular α_1-antitrypsin deposits in the hepatocytes; PiZ immunohistochemistry with PiZ antibody ATZ11

8.1.3 Diagnostics and treatment

Diagnosis: Evidence of conjugated jaundice in neonates and frequent bronchopulmonary infections in infants arouse suspicion of α_1-antitrypsin deficiency. In later life, this genetic cause needs to be ruled out in cryptogenic chronic hepatitis or cirrhosis. • Reduction in the α_1-globulin fraction in electrophoresis to < 2 rel.% is an important indicator of α_1-antitrypsin deficiency, because α_1AT is the main component of α_1-globulins (90%). Diagnosis is confirmed by determination of α_1AT in the serum. *Normal values* range between 190 – 330 mg/dl or 80 – 147 IU/ml. Half-life is about five days. Values < 20% of the normal level suggest a homozygous type, while a 40 – 70% reduction below normal points to a heterozygous type. Identification of the phenotype of α_1AT is possible. Alpha$_1$-AT is also found in tears, rhinal secretion, saliva, duodenal juice, bronchial secretion, cerebrospinal fluid and breast milk.

Liver histology points to cholestasis or changes in the biliary ductules. PAS-positive, diastasis-resistent, 1 – 40 µm sized globular deposits of α_1AT in the hepatocytes

are typical features, mainly found in the periportal area (zone 1), but only in homozygosity. (112, 113, 119, 125) (s. fig. 31.12) Inflammatory and fibrotic reactions are detectable in the vicinity, although α_1AT is not considered to be a toxic agent. Copper content in the liver is increased. In progressive fibrosis, there is generally a rise in P-III-P in the serum. The prognosis for α_1-antitrypsin cirrhosis is much poorer than for other types of cirrhosis; the mean survival rate is reported to be two years after a diagnosis has been established. In 10−30% of cases, hepatic carcinoma can be expected. (122, 128)

Causal treatment is not yet possible, but *gene therapy* may prove feasible, i. e. integration of a normal allele M into the genome of the patient's somatic cells. *Substitution* with α_1AT (e.g. infusion or aerosol inhalation of α_1AT) is less promising. An increase in α_1AT synthesis has been attempted by means of androgens and oestrogen antagonists (e. g. tamoxifen). • Smoking is strictly forbidden! In progressive cirrhosis and imminent liver failure, *liver transplantation* is indicated. (116, 121) The five-year survival rate in children was 83%. (114) Transplantation generally allows a phenotypical healing process to take place.

8.2 Amyloidosis

Amyloid is an insoluble protein-polysaccharide complex consisting of fibrillary protein, AP component and glycosaminoglycan; this AP protein is identical with the regular serum amyloid P (= SAP). Amyloid is mainly stored in the extracellular spaces. The substance shows a strong affinity to iodine (R. Virchow, 1854: "amyloid" = "starch-like") and Congo red (H. Puchtler et al., 1962). Under a polarizing microscope, amyloid shows a green staining with double refraction. Electron microscopy reveals a fibrillary structure with 7−10 µm fibres. Various amyloid types can be distinguished, depending on the different kinds of protein in the fibrils. Secondary amyloidosis is the most common form. • Autosomal dominant hereditary amyloidosis is transmitted by means of structurally altered transthyretin (TTR). Meanwhile, more than 60 mutations of TTR have been detected. The gene responsible is located on chromosome 18; a gene test is already available. (130, 133)

AA amyloid appears in a generalized form and is mainly stored perireticularly in the kidney, spleen and liver. This kind of amyloidosis occurs as (*1.*) a **congenital** form in cases of familial Mediterranean fever, (*2.*) an **idiopathic** (= primary) form without any associated basic disease, and (*3.*) a **reactive** (= secondary) form in chronic inflammations or tumours (e.g. Hodgkin's disease) as well as in drug abuse and AIDS.

AL amyloid possesses fibrils composed of fragments from Ig light chains of the *kappa* or *lambda* type. This amyloid is stored pericollagenically both in a generalized and organ-localized form. Deposits in organs, including the liver, may take the form of nodules (= amyloid tumour or paramyloid). AL amyloidosis may be: (*1.*) **idiopathic** (= primary) without any associated basic disease or (*2.*) **reactive** (= secondary) in cases of multiple myeloma, Waldenström's syndrome, Bence-Jones plasmocytoma and various type B cell tumours.

Further differentiation distinguishes between **AF amyloid** (ATTR) (amyloid types in familial amyloidosis), endocrine **EA amyloid**, **AS amyloid** (occasionally detected in old age in its isolated form in the heart and brain) and **AB amyloid** ($A\beta_2m$) (often observed in the osseous system during long-term dialysis).

8.2.1 Clinical picture and diagnosis

Clinically, pronounced *hepatomegaly* predominates; later on, splenomegaly usually develops as well. An increase in *alkaline phosphatase* is observed at an early stage. Severe courses of intrahepatic cholestasis have been observed. (129, 136, 138, 139, 143, 146, 147) These mostly involve obstructive cholestasis due to deposits of fibrils between the sinusoidal wall and hepatocytes, so that the canaliculi and the ductules are compressed. Hepatic enzymes and liver function tests are normal or deviate slightly from normal values. *Jaundice*, which appears in the late phases, is indicative of a poor prognosis. A decrease in *cholinesterase* is seen as a sign of increasing amyloid deposition with subsequent compromising of de novo synthesis. Factor X is often decreased. • In *CT scanning*, a reduced contrast medium enhancement in the area of amyloid storage may be detected (141, 142). *Scintigraphy* using ^{99m}Tc-sulphocolloid shows markedly decreased uptake in the spleen as compared to the liver, and also metastasis-like storage defects. (148) • The tracers ^{123}I-SAP or ^{99m}Tc-SAP show abnormal uptake into amyloid deposits. (135) In some cases, *involvement of other organs* − kidney (129, 132), heart, spleen (142, 144, 147), intestines, CNS, carpal tunnel syndrome − suggests the existence of amyloidosis. Sometimes, there is reddish-brown dyschromia. (133) • Rectum biopsy, when carried out expertly, provides diagnostic accuracy in 80−85% of cases. Aspiration biopsy from bone marrow or subcutaneous abdominal fat tissue only has an accuracy of 40−50%. (s. fig. 31.13)

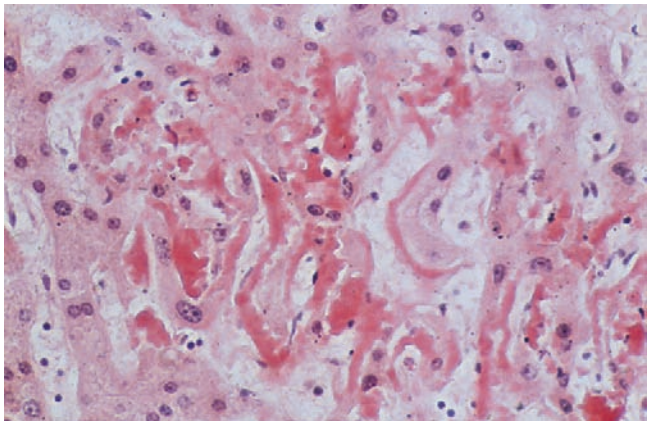

Fig. 31.13: Liver amyloidosis: Perisinusoidal amyloidosis with atrophic hepatocyte trabeculae (HE)

Morphology: Amyloid is also stored in the small portal vein branches of the liver. Depending on the guiding

fibres used by amyloid, it is possible to differentiate between perireticular and pericollagenous amyloidosis. The vascular amyloid involvement together with the solidity and fragility of the liver are the cause of occasional excessive *haemorrhagic tendency* (134); this condition is also given after liver biopsy. Therefore, **percutaneous liver biopsy** must be considered as a *contraindication.* (s. tab. 7.4) If liver histology is required, the sample should be obtained by **laparoscopic liver biopsy,** so that targeted treatment measures can be applied "with visual control" if complicative bleeding occurs. The biopsy specimen is pale pink. The liver surface appears pale and wax-like with coarse reddish marbling. (s. fig. 31.13)

8.2.2 Prognosis and treatment

The **prognosis** for amyloidosis is poor. In AL amyloidosis, fewer than 20% of patients had a five-year survival rate. A course of one to two years is regarded as a mean survival period. The outcome depends mainly upon renal and cardiac amyloidosis; the prognosis regarding the liver is determined by the development of portal hypertension or acute liver failure. (131, 149) Cytostatics are recommended for the **treatment** of benign or malignant B cell tumours in type AL amyloidosis, while melphalan and prednisolone are used for concomitant amyloidosis. However, therapeutic measures are generally limited. The mobilization of amyloid deposits can be attempted by administering dimethyl sulphoxide (1.5−10.0 g/day). • In secondary amyloidosis, treatment of the underlying disease is important; individual cases have been reported in which amyloidosis disappeared due to successful therapy. • In hereditary amyloidosis, the indication for *liver transplantation* is given, since the liver is the main site of transthyretin synthesis. Mutant transthyretin is no longer detectable in the serum after transplantation. (137, 140, 145, 147) Regression of extrahepatic amyloid deposits has also been reported.

8.3 α_1-antichymotrypsin deficiency

In genetically (autosomal dominant) induced α_1-antichymotrypsin deficiency, this inhibitor of serine proteases is not secreted in sufficient quantities from the hepatocytes or alveolar macrophages. Therefore α_1-antichymotrypsin is stored in these cells. The deposits can be detected as cellular inclusions by light microscopy. Patients suffering from this condition may develop chronic hepatitis and cirrhosis. (150−152)

9 Amino acid storage diseases

9.1 Hereditary tyrosinaemia

► This clinical picture was described for the first time by M.D. Baber in 1956; it was identified as an enzyme deficiency by B. Lindblad et al. in 1977.

Tyrosinaemia type I is very rare (incidence 1 : 100,000 births). It is caused by an autosomal recessive defect of fumarylacetoacetate hydrolase (localized on chromosome 15 q 23−25), which impairs tyrosine degradation. Tyrosine metabolites accumulate at the point where the metabolic process is compromised. This results in either an **acute** clinical course initiated immediately after birth and usually leading to a quick death or a **chronic** course in which patients reach adult age. (153, 154, 156, 157)

Tyrosinaemia type II is caused by reduced activity of tyrosine aminotransferase, resulting in greater concentration in the serum. This may develop into an independent **congenital-hereditary** clinical picture or physiological hypertyrosinaemia, which occurs in about 10% of newborns as a **neonatal-transitory** event.

9.1.1 Clinical picture

The gene mutation inhibits hydrolytic cleavage of fumarylacetoacetate into fumarate and acetoacetate. Consequently, the toxic precursors maleylacetoacetate and fumarylacetoacetate accumulate in the liver and kidneys. They possess a reactive double bond and can therefore react with macromolecules to assume the properties of alkylating substances. In addition, intracellular glutathione deficiency develops due to the stable complex formation with glutathione, favouring *lipid peroxidations.* Enhanced formation of δ-aminolaevulinic acid can also be observed during occasional attacks of acute intermittent porphyria (G. Mitchel et al., 1990).

The **acute course** in newborns is characterized by vomiting and diarrhoea as well as growth disturbances. Hepatosplenomegaly, hypoalbuminaemia, hypercholesterinaemia and hypoglycaemia as well as oedema/ascites and coagulopathy develop rapidly. A striking feature is the early and marked rise in the α_1-foetoprotein value in the serum. Excretion of succinylacetone (> 50 nmol/l) and delta-aminolaevulinic acid in the urine is noticeably increased. Methionine, phenylalanine and tyrosine are elevated in the serum. A slight rise in the transaminases and serum bilirubin occurs. Death is due to acute liver failure. There is histologic evidence of extensive necrosis, fatty infiltration, cholestasis and regenerative nodes in the liver. • If the newborn survives the first few months, the proximal renal tubule suffers complex damage, resulting in the clinical picture of **Fanconi's syndrome**: glucosuria, aminoaciduria, phosphaturia including osteomalacia, and renal tubular acidosis with polyuria.

The **chronic course** is characterized by a milder form. The general symptoms are less pronounced, and often they do not appear before the patient reaches school age. Hepatosplenomegaly is present. Laboratory findings include hypoalbuminaemia, coagulopathy and markedly elevated serum values of α_1-foetoprotein, methionine, tyrosine and phenylalanine. Activity of the fumarylacetoacetate hydrolase in leucocytes, fibroblasts and hepatocytes is increased. • The *liver* is granular and firm with a yellowish colour. Histologically, the picture is similar to that of galactosaemia (fatty changes, tubular transformation, cholestasis, periportal fibrosis, central

sclerosis). Within a few months, micronodular cirrhosis is detectable with early multifocal liver cell carcinoma. (153, 156, 159) • *Fanconi's syndrome* (see above) also develops in the chronic course. Occasionally, there are symptoms of peripheral neuropathy.

9.1.2 Treatment

The *prognosis* is determined by the early onset of acute liver failure in the newborn and, in the following months or years, by the development of hepatocellular carcinoma. (156, 160) • A *liver transplantation* is thus recommended as from the second or third year of life. (155) Dietary measures (avoidance of methionine, tyrosine and phenylalanine as nutritional components) have not proved particularly successful. • Good therapeutic results were achieved when *nitisinone* (2 mg/kg BW/day) was applied. This substance is an inhibitor of 4-hydroxyphenylpyruvate dioxygenase, which prevents the accumulation of succinylacetone. (158) Even the risk of HCC development was reduced by this substance.

9.2 Disturbances of the urea cycle

Six enzymes are involved in the urea cycle: (*1*.) carbamoylphosphate synthetase (CPS), (*2*.) ornithine transcarbamylase (OTC), (*3*.) argininosuccinate synthetase, (*4*.) argininosuccinate lyase, (*5*.) arginase, and (*6*.) N-acetyl glutamate synthetase − as well as enzymes involved in supply reactions. They may be compromised by a genetic defect. Up to now, six congenital disorders of the urea cycle are known. The cumulative frequency is estimated at 1 : 8,000. • The genetic disorder is autosomal recessive; only the ornithine transcarbamylase disorder (= OTC deficiency) shows a dominant chromosomal pattern of transmission (chromosome p 21.1). This is the most frequently detected intramitochondrial defect to date (1 : 14,000). (165) • Following preliminary detoxification of ammonia at the site of formation by reaction with glutamate (whereby glutamine is formed), definitive detoxification takes place in the urea cycle and by hepatic glutamine synthesis. • The **urea cycle** has two other functions: (*1*.) denovo synthesis of arginine and (*2*.) regulation of the acid-base balance by consumption of bicarbonate. (s. pp 62, 63) (s. figs. 3.12, 3.15)

The **clinical symptomatology,** which is almost the same in all enzymatic disturbances of the urea cycle, is caused by hyperammonaemia. • An *arginase defect* results in enhanced excretion of lysine, ornithine and cystine. Neurological symptoms such as athetosis and hyperreactivity followed by paresis and tetraplegia predominate.

In neonatal manifestation of *OTC deficiency*, lethargy, vomiting, refusal to take food, hyperventilation and hypothermia develop quickly. Death ensues in coma within a few days. Manifestation in infants or adolescents is based upon the residual activity of the defective enzyme. This course is also characterized by vomiting and lethargy. The clinical picture is aggravated by a protein-rich diet, whereas protein reduction improves the clinical situation. Without treatment, death occurs in a hepatic coma.

A defect in *argininosuccinate synthetase* coincides with markedly elevated citrulline values in the blood. Neither citrullinaemia nor a carbamylphosphate synthetase defect cause liver damage. • By contrast, an *argininosuccinase defect* leads to microvesicular steatosis and megamitochondria with dilatation of the ER. (s. p. 279)

Diagnosis is based upon hyperammonaemia, which is detectable either spontaneously or after the oral intake of proteins − or, most obviously, following intravenous infusion of amino acids. The respective amino acids are increased in the serum prior to the disturbed metabolic reaction. Argininosuccinate is only detectable in the urine. A striking feature in these patients is their thin, brittle hair. With defective ornithine transcarbamylase, there is an increase in orotic acid, uridine and uric acid in the urine, while the respective citrulline concentration is decreased. Determination of OTC activity in liver tissue verifies the diagnosis and facilitates a genomic analysis. The allopurinol test can be applied for the identification of heterozygosity (or the mild form of OTC deficiency). The liver shows steatosis, portal inflammation and portal fibrosis.

Treatment involves a low-protein diet (0.5−0.7 g/kg BW/day) with sufficient calories. Substitution of essential amino acids (in about the same quantity) is required. • Administration of benzoate (0.1−0.25 g/kg BW/day), arginine hydrochloride (1 mmol/kg BW/day) or sodium phenylacetate (0.3−0.5 g/kg BW/day) (phenylbutyrate may be more effective) facilitates nitrogen excretion via other metabolic pathways. (161−164) • With enhanced excretion of orotate or other metabolites of pyrimidine synthesis, administration of allopurinol leads to an increase in the excretion of nitrogen via metabolites from pyrimidine synthesis. Ammonia and urea precursors are eliminated by haemodialysis. In some cases, liver transplantation is indicated. (161−163)

9.3 Cystinosis

Autosomal recessive cystinosis is caused by an enzyme-induced blockage of cystine degradation, particularly in the RES lysosomes of the bone marrow, liver, spleen and kidneys. Especially in the stellate cells of the spleen and to a lesser extent of the hepatic lobule centres, hexagonal and rectangular cystine crystals are found, pointing at an early stage to cystinosis. There is evidence of hepatosplenomegaly and microvesicular steatosis. The clinical picture of the infantile type presents as a *Fanconi syndrome*. (s. pp 610, 611) The children affected die in the first five years of life.

9.4 Homocystinuria

This rare disease has an autosomal recessive inheritance pattern. It is based on a deficiency of cystathion-β synthase, so that homocystein and other metabolites (e. g. methionine) accumulate and are eliminated in increased quantities in the urine. There is evidence of disturbed mental development and a marked tendency to thrombosis in the arterial system. Liver steatosis (mainly in zone 1) and hepatomegaly with subsequent portal fibrosis can be seen.

10 Carbohydrate storage diseases

Carbohydrate storage diseases generally include: (*1.*) glycogenoses, (*2.*) galactosaemia, (*3.*) hereditary fructose intolerance, and (*4.*) fructose-1,6-biphosphatase deficiency.

10.1 Glycogenoses

Glycogenoses are congenital metabolic diseases with enzyme defects in which glycogen cannot be broken down to glucose. This results in normal or structurally changed glycogen being stored in the organs in ever-increasing quantities. To date, **ten types** (types I−X) have been defined. Types VIII−X are very rare and partly present as subgroups of the other types. • The **incidence** of types I−VII ranges between 1 : 20,000 and 1 : 40,000 births with geographic differences. A relative frequency was determined from 1,192 cases: type I (23%), type II (15%), type III (21%), type IV (2.5%), type V (6%), type VI (30%) and type VII (2.5%). • Transmission is **autosomal recessive.** With regard to the extent of the enzymatic disturbance or the involvement of regulatory enzymes, pronounced heterogeneity can be observed within the individual types. • **Liver disease** only develops in types I, III, IV and VI. *Hepatomegaly* is also found in type II and in the rare types IX and X. • Differential diagnosis of glycogenoses and their subtypes is not possible simply using liver histology. However, it can be achieved by means of additional *histochemistry*, quantitative determination of *glycogen* and *enzyme analysis.* (167, 172, 180, 181)

Gierke's disease (type I)

Hepatorenal glycogenosis was described by S. Van Creveld in 1928 and resolved in terms of pathological morphology by E.O.E. von Gierke in 1929. This genetic defect (localized on chromosome 17) causes the complete or partial absence of **glucose-6-phosphatase** activity **(subtype Ia)**. (173) • Enzymatic activity is only detectable after the application of detergents. There is a defect in the translocase of glucose-6-phosphatase at the endoplasmic reticulum membrane. This defect is also found in adults **(subtype Ib)**. • Disturbance of the microsomal phosphate/pyrophosphate translocase may occur **(subtype Ic)**. • Glucose-6-phosphatase is needed for the discharge of glucose from the liver when glucose-1-phosphate is supplied by glycogenolysis. This leads to an accumulation of **glucose-1-phosphate,** which in turn stimulates the neosynthesis of glycogen. However, glucose-1-phosphate is also broken down by glycolysis, with increased formation of lactate, acetyl-coenzyme A and glycerol-3-phosphate. The last two are basic products for the hepatic synthesis of fatty acids and triglycerides.

The **clinical picture** is characterized by hepatomegaly due to deposits of glycogen and triglycerides and by marked kidney enlargement (no splenomegaly as occurs in lipoidosis or cirrhosis). • Severe metabolic disorders such as hypoglycaemia and hyperlipidaemia (triglycerides, free fatty acids, cholesterol) as well as increased VLDL and LDL values are found, leading to the development of skin and tendon xanthomas, lactate acidosis, a slight increase in the transaminases, a tendency towards infection (due to leucopenia related to abnormal glucose-6-phosphatase transport), weakened skeletal muscles, bleeding tendency (due to abnormal platelet function), hyperuricaemia (with possible manifestations of gout, nephrocalcinosis, kidney stones) and osteoporosis. Hyposomia with fat pads, particularly in the buccal area, is often found. Diagnosis is additionally confirmed by a glucagon test (no or inadequate rise in the blood sugar level) as well as fructose or galactose tests and liver biopsy. (s. fig. 31.14)

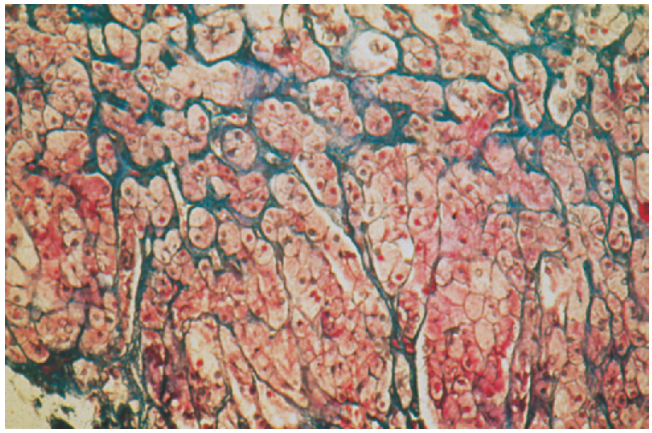

Fig. 31.14: Gierke's disease: phytocyte-like hepatocytes surrounded by a delicate network of fibrosis (Ladewig)

The occurrence of adenoma or HCC is associated with an increase in fatty acid synthesis. Subtype I b is characterized above all by susceptibility to infection with leucopenia. Recurrent oral ulcers are often a cardinal symptom. The *liver* shows evidence of enlarged, polygonal hepatocytes with bright cytoplasm and centrally located small nuclei due to glycogen storage. Occasionally, Mallory-Denk bodies and glycogen vacuolation of the nuclei are seen. The hepatocytes have a pro-

nounced (phytocell-like) cellular membrane. (168, 170, 171, 173–178)

Treatment consists of prevention or elimination of hypoglycaemia. In newborns and infants, this can be achieved by the continuous application of formula diets, if required via nasogastric tube feeding. The administration of allopurinol may also be advisable. A starch diet (e.g. uncooked corn starch) is then used to ensure that glucose is released and resorbed slowly in the intestinal tract. (166, 171) After puberty, the course of disease is usually less pronounced. In the long term, especially in adenoma-related complications, liver transplantation may be indicated. (179)

Pompe's disease (type II)

Generalized glycogenosis type II, also called Pompe's disease (J.C. Pompe, 1932), is caused by a deficiency of lysosomal acidic **α1,4 glucosidase.** This enzyme is responsible for the degradation of glycogen and maltose within the lysosomes. Glycogen is accumulated in all organs, particularly in the heart, but also in the liver. The disease mostly appears in the newborn (death due to heart failure, often within the first 12 months of life), but may also become manifest in infants and adults. • **Clinical symptoms** include weakened skeletal muscles, hyperlipidaemia, increased values of the muscle enzymes aldolase and CK, macroglossia as well as pronounced cardiomegaly and *moderate hepatomegaly.* Mental development is not impaired. The glucagon test is normal; hypoglycaemia does not occur. Diagnosis is confirmed by the detection of reduced enzyme activity in biopsy material obtained from the liver or muscle. Vacuoles can be demonstrated in the liver cells. • **Therapy** is based on the genetic engineering of alpha glucosidase (30 mg/kg BW every 14 days). In the later stages of life, the prognosis quoad vitam is determined by heart failure, pneumonia and respiratory insufficiency.

Cori's disease (type III)

Hepatomuscular glycogenosis (G.B. Forbes, 1952; G.H. Hers, 1959) is caused by a deficiency of **amylo-1,6-glucosidase** (debranching enzyme). Consequently, degradation of the glycogenic branched chains is impaired, yielding an abnormal residual molecule (limit dextrinosis). Gluconeogenesis from lactate is possible. In **Forbes subtype,** deposits of the markedly branched glycogen (with short external chains) are observed in the heart, liver and musculature; in **Forbes-Hers subtype,** they are found in the hepatocytes only. Further subtypes (up to III F) have been described.

Clinical symptoms include hepatomegaly, increased transaminases, hypoglycaemia (glucose may be released from glycogen when the terminal glucose fragments split off), hyperlipidaemia and moderate lactate acidosis. The glucagon tolerance test is positive (= no increase in blood sugar); the galactose test and the fructose test are normal. Liver histology shows numerous *glycogen vacuolizations of the nuclei* (s. fig. 31.1), slight steatosis and periportal fibrosis (also transition to cirrhosis and HCC) (169). Adenomas frequently appear. • The **treatment** consists of frequent small meals rich in carbohydrates and proteins plus additional dietary corn starch preparations.

Andersen's disease (type IV)

Liver-cirrhotic, reticulo-endothelial glycogenosis type IV (D.A. Andersen, 1956) is a defect of the branching enzyme **amylo-1,4-1,6-transglucosidase.** There is a disturbance in the formation of glycogen side-chains, which results in the production of a low-branched glycogen with long side-chains (amylopectinosis). This abnormal structure causes low solubility, resulting in the **deposition** of the malstructured glycogen in the hepatocytes at the periphery of the lobule in the form of eosinophilic or colourless, granular, PAS-positive substances. This abnormal glycogen has a cellulotoxic effect. It is also deposited in the spleen, lymph nodes and RES.

Clinical symptoms include hepatosplenomegaly and increased transaminases as well as an occasional rise in cholestasis-indicating enzymes. Histologically, the liver specimen shows a number of giant cells, cell necrosis and cholestasis. The abnormal glycogen stored in the hepatocytes can to a certain extent be removed by diastasis digestion, and it takes on a violet stain when in contact with iodine (in contrast to the usual reddish-brown colour); PAS staining is positive. Diagnosis can also be reached using rectal biopsy: macrophages with globular inclusions containing amylopectin are found. The further course is determined by portal hypertension with the formation of ascites and a haemorrhagic tendency. In addition, diminished reflexes, muscular atrophy and motor dysfunctions as well as dilative cardiomyopathy may be observed. The disease can also take a protracted course, so that the patient even reaches adolescence. • There is no specific course of **treatment**. Liver transplantation is indicated, whereby a surprisingly positive effect on extrahepatic complications and growth retardation can be expected. (179)

Hers' disease (type VI)

Hepatic glycogenosis (type VI) (G.H. Hers, 1959) is due to hepatic phosphorylase deficiency. Subtype VIa is caused by a lack of **phosphorylase-B kinase,** and it is transmitted by the x-chromosomal recessive route. Subtype VIb generally shows a deficiency in **glycogen phosphorylase,** and its transmission is autosomal recessive. In the musculature, the analogous enzyme is, however, intact. Nevertheless, there is pronounced genetic and phenotypical heterogeneity in most cases.

The **clinical picture** shows moderate hepatomegaly. The normal glycogen is stored in a focal form at the periphery of the lobules. There is a mixed pattern of small and large hepatocytes, giving the picture of an irregular mosaic. Hypoglycaemia occurs only during fasting. No other biochemical deviations are observed. The prognosis is relatively good when dietary instructions are adhered to. Mental functions are normal.

Type IX and type X

These benign glycogenoses are due to a defect in hepatic phosphorylase kinase or cAMP-dependent phosphorylase kinase. Glycogen is deposited unevenly in the hepatocytes; there is evidence of hepatomegaly with fine-droplet steatosis and occasional fibrosis. Hyperlipidaemia and elevated GPT values are present. In the course of time, the patient is able to catch up on physical growth, and hepatomegaly recedes.

10.2 Galactosaemia

Hereditary galactose intolerance (A. v. Reuss, 1908; F. Göppert, 1917) is caused by an autosomal recessive disorder in the galactose metabolism. • Type II is characterized by an enzyme defect in **galactose-1-phosphate uridyltransferase.** (Type I, caused by a defect of galactokinase, only affects the eyes and is therefore not discussed here.) Transferase deficiency affects several organs, particularly the liver, and results in severe damage. Galactonate and galactite develop as **toxic metabolites,** which are deposited in the tissues; galactite is also detectable in the urine. (182, 185)

The **prevalence** of the disease is 1:35,000 to 1:70,000 births. With the help of neonate screening, the defect can be discovered and treated at an early stage. • The enzyme coding for transferase is on **chromosome 9 p 13**; there are various mutations and pronounced polymorphisms. Accordingly, several **variants** displaying different kinds of enzymatic activities have been described in many different places (the Duarre, Rennes, Los Angeles and Chicago variants as well as the Caucasian and Negroid variants). Usually, the defect can only be detected by means of the **galactose tolerance test.** A healthy person oxidizes 30–50% of orally administered galactose to CO_2 in five hours, whereas in transferase deficiency, only 0–8% is oxidized. • Galactonate causes synthesis disorders of glycoproteins and glycolipids in the liver cell by the consumption of UDP. Due to the accumulation of **galactose-1-phosphate,** the consumption of phosphate is increased with a subsequent decrease in ATP and gluconeogenesis. • The deposits of galactose-1-phosphate in the proximal renal tubular cells lead to the development of *Fanconi's syndrome* (glucosuria, aminoaciduria, phosphaturia, acidosis). (s. pp 610, 611)

The **clinical picture** sets in immediately after birth, as soon as milk (including breast milk) is given. The symptoms are vomiting, diarrhoea, no weight gain and galactosuria. As early as the second week, pronounced hepatomegaly (or hepatosplenomegaly) is observed, often accompanied by jaundice and cholestasis. The findings correspond to those in haemolysis. Transaminases are elevated, liver functions are increasingly compromised, and metabolic acidosis is generally in evidence. Cataracts develop. • The diagnosis is confirmed by galactosuria and a rise in galactose and galactose-1-phosphate concentrations in the blood as well as reduced transferase activity in the erythrocytes. • Histologically, the *liver* shows mixed-droplet fatty changes, cholestasis with ductular proliferations, liver cell necrosis, collapse of reticular fibre structures as well as pseudoglandular and/or tubular transformation processes of the liver cell plates around the canaliculi. Within a period of three to six months, micronodular cirrhosis develops, followed by ascites and increasing liver insufficiency. (183, 184, 186)

Treatment is successful when the disease is recognized early enough and when nutrition is free of galactose and lactose, so that the prognosis is generally considered to be favourable. Organ damage can be halted or indeed prevented completely; occasionally, there is (partial) recession. The UDP galactose required for cell metabolism is supplied from UDP glucose. (186)

10.3 Hereditary fructose intolerance

Hereditary fructose intolerance is caused by an autosomal recessive hereditary defect of the enzyme **fructose-1-phosphate aldolase**. Whenever fructose is supplied, severe hypoglycaemia and functional disorders occur in the liver, kidneys and CNS. • The **prevalence** is estimated at 1:20,000 births. As with galactose intolerance, the gene which codes aldolase B is also localized on **chromosome 9.** This enzyme defect causes fructose-1-phosphate to accumulate in the liver and tissue. The cleavage of fructose-1,6-biphosphate is only slightly compromised since the enzymes aldolase A and C are available for this process. • The consumption of phosphate and ATP in the tissue results in various **functional disorders:** (*1*.) inhibition of gluconeogenesis in the liver and kidneys, (*2*.) increase in lactate in the serum with metabolic acidosis, (*3*.) decrease in protein synthesis in the liver, and (*4*.) functional disorders of the proximal tubular cells with development of **Fanconi's syndrome**. (s. pp 610, 611) (187, 189, 191)

The **clinical picture** is characterized by nausea, vomiting, diarrhoea, abdominal pain, dizziness and hypoglycaemia, not only following the intake of fructose (oral, intravenous), but also after sorbitol infusions (sorbitol is converted to fructose in the organism). Growth failure is observed in babies and infants. • During adolescence and in adults, a chronic course may develop, characterized by hepatomegaly, hypophosphataemia, hypoglycaemia, blood coagulation disorders and an increase in the transaminases as well as jaundice and moderate

cholestasis. Phosphaturia results in osteomalacia. Hypermagnesaemia is a striking feature. • *Histological findings* include a fatty liver with liver cell necrosis and increasing fibrosis; the liver cell plates show tubular transformation. Giant cells are often found. At a later stage, cirrhosis develops. (187–190) • When the intake of fructose (or sorbitol) is avoided, even patients with a homozygous genotype are symptom-free. The diagnosis is based upon the determination of liver aldolase B or on the *fructose tolerance test*.

Method: Oral administration of 200 mg/kg BW fructose in a 20% solution over a period of 30 minutes. After establishing the initial values, both glucose and phosphate are determined every 10 minutes; after 1 hour, the intervals are extended to 30 minutes. The test findings are pathological when serum glucose decreases to <40 mg/dl and phosphate to <1.5 mg/dl. Hypoglycaemia requires an immediate i.v. glucose infusion.

Treatment consists of completely avoiding fructose and commercially available cane sugar as well as excluding sorbitol from i.v. infusions. If this regime is strictly adhered to, prognosis is good and life expectancy unaffected in most cases.

10.4 Fructose-1,6-biphosphatase deficiency

Due to the autosomal recessively inherited reduction in fructose-1,6-biphosphatase activity in the liver, the gluconeogenesis of lactate, glycerine and glucogenic amino acids is inhibited. If fructose or glycerine are supplied, phosphate and bicarbonate are consumed in higher quantities and lactate production is increased. This results in metabolic acidosis.

The **clinical picture** is much less pronounced than in hereditary fructose intolerance (type aldolase B). When fructose is supplied or when infections develop, the affected children may suffer from metabolic disorders, such as hypoglycaemia, nausea, vomiting, diarrhoea, hyperventilation, convulsions, lactate acidosis and ketosis. The glucagon tolerance test is pathological (= no increase in blood sugar). • Histology shows a fatty liver. Diagnosis can be confirmed by measuring the fructose-1,6-biphosphatase activity in the liver tissue.

Treatment consists of completely avoiding fructose and, if necessary, in correcting metabolic acidosis, electrolytes and blood sugar values.

11 Lipid storage diseases

11.1 Wolman's disease

Xanthomatosis, an autosomal recessive inherited disorder, was described by A. Abramov, S. Schorr and M. Wolman in 1956. Probably, this rare clinical picture (about 40 cases have been reported to date) was already published by H. Dienst and H. Hamperl as early as 1927. Some cases may have been attributed erroneously to Niemann-Pick disease. • Wolman's disease is caused by a considerable reduction or complete absence of **intralysosomal lipase activity.** In acid pH, the lipase catalyzes the hydrolytic cleavage of cholesterol esters and triglycerides in the lysosomes. This enzyme defect results in an excessive storage of cholesterol esters and triglycerides in the abdominal organs, skin and nervous system. The mutant gene for isoenzyme A of acidic lysosomal lipase is located on **chromosome 10.** In homozygotes, lipase activity in the liver is reduced to less than 10% of the normal value, in heterozygotes to around 50%. • The **enzyme defect** is responsible for (*1.*) storage of cholesterol esters and triglycerides in the hepatocytes, Kupffer cells, macrophages and various other body cells, and (*2.*) disturbance of cholesterol homoeostasis in the blood, since the lysosomes release an insufficient quantity of free cholesterol into the cytosol. For this reason, the regulation of the cholesterol metabolism in the cytosol is considerably impaired: (*1.*) inadequate or no inhibition of the synthesis of cholesterol and the LDL receptor, and (*2.*) inadequate or no stimulation of cholesterol esterification with saturated fatty acids.

The **clinical picture,** which appears in early infancy, comprises vomiting, diarrhoea, growth failure and meteorism. Hepatosplenomegaly and anaemia develop rapidly. Multiple xanthomas of the skin appear. CT scanning shows enlarged adrenal glands (due to xanthomatous redifferentiation), often with finely spotted calcifications. Deposits of cholesterol esters, triglycerides and, to a certain extent, phospholipids are found in the spleen, liver, small intestine, lymph nodes, lungs, skin and nervous system. The foam cells detectable in the bone marrow differ from Pick cells in that they lack sphingomyelin. The transaminases are increased; liver function is markedly compromised. Death usually ensues within the first year of life. (192–194)

11.2 Cholesterol ester storage disease

► Cholesterol ester storage disease (CESD) was first described by D.S. Fredrickson in 1966. No more than 20 cases of this **very rare disease** have been reported to date, with twice as many girls being affected as boys. • Just like *Wolman's disease*, it is transmitted as an autosomal recessive mutation with the gene localization on **chromosome 10.** Due to this defect, degradation of cholesterol esters and triglycerides in the lysosomes is inhibited. Therefore, these lipids are stored in the hepatocytes, Kupffer cells and macrophages as well as in other somatic cells. Although CESD displays the same biochemical and metabolic disorders as Wolman's disease, it is less severe. It does not become evident until adulthood.

► *It was possible for us to describe this clinical picture in detail from our own observations of a 13-year-old girl. We detected a very high content of cholesterol ester in the biopsy sample and a deficiency of lysosomal α-naphthylacetate esterase in the fibroblast culture. (198) (s. figs. 21.5; 31.15, 31.16)*

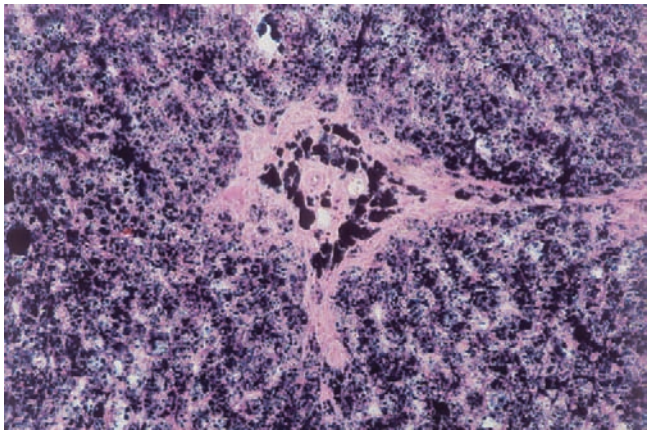

Fig. 31.15: Cholesterol ester storage disease. Fine-droplet fatty changes in the hepatocytes. Widely extensive small and larger lipid vacuoles in the liver cells and foam cells of the portal field (Sudan black) (s. fig. 21.5). Same patient as in fig. 31.16

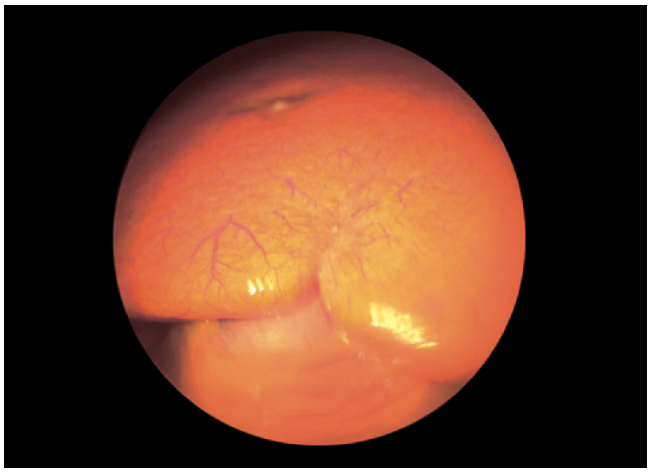

Fig. 31.16: Light yellowish-red, smooth surface in fatty liver due to cholesterol ester storage (13-year-old girl). Same patient as in fig. 31.15

Initially, the **clinical picture** is largely asymptomatic. The average age of the patients reported to date was 6.5 years at the time of diagnosis. Striking features are hepatomegaly and, occasionally, splenomegaly. (199) The cholesterol ester content of the liver can be up to 10% of the liver wet weight, while the total lipid content of the liver ranges between 22% and 28%. • *Laboratory findings* show a slight increase in the transaminases and bile acids as well as reduced liver function. In most cases, jaundice is observed. Markedly augmented concentrations of cholesterol, triglycerides and LDH are characteristic. (197) Foam cells can be found in the bone marrow. Diagnosis is confirmed by determination of lysosomal acidic lipase in the leucocytes and skin fibroblasts. • Corresponding to the high lipid content, the *liver surface* has an orange-yellowish colour, as does the liver specimen. (198) The lipid drops found in the hepatocytes and Kupffer cells are surrounded by a lysosomal membrane; they replace the cytoplasm and nucleus. In polarized light, the hepatocellular lipids show birefringence of the Maltese Cross type. The hepatocytes contain finely floccular, PAS-positive glycogen deposits. On the whole, the prevalent picture is that of mixed-droplet fatty changes. (198) In the further course, hepatic fibrosis or micronodular cirrhosis develop. Both the course and prognosis depend to a great extent on whether cirrhosis develops as well as what its concomitant complications are. (195, 196)

11.3 Cerebrotendinous xanthomatosis

This disease is caused by an autosomal recessive gene mutation (localization on chromosome 2) and leads to an enzyme defect in mitochondrial **steroid-27 hydroxylase.** The enzyme itself is responsible for the breakdown of cholesterol side-chains in bile acid synthesis. Such a defect results in the formation of **cholestanol,** a reduction product of cholesterol. It is deposited in various organs, particularly in the tendons and in the nervous system, because the substance cannot be broken down adequately. **Deposition** takes place conjointly with cholesterol. (202)

The **clinical picture** is characterized by xanthomas, particularly on the tendons (especially the Achilles tendon). Xanthomas consist of cholesterol and cholestanol. The enzyme defect also results in disturbed vitamin D metabolism. Osteoporosis is thus observed quite often, with a tendency towards spontaneous fractures. (201) Striking clinical features are cerebral functional disorders (from deviant behaviour to severe dementia, motor disturbances and convulsions) as well as peripheral neurological symptoms caused by cholestanol deposits. (203) High concentrations of apolipoprotein B and cholestanol are found in the CSF. (205) • **Treatment** is based on the administration of chenodeoxycholic acid (750 mg/day). Effectiveness is generally improved if HMG-CoA reductase inhibitors (e. g. pravastatin) are used concomitantly. (200, 204)

11.4 Abetalipoproteinaemia

In this autosomal recessive disease, the disorder does not involve the gene on chromosome 2, which is responsible for apoprotein assembly, but the **MTP gene** (microsomal triglyceride transfer protein), which is localized on chromosome 4 q 22−24. In the endoplasmic reticulum, MTP transfers cholesterol esters, triglycerides and phospholipids to the nascent apoprotein B. This process is a prerequisite for the transport of the complete **lipoproteins** (e. g. chylomicrons, VLDL) to the Golgi complex and their secretion into the blood via subsequent exocytosis. In the case of MTP deficiency, lipoprotein particles are not secreted, with the result that any superfluous **apoprotein B** is broken down in the endoplasmic reticulum. (206, 208, 211, 212)

Even in infants fed with high-fat milk, the **clinical picture** is characterized by malabsorption leading to fatty

stools and diarrhoea. This results in a deficiency of fat-soluble vitamins with all the respective complications. • Reduced elimination of lipids from the *liver* leads to hepatomegaly with large-droplet fatty changes, possibly causing the formation of fat cysts and larger fat pools. (s. fig. 31.2) Splenomegaly often occurs due to fat storage in the macrophages of the spleen as well as to portal hypertension, which may be caused by an increased resistance in the sinusoids in cases of fatty liver. (207, 209) Development of cirrhosis has also been reported. • As far as *laboratory findings* are concerned, a marked decrease in cholesterol (usually < 40 mg/dl) and triglycerides (usually < 10 mg/dl) is relevant for the diagnosis; VLDL (= pre-beta lipoproteins) are not found. The haemogram shows deformed (e. g. crenated) erythrocytes due to their altered membrane lipids. (210) • **Treatment** is based on the administration of triglycerides with medium-chain fatty acids (MTC diet) with strict avoidance of other fats. Fat-soluble vitamins have to be substituted (if necessary, orally in high doses).

11.5 Hypoalphalipoproteinaemia

► In 1961 D.S. Fredrickson et al. described an autosomal recessive disease called hypo- or analphalipoproteinaemia. This lipid storage disease is also known as **Tangier disease** − named after the small Tangier Island in Chesapeake Bay/Maryland − where it was first observed in two siblings. The cause is a gene mutation on chromosome 9q31 (= deficiency of the cholesterol-efflue regulatory protein). (213)

The most important **clinical feature** is the storage of cholesterol esters in the RES. The noticeably enlarged tonsils are of a distinctive yellowish or orange colour. There is evidence of lymphadenopathy and hepatosplenomegaly as well as polyneuropathy. PAS-positive foam cells or foamy Kupffer cells are found in the bone marrow, lymph nodes and liver. • *Laboratory findings* show an increase in triglyceride and a decrease in cholesterol and HDL. α_1-lipoprotein deficiency and moderate jaundice; occasionally, thrombopenia and leucopenia are observed. • The **prognosis** is generally good. Therefore, the disease is often diagnosed for the first time in adulthood. (213−215)

11.6 Debré's syndrome

This metabolic disorder with simultaneous storage of fat and glycogen in the liver was described by A.R. Debré et al. in 1934. Within a few weeks of birth, infants suffer from considerable hepatomegaly (without splenomegaly). There is evidence of hyperlipidaemia with hypercholesterolaemia, a tendency towards fasting hypoglycaemia, and disturbed glucose mobilization following exposure to insulin or adrenaline. • *It should be noted, however, that this form of glycolipidosis has still not been reliably classified.*

12 Sphingolipid storage diseases

The biochemical group of sphingolipids comprises: sphingomyelin, cerebroside, sulphatide, gangliosides, ceramide trihexosides, etc. Depending on the substance stored, differentiation is made between (*1.*) glucosyl ceramidoses (e. g. gangliosidoses, ceramide trihexosidosis, cerebrosidoses, sulphatidosis), (*2.*) phosphoryl ceramidoses (e. g. sphingomyelinosis), and (*3.*) mucopolysaccharidoses.

12.1 Gaucher's disease

► This form of glucosyl ceramidosis is the longest known lipid storage disease. It was described by P.C.E. Gaucher as early as 1882 and recognized as a separate entity with systemic character by F. Schlagenhaufer in 1907.

The condition is an autosomal recessive disease with reduced activity of the lysosomal **β-glucocerebrosidase.** (Instead of an enzyme defect, the cause may be the absence of cofactor **saposin C.**) The relevant gene is localized on **chromosome 1.** There is considerable genetic **heterogeneity.** While some gene mutations are responsible for more frequent neurologic and severe courses, others have been recognized, particularly in older patients, as causing a late onset and more moderate course of disease. The gene mutations are detectable with varying frequency in different ethnic groups. Due to the enzyme defect, sphingolipids are only broken down to the level of the glucocerebroside, whereby this glycolipid is subsequently stored. **Prevalence** is 1 : 30,000 − 1 : 50,000

Glucocerebroside is stored predominantly in the RES cells, but also in the spleen, hepatocytes, bone marrow and lymph nodes; rod-like tubules (20−40 nm) develop. • The storage of glucocerebroside leads to the formation of so-called **Gaucher cells.** These oval or polygonal cells are characterized by their light, striated ("crumpled") cytoplasm and a swollen cell body with a decentralized nucleus; occasionally, there are two or more hyperchromatic nuclei at the periphery. Apart from glucocerebroside, Gaucher cells also contain lysosomal acidic phosphatase. They are PAS-positive, 20−100 µm in size and grouped within the hepatic lobule; in some areas, the cells fill the sinusoids and are likewise found in the portal fields, where they originate from histiocytes.

Type I *(chronic visceral type):* This is the most common course and may manifest at any time in life; most often, however, it occurs between the ages of 20 and 40. The patients are able to lead relatively normal lives. Clinical features include pronounced *hepatosplenomegaly*, *ostealgia* with spontaneous fractures, backache and pain in the extremities, fever and *haematopoetic disorders* (microcytic anaemia, leucopenia, thrombopenia with haemorrhagic diathesis). The transaminases and the liver function values are usually normal; alkaline phos-

phatase is increased. Portal hypertension and ascites can occur due to obstruction or compression of the sinusoidal pathway. (224) After a course of several years, yellowish brown, patchy pigmentations (containing melanin) appear in the face, particularly at the root of the nose, and on the legs. Yellowish grey pingueculae may be present around the eyes (nasal or bilateral). Diagnosis is confirmed by the detection of acidic phosphatase in the cells and a reduced activity of glucocerebrosidase in leucocyte suspensions or fibroblast cultures. Meanwhile, the various types of Gaucher's disease can be identified by PCR. There are no neurologic symptoms. The cholesterol level is normal. (221, 223, 226) • Prognosis is determined by haematological complications, an increased susceptibility to infections and a higher rate of lymphoproliferative diseases. Transition to cirrhosis has often been observed, as have bleeding oesophageal varices. (216, 224, 228, 230, 232)

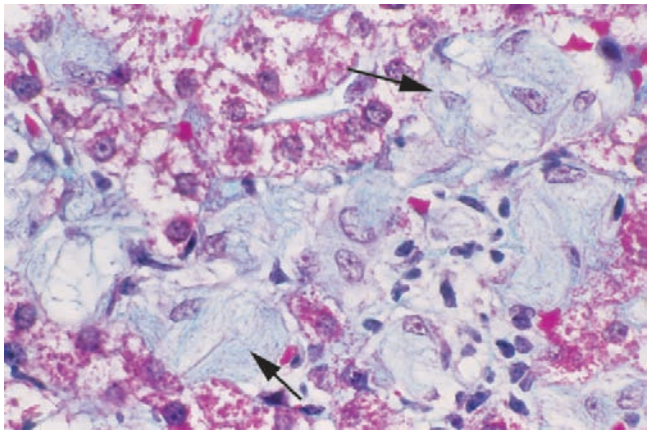

Fig. 31.17: Gaucher's disease. Gaucher cells (sphingolipid-storing macrophages) within the liver parenchyma (arrow), shown here as pale-blue cells with an internal structure similar to cigarette paper (PAS)

► *We were able to observe a family with 6 children, 4 of whom (3 boys and 1 girl) suffered from Gaucher's disease. I examined one of the children laparoscopically. All 4 children showed pronounced hepatosplenomegaly with symptoms of abdominal organ displacement; splenectomy was carried out in good time. (231) (s. fig. 31.17)*

For successful **treatment,** *β-glucocerebrosidase* gained from the placenta or recombinant imiglucerase can be used as an intravenous infusion every second week. (217–219, 222, 225, 227) • A novel form of oral treatment with N-butyldeoxynojirimycin over a period of up to 12 months produced good results. Further trials with this substance, which inhibits the synthesis of glucocerebrosides, are justified. Diarrhoea was a frequent side effect (79%). (220) • Following *liver transplantation*, there was a regression in glucosylceramide deposition in the extrahepatic organs – the metabolic defect was not corrected however. (236) Long-term follow-up showed good results after *bone-marrow transplantation*. (229)

Type II *(acute infantile neurological type):* Type II becomes manifest within the first few months of life. Splenomegaly followed by hepatomegaly can be observed at a very early stage. A striking feature is the cerebral and neurological symptomatology. The affected child usually dies within the first year of life due to bronchopulmonary infection.

Type III *(chronic juvenile neurological type):* At the onset of the disease, splenomegaly and, subsequently, hepatomegaly are observed. Gaucher cells are found in the bone marrow at an early stage. There are severe cerebral and neurological disorders.

12.2 Fabry's disease

The enzymatic defect in glycoprotein and lipid metabolism in this disease, which is based on X-chromosomal transmission, is most likely to be **α-galactosidase-A deficiency.** Storage products consist of sphingolipids, mainly GL-3. They are principally stored in the kidneys, vascular walls and smooth musculature, but also in the **liver** (stellate cells, portal macrophages, vascular endothelium). These affected cells take on a yellowish-brown colour. The hepatocytes are finely vacuolated; the Kupffer cells and macrophages are hypertrophic and contain cholesterol and lipofuscin. • The clinical picture, which usually begins with swelling of and burning pains in the limbs as well as skin changes and corneal opacity, was simultaneously described by J. Fabry and by W. Anderson in 1898. • *Treatment* with recombinant agalsidase beta has proved successful.

12.3 Gangliosidosis

Among other things, gangliosidosis may also affect the liver. The deposition of gangliosides (particularly of the G_{M1} and G_{M2} type) in the liver causes hepatomegaly to develop. In 1964 B.H. Landing et al. described *familial neurovisceral lipidosis* as a disease in its own right, although this entity had probably been observed by J. Caffey as early as 1951. The type of metabolic disorder was defined by J.S. O'Brien et al. in 1965. It is in fact an autosomal recessive **G_{M1} gangliosidosis** of the infantile generalized type I. The disease is caused by **β-galactosidase A, B** and **C deficiency**, so that the cleavage of the terminal galactose of both the ganglioside G_{M1} and the mucopolysaccharides is inhibited. These abnormal products are then stored. Foam cells with PAS-positive substances develop, which in turn involves the total RES and hence the stellate cells and portal histiocytes as well. Progressive hepatomegaly is present. Familial neurovisceral lipidosis causes the death of the child in the first or second year of life. (Type II G_{M1} gangliosidosis is considered to be a juvenile type without visceral involvement.)

G_{M2} gangliosidosis is caused by a deficiency of **hexosamidase A** and **B**. It was described for the first time by K. Sandhoff et al. as *Sandhoff's disease* in 1968. This biochemical variant (type II) largely corresponds to Tay-Sachs disease, which is also autosomal recessive. Renal

globoside (ceramide trihexoside) is stored in the visceral organs, particularly in the liver and spleen. Hepatomegaly is present, occasionally with splenomegaly. The lysosomes within the hepatocytes become considerably larger until they are as big as the nucleus and show lamellar structures.

12.4 Niemann-Pick disease

► In 1914 A. Niemann described a type of storage disease which, however, he did not recognize. In 1926 L. Pick differentiated this clinical picture from Gaucher's disease and called it "lipid cellular splenomegaly". The substance predominantly stored was identified as sphingomyelin by E. Klenk in 1934. Thus, this condition is a kind of phosphoryl ceramidosis.

The disease is based upon a deficiency of the lysosomal enzyme **sphingomyelinase,** mainly in the RES cells. This autosomal recessive enzyme defect results in **sphingomyelin** being stored in the liver (hepatocytes, Kupffer cells, portal macrophages), spleen, bone marrow and lungs. These pale storage cells, the so-called **Pick cells** (20−40 μm) display a mulberry-like, alveolate structure, and, in addition to sphingomyelin, contain cholesterol and triglycerides as well as pigment ceroid (s. fig. 21.6). The cell inclusions are granular and double-refractory. Due to vacuolation, the Pick cells look like foam cells. (234) • Meanwhile, five biochemical and **clinical types** have been differentiated (types A−E); type A is equivalent to the classical Niemann-Pick disease observed in infants. To date, some 200 cases have been reported.

Type A is already present in infancy. The following symptoms rapidly develop: anorexia, vomiting, weight loss, growth retardation, hepatosplenomegaly, lymphadenopathy, profuse sweating, a brownish-yellow waxy complexion and a pronounced cerebral and neurological symptomatology (muscular hypotonia, areflexia, deafness, loss of sight). In about 5 % of cases, cherry-red spots with yellowish prominence can be found on the fundus of the eye. Liver cell damage and cell necrosis as well as cholestasis develop. The affected child dies before reaching the age of 2 years. (236, 240)

Type B is often characterized by neonatal cholestasis, which disappears in the later course of disease. The condition becomes manifest in childhood and progresses slowly. Hepatosplenomegaly develops, as does cirrhosis with portal hypertension. (237) The lungs occasionally display a miliary picture due to the interstitial storage of sphingomyelin; chronic bronchitis is often observed. No neurological or cerebral symptoms are in evidence. The haemogram shows anaemia and thrombopenia. • *Treatment* with allogeneic bone marrow transplantation has been reported. (238)

Type C is characterized by a slow course with neurological symptoms and gradual mental deterioration. A rare event is the development of neonatal cholestasis (235, 239) or HCC (233).

Type E is also known as the **sea-blue histiocytes syndrome** and is considered to be a special form of Niemann-Pick disease found in adults. The stored, ceroid-like, acid-proof and PAS-positive pigment shows a sea-blue colour on Giemsa staining. Hepatosplenomegaly is present; thrombopenia is likewise in evidence due to bone marrow involvement. Cirrhosis may develop. The prognosis is considered to be good. (235)

12.5 Mucopolysaccharidoses

These are congenital disturbances in the enzymatic degradation of acidic mucopolysaccharides (MPS) by the lysosomes. *Four types of mucopolysaccharides* (glycosaminoglycam, dermatan sulfate, heparan sulfate, chondroitin-6 sulfate) are stored, ultimately compromising all organs and tissues. In addition, ganglioside is stored in the CNS. Depending on the respective disease, varying distribution patterns can be found, with storage forms occurring individually or in combination. To date, **ten individual forms** have been identified; others, however, have remained unclassified. In all forms, there is increased elimination of the above-mentioned MPS in the urine.

Six out of a total of ten forms are accompanied by **liver involvement** *(storage in the hepatocytes, Kupffer cells and portal macrophages as well as vacuolization of the cytoplasm)* and **hepatomegaly**. The liver is firm and has a bluish-yellow or greyish-yellow colour. The stored mucopolysaccharides are PAS-positive, but not diastasis-resistant. During a longer course, *fibrosis* develops and *cirrhosis* may occur.

Some forms overlap in symptomatology and cannot be distinguished by enzyme or urinary assays. • A **new therapy** for α-L-iduronidase deficiency using enzyme replacement (= *laronidase*) as i.v. infusion has proved safe and efficacious (J. Wraith et al., 2004).

Pfaundler-Hurler syndrome *(type I):* This syndrome is caused by an α-L-iduronidase deficiency (M. v. Pfaundler, 1920; G. Hurler, 1920). It is autosomal recessive and panethnic, with an incidence of approx. 1: 100,000 live births. A major cause of morbidity and mortality is respiratory insufficiency together with cardiac compromise (valvular dysfunction).

Ullrich-Scheie's syndrome *(type V):* This is also characterized by an α-L-iduronidase deficiency, but it does not appear before school age (O. Ullrich et al., 1943; H. G. Scheie et al., 1962). Further symptoms are above-normal growth and head size, chronic otitis, chronic rhinitis, hepatosplenomegaly, corneal clouding, ankylosis and mental retardation.

Hunter's syndrome *(type II):* Initially, this disease was erroneously classified as type I. Its nosological independence and X-chromosomal recessive transmission were recognized in 1964 (A. Nja). The disease is based on a deficiency of L-iduron-sulphate sulphatase and sulphoiduronate sulphatase. The syndrome may appear in a moderate or severe form.

Sanfilippo's syndrome *(type III):* This syndrome exhibits a similar clinical picture as described above with at least two different enzyme defects: heparan-sulphamidase deficiency (type A) and N-

acetyl-α-D-glucosamidase deficiency (type B) and possibly types C or D as well (S. J. Sanfilippo et al., 1963).

Morquio's syndrome *(type IV):* This syndrome is a result of a galactosamine-6-sulphatase deficiency (type A) or a beta-galactosidase deficiency (type B) (L. Morquio, 1929; J. F. Brailsford, 1929).

Maroteaux-Lamy syndrome *(type VI):* This syndrome is due to an arylsulphatase B deficiency. It appears in a severe or moderate form (P. Maroteaux et al., 1965).

12.6 Mucolipidoses

Mucolipidoses are characterized by a **combined metabolic disorder** of mucopolysaccharides, lipids, and glycoproteins. Lysosomal storage and foamy swollen Kupffer cells with hepatomegaly may be seen. In some of the numerous types, the underlying enzymatic defects have not yet been detected. • Type II is also called **Leroy syndrome** (J.G. Leroy et al., 1967). Due to distinctive cytoplasmic inclusions in fibroblast cultures, this disorder is also known as **"inclusion cell disease"** (J.G. Leroy et al., 1971). Foamy altered stellate cells, macrophages and also epithelioid foam cell granulomas are found.

Fucosidosis, being a so-called mucolipidosis, can be grouped among the mucopolysaccharidoses. It is due to α-fucosidase deficiency (localized on chromosome 1p 34). The storage products are deposited in the form of granular or lamellar inclusions in the lysosomes. This autosomal recessive disorder was first observed by P. Durand et al. in 1967 and clarified by F. Van Hoof et al. in 1968. Besides hepatomegaly, splenomegaly also sometimes occurs.

Mannosidosis, first observed by P.-A. Öckerman in 1967, is caused by α-mannosidase deficiency. The gene defect (autosomal recessive) is localized on chromosome 19p, 13, 2-q12. Hepatosplenomegaly, steatosis and perisinusoidal fibrosis are in evidence. PAS-positive vacuoles, consisting of lysosomally stored substances, are found in the cytoplasm of the hepatocytes.

Mucosulphatidosis is caused by arylsulphatase deficiency (types A, B and C). Metachromatic granules are found mostly in portal macrophages and less frequently in hepatocytes or Kupffer cells.

Lafora's disease (H. Unverricht et al., 1891) is seen as an autosomal recessive enzyme defect. A striking feature are the severe CNS disorders. The myocardium, liver and musculature are also involved. The affected hepatocytes are similar to *ground glass cells* and mostly found at the periphery of the lobules. The coarse granular and lamellar cytoplasmic inclusions are PAS-positive glycoprotein-mucopolysaccharide particles, also known as *Lafora bodies* (C.R. Lafora, 1911). Liver biopsy shows signs of unspecific reactive hepatitis and fibrosis. Death occurs in infancy.

13 Mucoviscidosis

Mucoviscidosis or **cystic fibrosis (CF)** is indeed one of the most common autosomal recessive diseases. It is characterized by the production of a viscous secretion in the excretory glands. Accordingly, pancreatic cystic fibrosis can be observed in the pancreatic area and cylindrical bronchiectases in the pulmonary area. The inspissation of bile and mucus leads to obstruction of the bile canaliculi and subsequently to cholestasis. The gene product is characterized as cystic fibrosis transmembrane regulator (CFTR). (246) The gene defect, which is located on chromosome 7, causes a disorder of the intracellular transport of chloride ions (probably also of chloride ion secretion) and thus triggers the occurrence of CF. The incidence of mucoviscidosis is about 1 : 2,000−4,500.

Liver involvement: In protracted disease, the liver becomes involved in 20−25% of cases. Rarely, a symptomatic hepatobiliary disease, mostly in the form of fatty liver but also as focal biliary cirrhosis, occurs in the first few weeks of life. Proliferation of the ductuli and dilatation of the intralobular bile ducts can be detected. (241, 242) As a result of this, crumbly products are deposited and the lumina are completely sealed off. These retained crumbly products have not yet been differentiated specifically; they are, however, PAS-positive and mucin-negative and consist mainly of precipitated protein. Hepatomegaly is due to macrovesicular steatosis. Inflammatory changes in the bile canaliculi and the small bile ducts with cholestasis and infiltration of the portal fields are often observed. (244, 248) With progressive proliferation of the ductules and liver fibrosis, there is a rise in γ-GT, AP and 5' NU. In the further course, a slight increase in the transaminases as a sign of hepatocellular damage is detectable. A score can be used for the sonographic diagnosis of liver involvement in CF. (252) Scintigraphy may be applied for the quantification of impaired secretion. (250) Liver function is assessed using ChE, Quick's value, GEC (s. p. 114) and ICG tests (s. p. 114). Gene mutation is easily confirmed by direct genetic screening with allele-specific oligonucleotides. The inflammatory and reactive-fibrosing processes may ultimately result in necrotizing cholangitis and multilobular biliary cirrhosis, possibly with bleeding oesophageal varices; frequency is reported to be 5−20%. (244, 248, 249)

Treatment: The only effective therapy is gene replacement. (247, 253) Symptomatically, liver damage and cholestasis can be reduced by UDCA. (243) It is also advisable to achieve an optimum nutritional status and to use essential phospholipids as long-term therapy. (251) In severe cases, there may possibly be an indication for liver transplantation or indeed combined liver-lung transplantation. (245)

14 Zellweger's syndrome

This **cerebro-hepatorenal syndrome** was described by P. Bowen, C.S. Lee, H. Zellweger and R. Lindenberg in 1964 on the basis of observations originally made by Zellweger. It is an autosomal recessive disease, with a lack of peroxisomes due to a mutation of the mRNA peroxisome-assembly factor 1. In this complex syndrome, there are various malformations with *disturbances in the amino-acid balance and β-oxidation of long chain fatty acids as well as iron metabolism resulting from a disorder*

of bile acid synthesis at the side chain. The blood shows hypersiderinaemia, and there are increases of different intensity regarding alanine, lysine, isoleucine, methionine, phenylalanine and serine. Signs of substantial cholestatic liver damage, siderosis, portal inflammation, fibrosis and the potential development of cirrhosis are evident. (254–258) (s. p. 242)

15 Porphyrias

15.1 Definition

Porphyrias are metabolic disorders caused by hereditary enzyme defects or acquired disorders of haem synthesis. These result in the increased formation of porphyrins and porphyrinogens and their precursors, which are either stored in the tissue or excreted in the urine or faeces. The term porphyria is derived from the Greek word "porphuros", which means purple and describes the purple-red crystalline porphyrins. Depending on the underlying enzyme defect or the respective aetiopathogenesis, porphyrias can become clinically manifest with neurological, photocutaneous, cerebral, cardiovascular, abdominal or hepatic symptomatology.

15.2 Classification

► **Primary porphyrias** are caused by hereditary enzyme defects in haem synthesis. They can be differentiated clinically into **acute** and **chronic** porphyrias as well as pathogenetically into **hepatic** and **erythropoietic** porphyrias. • **Secondary porphyrias** are symptomatic porphyrias present in various diseases or caused by poisoning or chemical substances, particularly alcohol. • Depending on the preferred **manifestation site** of the enzyme defect, either in the hepatocytes or in the erythrocytes (bone marrow), the porphyrias are subdivided into hepatic, erythropoietic and hepatoerythropoietic forms. However, this classification is not always strictly applicable. • Based on the **course of disease,** acute and chronic forms may be differentiated in primary hepatic porphyrias. The acute form is characterized by a congenital regulatory disturbance of porphyrin and haem synthesis together with the induction of ALA synthase within the hepatocyte. The acute forms are less frequent, but associated with a higher risk. Only acute hepatic porphyrias show convulsive gastrointestinal and neuropsychiatric symptoms. Chronic hepatic porphyrias are due to congenital or acquired enzyme defects; they are the most frequent forms and always involve liver damage. • Only the erythropoietic forms are accompanied by acute phototoxic reactions. Skin changes have been observed in variegate porphyria and porphyria cutanea tarda. (s. tab. 31.10)

Primary porphyrias may arise from a *hereditary defect* in any of the **eight enzymes** involved in haem synthesis: (*1*.) δ-aminolaevulinic acid synthase (which causes a sideroblastic anaemia), (*2*.) porphobilinogen synthase, (*3*.) porphobilinogen deaminase, (*4*.) uroporphyrinogen III synthase, (*5*.) uroporphyrinogen decarboxylase, (*6*.) coproporphyrinogen oxidase, (*7*.) protoporphyrinogen oxidase, and (*8*.) ferrochelatase. • A most important **branching point** in haem synthesis is the transformation of porphobilinogen (by PBG deaminase) into pre-uroporphyrinogen. The latter is transformed by uroporphyrinogen III synthase into uroporphyrinogen III; in the case of reduced enzyme activity, uroporphyrinogen I is spontaneously formed and subsequently eliminated in the urine. (300) (s. fig. 3.2)

► **Secondary porphyrias** are symptomatic and hepatic porphyrias. (300) They are coproporphyrinurias with simultaneously increased protoporphyrin concentrations in blood plasma. For prophylactic, prognostic and therapeutic reasons, it is important to differentiate between primary porphyria and secondary coproporphyrinuria. • Only in coproporphyrinuria caused by lead intoxication and in tyrosinaemia can δ-aminolaevulinaciduria be found at the same time. The alcohol-liver-porphyrinuria syndrome is the most important form of secondary porphyrinuria. In chronic alcohol abuse, secondary coproporphyrinuria may develop into chronic hepatic porphyria. (272) • *Some 30% of patients suffering from chronic liver disease later develop pathological coproporphyrinuria*. (s. tab. 31.11)

15.3 Biochemistry

Glycine and **succinyl coenzyme A** are the *initial substrates* for porphyrin synthesis, from which δ-aminolaevulinic acid (ALA) is formed with the help of ALA synthase. This initial biosynthetic step takes place in the mitochondria. Porphobilinogen (PBG) develops in the cellular cytosol due to the connection of 2 mol ALA. Uroporphyrinogen is then formed from 4 mol PBG and subsequently decarboxylated, thus producing coproporphyrinogen. Further decarboxylation occurs within the mitochondria, leading to the production of protoporphyrin. Due to its lipophilic qualities, the latter is not filtered by the kidneys and is therefore subject to enterohepatic circulation. With the help of ferrochelatase, iron is stored and haem is formed. • **Haem** is the *final product* of porphyrin synthesis, which may take place in all cells. It is required as a prosthetic group by enzymes or pigments (catalase, cytochrome, haemoglobin, myoglobin, etc.). Via feedback, haem regulates the limiting enzyme *ALA synthase* and thus the whole porphyrin synthesis. The activity of ALA synthase is increased by various drugs (s. tab. 31.13), chemical agents and endogenous metabolic products. • Induction of ALA synthase can be suppressed by glucose. Due to the short half-life of

Primary porphyrias	Abbreviations	Enzyme defect	Hereditary transmission
Erythropoietic porphyrias			
1. ***Congenital erythropoietic porphyria*** *(Günther's disease)*	CEP	Uroporphyrinogen III synthase	autosomal recessive
2. ***Erythropoietic protoporphyria***	EPP	Ferrochelatase	autosomal dominant
Hepatic porphyrias			
1. ***Acute hepatic porphyrias***			
• Acute intermittent porphyria	AIP	Uroporphyrinogen I synthase	autosomal dominant
• Variegate porphyria	VP	Protoporphyrinogen oxidase	autosomal dominant
• Hereditary coproporphyria	HCP	Coproporphyrinogen oxidase	autosomal dominant
• Doss porphyria	DP	Aminolaevulinic acid dehydratase	autosomal recessive
• Porphobilinogen synthase defect	PS	Porphobilinogen synthase	autosomal recessive
2. ***Chronic hepatic porphyrias***			
• Porphyria cutanea tarda	PCT	Uroporphyrinogen III decarboxylase	autosomal dominant
• Hepatoerythropoietic porphyria	HEP	Uroporphyrinogen III decarboxylase	autosomal recessive

Tab. 31.10: Primary (erythropoietic, hepatic, hepatoerythropoietic) porphyrias

ALA synthase of 70−80 minutes, inhibition or induction of this enzyme quickly affects haem synthesis. Haem deficiency due to an enzyme defect causes an increase in δ-aminolaevulinic acid. Free haem is either integrated into various apoproteins or intervenes as a haem repressor with the nuclear gene chain, which leads to the formation of specific mRNA for ALA synthase. • Synthesis and consumption of haem are synchronized precisely. The organism produces some 300 mg haem per day, with only 1% being excreted unused in the urine or faeces. (264, 266, 291, 300) (s. p. 38) (s. tab. 3.3)

Secondary coproporphyrinurias and protoporphyrinaemias

1. **Poisoning**
   - Alcohol
   - Heavy metals
     - lead, arsenic, iron, gold
   - Chemical agents
     - benzenes, hydrocarbons, *etc.*
2. **Liver diseases**
   - Acute and chronic hepatitis, alcohol-induced fatty liver, cirrhosis, cholestasis, haemochromatosis
3. **Blood diseases**
   - Haemolytic anaemia, sideroachrestic anaemia, aplastic anaemia, pernicious anaemia, leukaemia, Hodgkin's disease, *etc.*
4. **Infectious diseases**
5. **Diabetes mellitus**
6. **Hereditary metabolic defects**
   - Benign recurrent cholestasis
   - Dubin-Johnson syndrome
   - Rotor's syndrome
   - Tyrosinaemia type I
7. **Neoplastic diseases**
8. **Cardiac infarction**
9. **Pregnancy**
10. **Starvation**
11. **Iron metabolism disturbances**
12. **Medication** (s. tab. 31.13)

Tab. 31.11: Secondary (symptomatic, acquired) disorders of porphyrin metabolism

► The colourless porphyrinogens easily convert to coloured *red-fluorescent uroporphyrins* at 366 nm when there is sufficient oxygen. This gives the urine a red colour, which becomes darker (burgundy red) when left in contact with air. The *red-fluorescent specimen* obtained by liver biopsy in chronic hepatic porphyria is impressive. (s. pp 153, 166) (s. fig. 7.10)

15.4 Genetics

Primary porphyrias are genetically determined, whereby their expression varies in intensity, i.e. there is either a reduction in or instability of the enzyme affected by gene mutation. A total loss of enzyme activity or a lack of enzyme protein is inconsistent with the viability of the organism. Transmission is autosomal dominant in five forms of porphyrias, but autosomal recessive in congenital erythropoietic porphyria (CEP), hepatoerythropoietic porphyria (HEP) and the so-called Doss porphyria. (s. tab. 31.10) • Any type of porphyria may be caused by various gene mutations, which results in pronounced genetic heterogeneity. Two different types of porphyria may even become manifest within one family. The penetrance of the relevant gene mutation is low, so that about 80% of the persons involved are without clinical or laboratory findings. The probability of the enzyme defect being passed on to a child is about 50%. (267, 275, 281, 299, 300)

Not only genetic factors figure in the manifestation of porphyria, but also endogenous and exogenous causes such as alcohol (272), medicaments, stress situations, fasting, intoxication, metabolic products, effects of light and chemical agents (e.g. polyhalogenized biphenyls, dioxin). • In a well-known case in Turkey, about 4,000 people contracted hepatocutaneous porphyria after eating wheat contaminated with the fungicide hexachlorobenzene. (s. p. 581)

15.5 Clinical aspects

In cases of suspected hepatic porphyria, **instant orientation** is gained by examining the urine for evidence of δ-aminolaevulinic acid and porphobilinogen − using the *Watson-Schwartz test* (C.J. Watson et al., 1941) or *Hoesch test* (K. Hoesch, 1947) − as well as for the presence of porphyrins. (Hoesch test: 2 ml Ehrlich's reagent + 3 drops of urine = pinkish-red discoloration after shaking, revealing positive evidence of porphobilinogen.) • Further **differentiation** of porphyrins excreted in the urine and faeces or present in plasma and erythrocytes as well as of the distribution pattern of porphyrin metabolites is necessary; for this purpuse, thin-layer chromatography, ion-exchange chromatography and HPLC are available. • Identification of asymptomatic **gene carriers,** which is important for preventive measures, can be achieved by determining the enzymes involved in porphyrin synthesis.

The **symptomatology** of porphyrias is of such complexity and variability that there is always a danger of misinterpretation. Wrongly indicated laparotomy due to suspected acute abdomen entails a high risk! The initiation of incorrect treatment measures may also have grave consequences. Owing to the extremely complex interdisciplinary symptomatology, an emergency due to porphyria must be interpreted in terms of surgery, internal medicine or neurology, and depending on the results obtained, the patient is referred to the corresponding department. • *Porphyria can imitate numerous diseases.*

15.6 Erythropoietic porphyrias

The enzymatic metabolic defect is mainly restricted to the erythrocytes or bone marrow. Two distinct clinical pictures can be differentiated: *congenital erythropoietic porphyria (CEP)* and *erythropoietic protoporphyria (EPP)* (W. Kosenow et al., 1953; I.A. Magnus et al., 1961). From a hepatological point of view, only EPP is important. (s. tab. 31.12)

15.6.1 Congenital erythropoietic porphyria

This clinical picture is also termed *Günther's disease* (H. Günther, 1911). About 200 cases have been described so far. An autosomal recessive deficiency of uroporphyrinogen III synthase (chromosome 10q25) results in augmented accumulation of porphyrin isomers of type I, which cannot be used biologically. This causes an increase in uroporphyrin with the occurrence of fluorocytes (mostly erythrocytes, but sometimes also hepatocytes). A red discolouration of urine has already been observed in infants. Exposure to the sun leads to severe burns with the formation of blisters (the content of which may fluoresce) and necroses. Pronounced mutilations on the face and hands develop in the course of time.

	CEP	EPP
Urine		
Total porphyrins	+++	N/v
Uroporphyrin	++	N/v
Coproporphyrin	+	N/v
Porphobilinogen	N	N
ALA	N	N
Faeces		
Total porphyrins	++	+
Uroporphyrin	+	N/v
Coproporphyrin	+	v
Protoporphyrin	+	++
Erythrocytes		
Total porphyrins	+++	+++
Uroporphyrinogen I	+++	N/v
Coproporphyrin	++	N/v
Protoporphyrin	++	+++
Plasma		
Total porphyrins	++	+

Tab. 31.12: Constellation of findings in erythropoietic porphyrias (v = variable, N = normal)

The teeth are discoloured reddish-brown due to the deposition of porphyrins in dentine. Haemolytic anaemia with splenomegaly may develop. • *CEP causes neither neurologic disorders nor (substantial) liver damage.*

15.6.2 Erythropoietic protoporphyria

An autosomal dominant ferrochelatase deficiency causes enhanced accumulation of protoporphyrin within the erythrocytes. The gene for ferrochelatase has been detected on chromosome 18q21. EPP is considered to be the third most frequent porphyria (1 : 100,000). (306) Red-fluorescing erythrocytes form as a result of the high porphyrin content. EPP becomes manifest already in childhood, but the course of disease may be latent for a long time. Exposure to the sun causes skin reactions, such as painful erubescence, oedema and blisters. After long-term insolation, permanent skin infiltrations are observed. Prognosis is generally good. • The liver is damaged by increasing deposits of porphyrins. Cholestasis as well as liver fibrosis and cirrhosis may become manifest. Pigment gallstones can occur. Acute liver failure with a fatal outcome has also been reported. (265, 284, 298) • **Treatment** consists of the administration of β-carotene and vitamin E in order to capture the oxygen radicals which are present in greatly increased numbers in the skin following exposure to sunlight and thus to prevent the triggering of phototoxic reactions. Administration of chenodeoxycholic acid, cholestyramine and glucose (>300 g/day) is likewise recommended. Liver transplantation may be indicated. (263, 267, 269, 271, 282, 287, 289, 294, 295, 297, 300, 308, 310)

15.7 Hepatic porphyrias

Hepatic porphyrias show the following *characteristics:* (*1.*) intermittent course, (*2.*) increased ALA synthase

activity, and (*3.*) acute attacks induced or manifesting during the latency period due to numerous causes such as alcohol (272), hunger, carbohydrate deficiency, hormones, stress, intoxication, metabolic products and medicaments. (s. tab. 31.13)

Alcohol	Lofepramine
Allopurinol	Medrogestone
Amiodarone	Meprobamate
Barbexaclon	Mesuximide
Barbiturates	Methyldopa
Benegrid	Metoclopramide
Carbamazepine	Metronidazole
Carbromal	Nalidixic acid
Chloramphenicol	Nicethamide
Chlordiazepoxides	Nifedipine
Chlormezanone	Nitrofurantoin
Chloroquine	Oral contraceptives
Chlorpropamide	Oestrogens
Clonazepam	Oxazepam
Clonidine	Paramethadione
Cyclophosphamide	Pentazocines
Danazol	Pentetrazol
Dapsone	Phenacetin
Diazepam	Phenoxybenzamine
Dichloralphenazone	Phensuximide
Diclofenac	Phenylbutazone
Dimenhydrinate	Phenytoin
Ergotamine preparations	Piroxicam
Ethosuximide	Primidone
Eucalyptus oil	Progesterone
Fern extract	Pyrazinamide
Flufenamic acid	Pyrazolone derivatives
Frusemides	Pyrimethamine
Gestagens	Ranitidine
Glibenclamide	Rifampicin
Gliquidone	Spironolactone
Glutethimide	Steroids
Griseofulvin	Sulphonamides
Halothane	Sulthiame
Hydralazine	Theophyllines
Ibuprofen	Tolbutamide
Imipramines	Trimethadione
Ketoconazole	Valproic acid
Lidocaine	*etc.*

Tab. 31.13: Drugs which are able to trigger acute hepatic porphyria (AIP, VP, HCP) with differing degrees of risk (s. tab. 31.10)

	AIP	VP	HCP	PCT
Urine				
Total porphyrins	++	++	++	+++
Uroporphyrins	++	+	+	++
Coproporphyrins	++	++	++	+
Porphobilinogen	+→+++	+→+++	+→+++	N
β-ALA	+→+++	+→+++	+→+++	N
Faeces				
Coproporphyrin	N/v	+	+++	N/v
Protoporphyrin	+	++	+	N/v
Uroporphyrin	N/v	+/v	N/v	+
Plasma				
Total porphyrins	N/v	N/v	+→++	+→++

Tab. 31.14: Porphyrin and porphyrin precursor content in urine and faeces for the differentiation of hepatic porphyrias (v = variable, N = normal) (s. tab. 31.10)

The *diagnosis* is based upon the clinical symptomatology and the excretion pattern of the porphyrins or their precursors in the urine and faeces as well as their concentrations in the erythrocytes and plasma. (s. tab. 31.14)

► Patients suffering from hepatic porphyria receive a **porphyria pass** as well as all important **information** referring to the disease; in particular, they must be informed about risk factors leading to manifestation, e. g. avoidance of porphyria-inducing drugs (s. tab. 31.13) and xenobiotics, alcohol abstinence. A diet rich in carbohydrates is important as a preventive measure, because this reduces ALA activity in the liver. • It is essential to examine other **family members** in order to recognize any potential genetic carriers or to identify a relative afflicted by porphyria which is still in the latency phase. Such persons are likewise informed about the disease, particularly with respect to potential manifestation factors, and they also receive a porphyria pass.

Acute hepatic porphyrias

There are pathophysiological and clinical transitions between all forms of acute hepatic porphyrias. Due to the complex clinical symptomatology, including abdominal, neurological and cardiovascular findings, misinterpretations are possible, and often the diagnosis is made too late. Skin symptoms are observed when porphyrins have accumulated in the tissue, causing reactive oxygen intermediates to form following exposure to sunlight. *In all acute hepatic porphyrias, the excretion of δ-aminolaevulinic acid, porphobilinogen and porphyrins is increased in the urine.* However, no augmentation in the excretion of PBG is observed in defective PBG synthase. Even genetically identified acute hepatic porphyrias may remain latent for a considerable period of time. Their frequency is given at about 5:100,000, with remarkable geographic variations. There are four (or five) different forms of acute hepatic porphyria. (300, 303) (s. tab. 31.10)

15.7.1 Acute intermittent porphyria

Acute intermittent porphyria (AIP) is the second most frequent form of porphyria; it occurs three to four times more often in women than in men. The peak rate is between the ages of 20 and 40 years. This autosomal dominant form is responsible for a deficiency in porphobilinogen deaminase (uroporphyrinogen I synthase). The genetic defect of this enzyme is localized on chromosome 11q24. Several genetic variants (>4) exist. The frequency of gene defects shows geographic variations. **Manifestation** of AIP is also caused by numerous exogenous or endogenous factors (see above). In women, the first clinical manifestation is often associated with the premenstrual phase. Some women suffer from cycle-related episodes of the disease. The overall frequency of AIP is 5−10: 100,000. • There are **five cardinal symptoms,** which may appear with widely differing intensity: (*1.*) cardiovascular findings such as

tachycardia, hypertension and changes in the ECG, (*2*.) intermittent and diffuse abdominal pain, often localized in the lower abdomen, which may take the form of colic, including vomiting and ileus-like features, (*3*.) severe obstipation, largely unresponsive to treatment (onset mostly during puberty), (*4*.) peripheral neurological and muscular disorders, which may result in pareses or paralyses, and (*5*.) neurotic or psychotic behaviour (confusion, depression, anxiety, hallucinations, delirium and coma). In cases of hyponatraemia (insufficient ADH secretion), encephalopathy may become manifest. If cardiovascular and psychiatric symptoms coincide, the picture may be misinterpreted as a thyrotoxic crisis, particularly because the serum values of T_3 and T_4 are increased in approximately 20% of patients suffering from AIP. The causes of the clinical symptoms are thought to be abnormal biochemical stimuli, triggering dysregulations in peripheral and autonomic innervation. However, there are no skin changes in AIP, and there is no photosensitivity. (261)

Occasionally, the **liver** is also involved: slight increases in the transaminases and bilirubin, and possibly impaired excretory functions as well. Initially the hepatocytes reveal ultrastructural changes, and later slight steatosis and siderosis. The liver bioptate shows no signs of red fluorescence. In AIP, there is a high risk of *cirrhosis* and *liver cell carcinoma* developing. (259, 262, 268, 285) In some patients suffering from such conditions, liver transplantation has proved successful.

Acute attacks often coincide with oliguria and hyponatraemia. It is relatively simple to demonstrate the enhanced excretion of porphobilinogen in the urine using the Watson-Schwartz test or Hoesch test. Urinary excretion of ALA is increased. In about 60% of cases, the urine takes on a burgundy-red colour as a result of uro- and coproporphyrin when allowed to stand (due to the action of light and O_2). For evaluating prognosis and preventive measures, it is important to consider the different **AIP disease phases:** (*1*.) enzymatic defect, (*2*.) compensated latency period with moderate excretion of porphyrins, (*3*.) decompensated latency period with stronger excretion of porphyrins and discrete clinical symptomatology, and (*4*.) resultant clinical period with acute porphyria syndrome. When PBG is discharged in small amounts during remission, no acute episodes need to be feared. • **Misdiagnoses** include: acute abdomen, ileus, pancreatitis and peritonitis (= *beware of laparotomy*!), poliomyelitis, psychosis, hysteria, hypertonic crisis, thyrotoxic crisis, heart attack, etc. • A **prognosis** is difficult to make regarding an acute attack, which may be life-threatening. An acute sporadic attack has to be treated immediately. Morphological damage to nerves and also demyelination can develop after a long period of paralysis following an acute attack. The regression of these forms of damage often takes many months. Residual defects may remain, mostly in the hands and feet. If corresponding preventive measures are carried out to curtail the factors triggering the disease, the prognosis is quite favourable.

Treatment consists of i.v. glucose infusions (2 × 2,000 ml, 20% per day) plus administration of **haemarginate** (3 mg/kg BW/day on four consecutive days and at varying sites of injection) (s. p. 893), possibly with simultaneous administration of metalloporphyrin (for the inhibition of haemoxygenase) (292) as well as intensive care measures and administration of cimetidine (283) or iron. In cases with peripheral or CNS symptoms, prednisolone (100 mg/day) may be administered in addition. (260, 280, 286, 287, 303, 304, 309)

15.7.2 Variegate porphyria

Variegate porphyria is caused by protoporphyrinogen oxidase deficiency. Transmission is autosomal dominant (chromosome 1 q 23). The frequency is 1 : 100,000. • The **clinical picture** is characterized by skin changes similar to PCT (men > women), abdominal pain and internal as well as neurological symptoms similar to AIP (women > men). Therefore, VP is also called "mixed porphyria". Skin symptoms (increased photosensitivity, vulnerability, formation of blisters, pigmentation, hypertrichosis) are observed in 85% of patients, beginning mostly between the ages of 20 and 30 years. Constipation, vomiting and hypertension are even more common in PV than in AIP. Growth is retarded. • **Laboratory findings** show relatively high increases in total porphyrins, porphobilinogen and ALA, particularly in acute attacks. Enhanced excretion of coproporphyrin in the urine and faeces is observed, even in the preclinical period. The following are considered to be characteristic features of VP: (*1*.) increased excretion of porphyrin-peptide (x-porphyrin) in faeces and (*2*.) maximum absorption of porphyrins in the plasma at 626 nm, while the maximum is 619 nm in all other porphyrias. • The liver biopsy sample shows no red fluorescence. No significant or even characteristic histological liver changes are in evidence. • Factors triggering an acute attack correspond to those found in AIP. The prognosis is quite good when such noxae are avoided. (270, 288)

15.7.3 Hereditary coproporphyria

This is an autosomal dominant coproporphyrinogen oxidase deficiency (chromosome 3 q 12). The frequency is 1 : 100,000. An increase in porphyrins, coproporphyrin and porphobilinogen is found in the urine. This form of acute hepatic porphyria is very rare. Its clinical course is largely identical to that of AIP, whereby acute gastrointestinal and neuropsychiatric symptoms predominate. However, they are less pronounced and cannot be detected with the same frequency as in AIP and VP. Skin symptoms (in about 30% of cases) include photosensitivity, pigmentation and hypertrichosis. The liver displays red fluorescence due to the accumulation of porphyrin (s. fig. 7.10), but no morphological damage. Coexistence with PCT has been described. (273)

15.7.4 Doss porphyria

This rare, autosomal recessive form of porphyria is based on 5'-aminolaevulinic acid dehydratase deficiency (chromosome 9q34). A symptomatic disease only occurs in homozygotes or double heterozygotes. No more than seven cases have been described to date. Urinary excretion of ALA and coproporphyrin is increased; greater amounts of protoporphyrin accumulate in the erythrocytes. Neuropathy develops, as in AIP. Repeated severe neurological crises may necessitate liver transplantation. Heterozygotes are considerably endangered by lead, because lead inhibits ALA and thus triggers the manifestation of porphyria (= *plumboporphyria*).

15.7.5 Porphobilinogen synthase defect

This rare form shows a very varied symptomatology. The disease may become manifest during puberty with severe pain or it may present later in life (beyond the fifth decade) with the clinical picture of moderate polyneuritis. ALA as well as uroporphyrins and coproporphyrins are augmented in the urine, whereas no abnormalities are evident in faeces or plasma.

Chronic hepatic porphyrias

Chronic hepatic porphyrias appear in two variants: (*1.*) porphyria cutanea tarda and (*2.*) hepatoerythropoietic porphyria. Chronic hepatic porphyria develops in about 10% of patients suffering from chronic liver disease. (s. tabs. 31.10, 31.11)

15.7.6 Porphyria cutanea tarda

This is the most common form of porphyria (prevalence 20−50 : 100,000). The primary enzyme defect is a *uroporphyrinogen III decarboxylase* deficiency. The coded gene for the enzyme defect is on chromosome 1q34. Porphyria cutanea tarda (PCT) is characterized by pronounced genetic heterogeneity. • As far as its transmission is concerned, two forms can be differentiated: (*1.*) **familial form** (type 2), which is characterized by an autosomal dominant transmission route with heterozygote enzyme deficiency (about 50% enzyme activity) in all tissues (e. g. erythrocytes, liver, fibroblasts) − other family members are frequently affected as well; (*2.*) **acquired form** (type 1), which is also autosomal dominant, displays a uroporphyrinogen decarboxylase deficiency, but only in the liver (normal enzymatic activity in erythrocytes). This "sporadic" type occurs about four to six times as often as the familial form. • Probably, there is also a third form of PCT, namely another familial form, in which, however, the inheritance is not confirmed. The URO-D in the hepatocytes is normal (100%) in these patients. The enzyme defect seems to be limited to the liver. (275) It is (not yet) possible to differentiate clinically between these forms.

► As is the case with all hepatic porphyrias except HEP, additional **realization factors** such as (*1.*) alcohol, (*2.*) oestrogens, and (*3.*) haemodialysis (together with a genetically induced enzyme defect) are required for the clinical manifestation of PCT. Alcohol may cause the manifestation of PCT due to the induction of MEOS and an alcohol-related increase in iron in the liver. However, (*4.*) pharmacons (particularly lipophilic medicaments) may also be responsible for PCT, e. g. barbiturates, diazepam, hydantoin, rifampicin, antipyrin, cyclophosphamide (290), hexachlorobenzene. (s. p. 581) These substances also trigger induction of the cytochrome P 450 system, but no regulatory disorder of haem synthesis, so there is no compensatory increase in ALA synthase. A rise in PBG and ALA is therefore not detectable in the urine; this means that no neuropsychiatric symptoms appear in PCT. • Apart from that, there are substances which act as **provocation factors**, e. g. xenobiotics, steroids, lead, iron and mercury. Iron interferes with porphyrin metabolism by inhibiting uroporphyrinogen decarboxylase and probably the subsequent ferrochelatase as well, so that PCT becomes manifest. While these factors trigger an acute life-threatening porphyric process in acute hepatic porphyrias, they are generally well tolerated in PCT (e. g. all drugs), at least for a short period of time. (274, 276, 300)

While PCT was formerly observed with greater **frequency** in men (peak rate between the third and fifth decade), the ratio between the sexes nowadays is assumed to be 1:1, with women occasionally being even more prone to the disease. This situation is possibly attributable to oestrogen intake (e. g. contraceptives) and increased alcohol consumption among women. Obviously, hepatic siderosis is a prerequisite for the expression of the "sporadic" form. The frequency of PCT is estimated to be 1% of the population in the age groups 30−70 years.

Predisposition: The additional liver damage caused by the accumulation of urocarboxyporphyrins (UCP) and heptacarboxyporphyrins (HCP) in hepatic tissue is a prerequisite for clinically manifest PCT. Several forms of **liver disease** increase susceptibility to PCT, e. g. viral hepatitis B, and more particularly type C (277, 278, 293, 300), fibrosis, siderosis, cirrhosis, alcohol-induced liver disease and liver cell carcinoma. Chronic liver disease is found in 30−40% of patients with PCT. (276, 296)

The **pathogenesis** of PCT is thus caused by a combination of *four factors:*

1. Defective uroporphyrinogen decarboxylase
2. Realization and provocation factors
3. Accumulation of UCP and HCP in the liver
4. Liver disease

Clinical picture: The clinical picture is characterized by skin lesions and liver damage. • The **skin changes** usually begin between the ages of 40 and 70 years. Photosensitivity is due to porphyrin deposits in the skin, where

their distribution pattern resembles that in the urine and serum. The lesions are so typical that there are hardly any misinterpretations. They are found at locations exposed to light such as the dorsal surface of the hands and fingers, face, neck, auricle and hairless areas of the head. The following forms are evident: (*1.*) vesiculated erosive blisters of 2 mm to 3 cm in size, which "migrate" when subjected to pressure (= *Nikolsky's phenomenon*), (*2.*) bloody scabs after the blisters have ruptured, with a poor tendency to heal, but only a slight inclination to secondary bacterial infection, (*3.*) healed vesiculated erosive blisters with atrophic scar formation and blotchy hyperpigmentation or depigmentation as well as whitish epithelial cysts, the size of a pinhead, filled with pearly bodies, (*4.*) hypertrichosis and facial cyanosis as well as melanotic hyperpigmentation, (*5.*) chronic actinic skin changes (premature skin ageing, cutis rhomboidalis, elastosis), and (*6.*) pseudosclerodermia on skin areas exposed to sunlight. (s. figs. 4.13; 31.18)

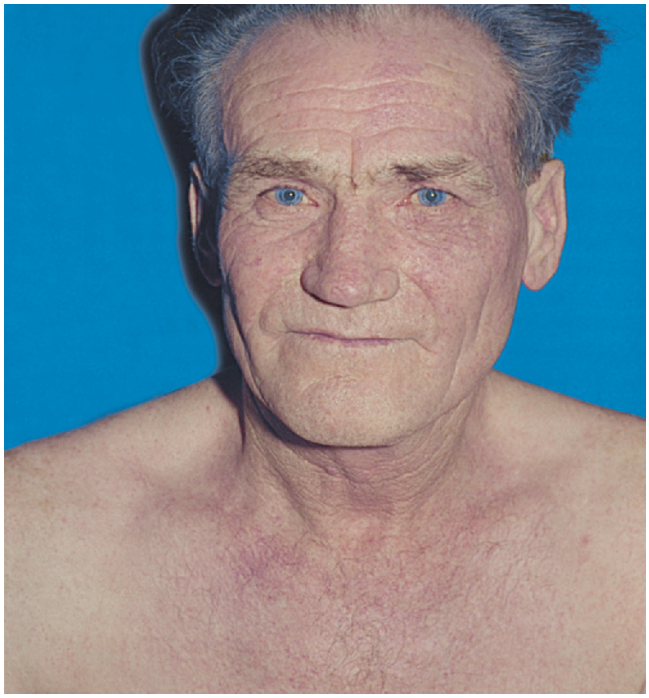

Fig. 31.18: Skin changes (face, front part of the neck) in porphyria cutanea tarda (s. fig. 4.13)

Liver damage is clinically recognizable as hepatomegaly. There is a rise in the transaminases, GDH, γ-GT, serum iron (increased saturation of transferrin) with secondary polycythaemia, and occasionally alkaline phosphatase. Reduced liver function results from decreased cholinesterase. • Depending on the duration and progression of PCT, changes in the *liver surface* range from a faded lobular pattern on a reddish-brown coloured liver to finely granulated areas with fine whitish fibrosis on a diffuse blue-grey surface colour (due to porphyrin deposits) and brownish speckled areas as well as flat tuberous surfaces with scar formation. (s. fig. 31.19)

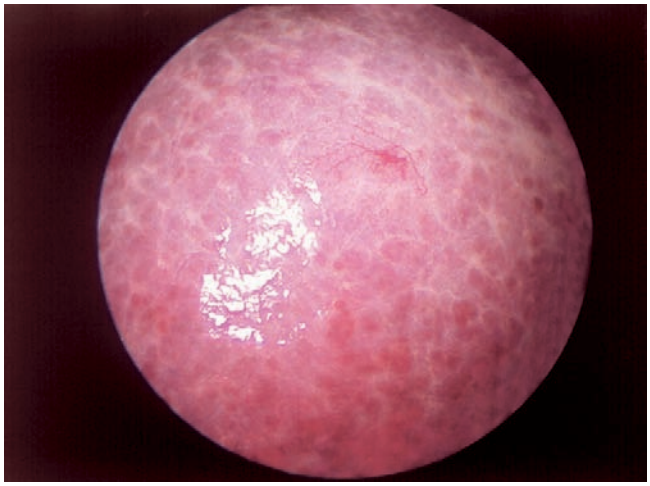

Fig. 31.19: Chronic active hepatitis in PCT: dispersed light reflection, capsular fibrosis with pronounced net-like fibrosis. Spider-like subcapsular neovascularization (s. fig. 33.13)

Histology shows deposits of needle-like porphyrins and large-droplet fatty changes in the hepatocytes, moderate iron deposits in the hepatocytes and Kupffer cells, and signs of non-specific reactive hepatitis. (s. fig. 31.20)

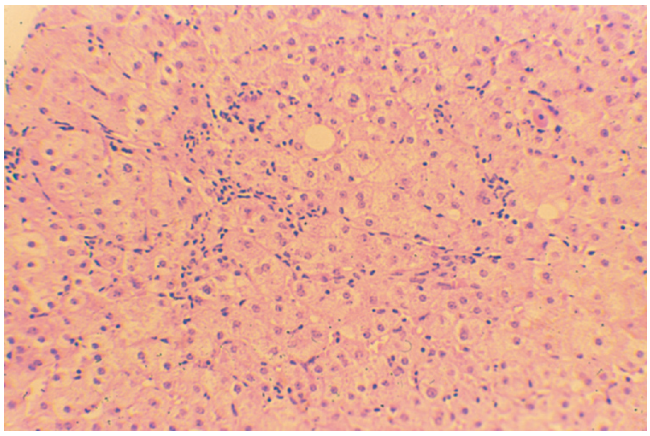

Fig. 31.20: Chronic porphyria: uncharacteristic lobular hepatitis histologically correlating to increased liver enzyme levels (HE)

In the bluish-grey areas, the porphyrin content is three to four times higher than in the lighter liver sections. In *UV examinations* (366 nm), to which every liver bioptate should be subjected, the red fluorescence has different intensities, facilitating classification into *PCT groups A–D:* types A and B (no clinical symptoms) show dot-like fluorescence, type C (latent PCT) has mostly reticular fluorescence, and type D (manifest PCT) is characterized by homogeneous red fluorescence. (s. fig. 7.10!) (s. pp 153, 166) • In the further course, scar tissue or micronodular cirrhosis develop. There is a greater risk of hepatocellular carcinoma (15–25%) (due to concomitant HBV or HCV infection as well as haemochromatosis?). (296, 300–302)

Diagnosis of PCT is based upon *skin changes* with characteristic anamnesis and *detection of porphyrins* in the urine and faeces. (s. tab. 31.14) There is evidence of

increased uroporphyrins, particularly heptacarboxyporphyrin and coproporphyrin in the urine as well as elevated uroporphyrin in the faeces and plasma. Dark red discolouration of the urine resulting from enhanced release of porphyrin (>15 µmol/day) has often been observed. Subclinical forms can be detected three times more frequently using targeted diagnostics! • In most cases, *HLA-A3* and *HLA-B7* (as in haemochromatosis) are present in the serum. *Serum iron* is elevated. The undoubtedly important role of **iron** in the pathogenesis of PCT has still not been clarified. Mutations of the HFE gene in haemochromatosis (C 282 Y, H 63 D) are also much more numerous in PCT. Probably, these (and other?) mutations cause an enhanced resorption of iron from the intestine. (275, 301) • There is a close coincidence of PCT and **HCV** infection (mainly genotype I b). A great geographical variation in frequency has been reported, from which an average of HCV positivity in PCT patients of about 45% can be determined. Interactions between the two diseases have not been clarified as yet; however, it is striking that both conditions generally show siderosis as well as being influenced to a particularly unfavourable degree by alcohol. (275, 278, 279, 293)

Sonography: Even at an early stage of porphyria, ring-shaped foci with marginal hyperechoic ring and central hypoechoic reflexes can be detected. (s. fig. 31.21)

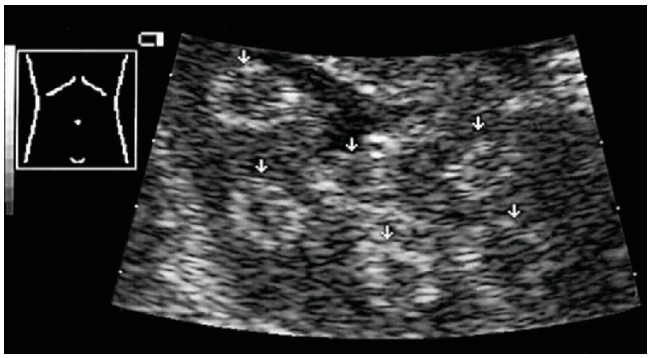

Fig. 31.21: Chronic hepatic porphyria: Sonographically, multiple, ring-shaped foci with marginal hyperechoic ring and central hypoechoic reflexes. (Completely reversible after alcohol abstinence)

These foci result from porphyrin deposition and are often discovered by chance since there is no evidence of a chronic porphyria. They can be mistaken for tumours or metastases; neither the foci nor their neighbouring parenchyma are hypervascularized. With abstinence of alcohol and avoidance of oestrogens, they are completely reversible. In a chronic porphyria, there may be a uniform increase in density due to diffuse porphyrin storage. (s. p. 544)

Treatment comprises avoidance of *trigger factors* (alcohol, oestrogens, sunlight, etc.) and application of sunblocks. *Venesection* with its withdrawal of iron results in inhibition of ALA synthetase, while uroporphyrinogen III decarboxylase activity is increased. This blood-letting therapy is based on 500 ml per week, after 4–6 weeks at monthly intervals. As an alternative, plasmapheresis has also been applied. *Chloroquine* (2 x 50 mg/day or 2 x 125 mg/week) is administered every second day for 6–12 months; it combines biochemically with porphyrins in the hepatocytes, thus improving the elimination of porphyrins from the liver. Venesection and chloroquine therapy may be carried out jointly. *Alkalization* of the renal metabolism enhances the elimination of porphyrins via the kidneys. (287)

15.8 Hepatoerythropoietic porphyria

This is a very rare form of chronic hepatic porphyria. As with PCT, the enzymatic defect is a deficiency of uroporphyrinogen decarboxylase. But the genetic defect is homozygous. It may also be caused by exogenous factors. Hepatoerythropoietic porphyria manifests in early childhood with high photosensitivity, scleroderma, hypertrichosis and anaemia. • The *liver* shows red fluorescence. Histologically, siderosis and non-specific hepatitis are found. Development of cirrhosis is possible. • No effective *therapy* is known. (305, 307)

16 Wilson's disease

► The first clinical and morphological description of the disease was published by S. A. K. Wilson in 1912. (383) This article did not mention the previous reports by K. F. O. Westphal (1883) and A. von Strümpell (1898); they distinguished between the neuropsychiatric picture of the disease, assigning to it the rather unfortunate term *"pseudosclerosis"*, derived from multiple sclerosis, which was already clearly defined at that time. • In 1921 H. C. Hall maintained that Wilson's disease and pseudosclerosis were actually one and the same thing and that it was even hereditary; he coined the term *"hepatolenticular degeneration"*. • The *storage of copper* in the liver and brain was postulated by A. Rumpel in 1913 and confirmed by F. Haurowitz in 1930. The brown-green colour of the corneal ring described by B. Kayser (1902) (339) and B. Fleischer (1902) (320, 321) is due to copper deposits, as was confirmed by W. Gerlach in 1934. J. N. Cumings (1948) identified copper as the cause of disease. *Hypercupriuria* was first described by B. M. Mantelbrote et al. (1948) and *hypocupraemia* by A. G. Bearn et al. (1952). This abnormality in copper metabolism was clarified by the detection of *hypoceruloplasminaemia* (I. H. Scheinberg et al., 1952). In 1960 A. G. Bearn demonstrated the recessive autosomal transmission.

The *first therapeutic attempts* to eliminate the increased copper content of the tissue by way of chelating agents go back to J. N. Cumings (1948) and D. Denny-Brown et al. (1951). For this purpose, *dimercaprol* and *ethylenediaminetetraacetic acid* (EDTA) were used. Treatment with *potassium sulphide* was recommended by M. M. Wintrobe et al. in 1954. The final breakthrough came with examinations carried out by J. M. Walshe (1956), who recognized the copper-binding properties of *β,β-dimethylcysteine* (penicillamine). Another copper-chelating substance, *trientin-dihydrochloride*, was introduced by J. M. Walshe in 1969. (379) The therapeutic effectiveness

of zinc in patients suffering from Wilson's disease had been reported as early as 1961 (G. Schouwink), while the therapeutic principle itself was described later on by T. U. Hoogenraad et al. (1978). (325)

16.1 Definition

Wilson's disease is a genetically determined, autosomal recessive copper storage disease with a reduced discharge of copper into the bile. Due to pathological copper deposits in the liver and brain as well as various other organs, sequelae develop above all in the liver and CNS. The other affected organs are generally involved in the disease as late manifestation. The chromosomal defect is still not fully clarified.

16.2 Frequency

The incidence rate is 1:100,000 inhabitants/year. The prevalence of this disease in patients with manifestation is estimated at 1:30,000 and in heterozygote symptom carriers at 1:100 to 1:200 of the population, i. e. 5−30 patients/1 million inhabitants. • Wilson's disease appears in childhood, adolescence and early adulthood. Initial occurrence before the 5th or after the 35th year of life is considered to be an exception. However, mild courses of disease have also been diagnosed beyond the age of 50. (314, 316) Most patients develop the first clinical symptoms around the age of 15. Geographical or race-related differences in frequency have not been reported. However, a higher incidence is found in regions with strong consanguinity (e. g. Sardinia, Israel).

16.3 Pathogenesis

The metabolic defect in Wilson's disease is located in the liver on chromosome 13 (M. Frydman et al., 1985), close to the esterase-D locus (ATP 7B). (312, 318, 329, 338, 352) Apparently, this is a genetically determined disturbance of **hepatobiliary copper** discharge due to a *defect in lysosomal copper-transporting ATPase*, which is localized in the trans-Golgi network. As a result, apoceruloplasmin cannot be loaded with copper, and is therefore degraded. The reduced secretion of ceruloplasmin explains the low copper level in the serum (D. J. Frommer, 1974). So far, more than 250 different mutations of Wilson's gene have been described. (320, 351) This disorder may be located (*1.*) in the area of the sinusoidal membrane (transfer into the blood via ceruloplasmin) or (*2.*) in the area of the canalicular membrane (transfer into the bile via copper-binding ATPase). About 80% of the copper absorbed enterally is excreted via the bile, while in Wilson's disease, the biliary excretion is reduced to 10−20%. Neither reabsorption of copper excreted via the bile nor intestinal copper resorption are increased. (325, 340, 363, 376, 384)

In the first three to four months of life, the **newborn** usually shows findings concerning copper metabolism which correspond to those found in Wilson's disease. It is therefore assumed that the conversion of foetal copper metabolism to that normally found later on in life is effected by a control gene. From birth onwards, 10−20 mg copper are accumulated every year. It usually takes 6−15 years before clinical symptoms become apparent as a result of the cumulative copper deposition. • In **siblings,** there may be considerable differences regarding clinical and laboratory findings. It can therefore be concluded that *exogenous* or *endogenous factors* modify the genetically determined process or alter the gene expression within the families involved. In other words, there is *genetic heterogeneity.*

Copper: The daily intake from food is 0.8−2.0 mg; it is released into the portal vein via copper-transporting ATPase. The transport of copper, which is toxic in its free form, is effected by the binding to ceruloplasmin, albumin and transcuprin. • Copper is bound to reduced glutathione and metallothionein in the hepatocytes and distributed to various organelles or incorporated into enzymes. The biological effects of copper are manifold and essential for some cellular functions. (s. p. 54) Copper is toxic not only in its free form, but also in cases of overload (e. g. cirrhosis in childhood due to the consumption of water from copper pipes). Copper homoeostasis is regulated via biliary excretion (normal value: about 1.2−2.0 mg/day), so that the **normal value** in serum is 75−130 µg/dl. (314, 316, 363, 374, 377) (s. p. 108)

Ceruloplasmin binds eight copper atoms per molecule and is of an intense blue colour. The coding gene is localized on chromosome 3. (385) Ceruloplasmin is the most important transport protein for copper in circulating blood (about 75−95% binding capacity). Another important function of this protein is the catalysis of oxidative metabolic reactions; it also possesses antioxidative features for the elimination of reactive oxygen intermediates. (s. tab. 3.25) The **normal value** in serum is 20−35 mg/dl.

The **reduction** in the serum value of ceruloplasmin led to the assumption that a primary synthesis disturbance was of particular pathogenic importance. There are several observations which contradict this hypothesis, suggesting that the *disturbance in ceruloplasmin synthesis* is probably a secondary consequence of the underlying metabolic defect. The introduction of copper into ceruloplasmin is possibly inhibited as a result of a dysfunctional apoprotein of ceruloplasmin.

Transcuprin is another transport protein for copper in circulating blood (about 7% binding capacity). • Apart from being bound to glutathione and metallothionein, copper may also be bound intracellularly to *another protein* that has only recently been detected.

16.4 Pathophysiology

The **toxicity of copper** is due to (*1.*) its binding to SH groups of cysteine, which is converted into an irreversible form by oxidation, and (*2.*) the fact that it is responsible for the formation of reactive oxygen intermediates (e. g. hydroxyl radicals), resulting in lipid peroxidation, which, in turn, is responsible for functional and structural disturbances of biomembranes. (334) In addition, a reduction in vitamin E likewise encourages the occurrence of lipid peroxidations. • Initially, copper is diffusely distributed in the cytosol and then stored in the hepatocellular lysosomes. At this point, it can also be demonstrated histochemically. Rapid transfer from the cytosol to the lysosomes may lead to increased lipid peroxidation with cell necrosis. (363, 374) • *Abrupt discharge of copper from the lysosomes into the bloodstream results in intravasal haemolysis and widespread liver cell necroses with fulminant hepatitis.*

16.5 Morphological changes

16.5.1 Hepatic manifestation

Electron-microscopically, the *mitochondria* – an essential target of toxic copper – show swelling and altered morphology, separation of external and internal membranes, greater density of the matrix, and granular or vacuolic inclusions. The *peroxisomes* are characterized by their increase in size and altered form as well as a granular matrix. Secretion of lipids is reduced. These structural changes result in discernible steatosis of the hepatocytes. • The *lysosomes* grow in number and size; finally, they decay and release lysosomal enzymes. (341) Apart from that, structural changes in the endoplasmic *reticulum* and *cytoskeleton* can be observed (= **stage I**).

Histologically, fine-droplet, ultimately also large-droplet, *steatosis* in the peripheral lobules and the occasional formation of *Mallory-Denk bodies* can be found. There are no signs of fat cysts. Steatosis subsides with increasing duration of disease. The lysosomes, meanwhile overloaded with copper, are deposited mainly at the bile pole of the hepatocytes and resemble lipofuscin-like pigment bodies. Degenerative changes in the hepatocytes, hypertrophy of Kupffer cells and an accumulation of glycogen in the nuclei with formation of *glycogen vacuolations* can be observed. (s. fig. 31.1) • Rapid intracellular redistribution of copper causes extensive cell necrosis. In this intermediate stage (= **stage II**), inflammatory reactions (very probably of an autoimmune nature) and proliferations of the bile ducts are evident. The histological picture – as well as the laparoscopic image of the liver surface – may at this point correspond to that of chronic hepatitis. (s. fig. 31.22) • The late stage shows increased fibrogenesis with variable fibre contents, and micronodular cirrhosis develops (= **stage III**). (s. figs. 31.23–31.25)

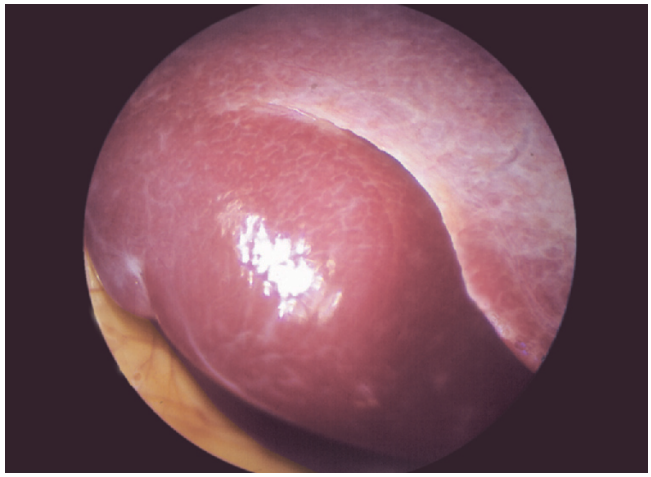

Fig. 31.22: Chronic hepatitis in Wilson's disease. Pronounced "simian cleft" with barely recognizable hepar succenturatum (s. p. 19)

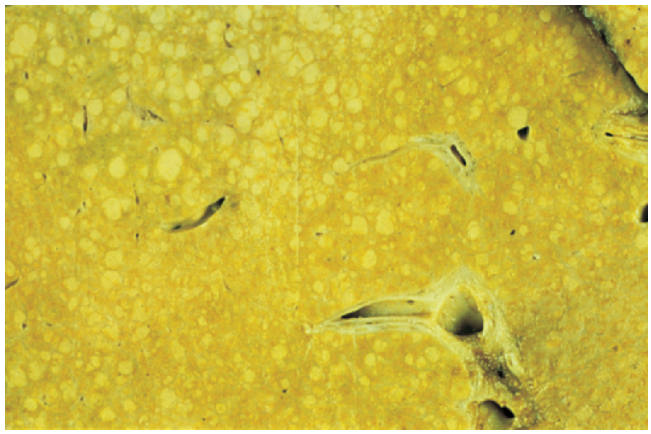

Fig. 31.23: Explanted liver with micronodular (regenerative-weak) cirrhosis in Wilson's disease (18-year-old woman presenting with acute liver failure) (Sirius red)

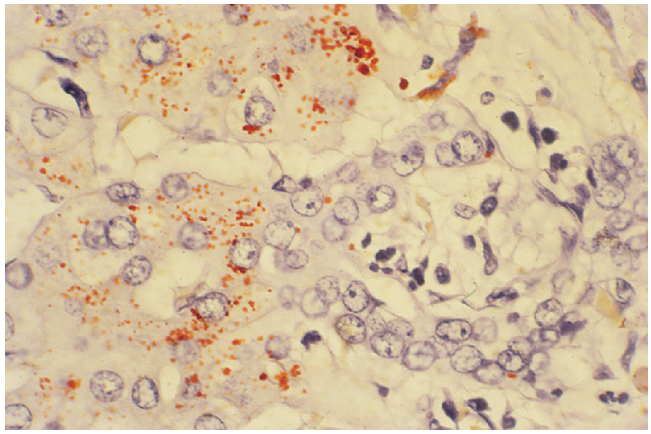

Fig. 31.24: Cirrhosis in Wilson's disease. Numerous copper deposits in periportal liver epithelia (Rhodanine)

The *distribution of copper* in the liver is concentrated in the peripheral lobules, but its pattern is irregular (even in advanced stages): there may be areas with very high copper concentrations adjacent to regions which are almost copper-free; this can give rise to false-negative findings during histochemical examination of the liver

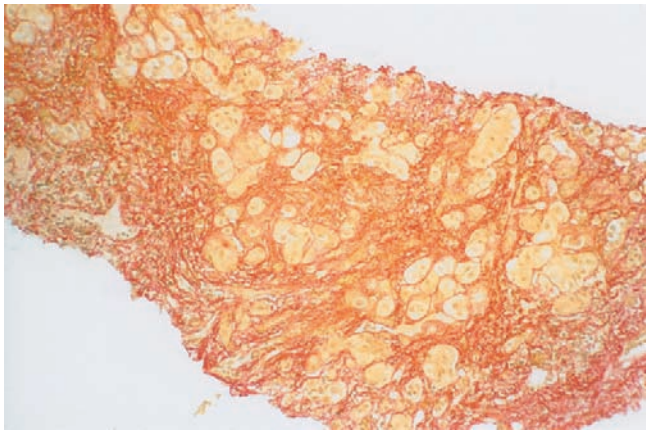

Fig. 31.25: Active progredient liver cirrhosis in Wilson's disease

bioptate. The *detection of copper* is possible using (unreliable) rhodanine staining, while the copper bound to metallothionein in the lysosomes is demonstrable by orcein staining (likewise not reliable). The copper content of the liver (normal 15−55 µg/g dry weight) may rise to 3,000 µg/g. If untreated, Wilson's disease shows rapid progression, also in the liver. (332, 340, 347, 373) With an effective therapeutic reversal of copper deposition, the copper balance can be restored. The patient returns to an asymptomatic course of Wilson's disease. However, the organ damage that has hitherto occurred is irreversible (= **stage IV**).

The **morphological spectrum** may therefore range from steatosis, acute hepatitis, fulminant course, chronic hepatitis, aggressive episodes in chronic hepatitis and liver fibrosis through to micronodular cirrhosis. Complete cirrhosis can exist in children aged four to five years. (312) • The development of *hepatocellular carcinoma* is extremely rare (353); it is assumed that copper has a protective effect against malignant transformation. (380, 382)

16.5.2 Extrahepatic manifestations

After complete saturation of the copper-binding capacity of the liver, the copper absorbed from food can no longer be taken up by the liver. This means that copper is stored in the brain, skeletal system, heart, cornea and kidneys − as is also the case when copper stored in the hepatocytes is released into the circulation on a large scale due to extensive liver cell necrosis. (332, 346)

CNS: Copper deposits affect the whole CNS. Degeneration and tissue loss as well as atrophy of the lenticular nucleus prevail. Occasionally, there are also small necrotic foci with a diffuse spot-like distribution. Microcavernous lesions occur due to the destruction of nerve cells. Myelinized fibres and oligodendrocytes are present, but there is also cellular hyperplasia and hypertrophy of astrocytes rich in protoplasm. The cerebral changes detectable in CT scanning do not correlate with the degree of severity of the functional disturbances (311, 328); however, there is a close correlation between the lesions detected by MRI and certain neurological findings. (313, 341, 359, 369) (s. p. 633)

Eyes: Copper is deposited in the form of a copper-sulphur complex in Descemet's membrane on the back of the cornea. Fine copper granules are observed, first as moderate colour changes at the upper limbus, then also at the lower limbus, with the ultimate development of a complete brown-green ring, 1−3 mm in width. (330, 331, 339) The **Kayser-Fleischer corneal ring** is nearly always found in adults with principally neurological and psychological problems, whereas it is generally not present in juveniles with a hepatic course. The best way to detect this ring is by slit-lamp examination. (s. fig. 4.17) The existence of such a ring is not in itself proof of Wilson's disease, as it is also found in CDNC-induced cirrhosis and primary sclerosing cholangitis; its absence does not, however, exclude Wilson's disease. • Occasionally (10−20%), a **sunflower cataract** is observed on the lens (E. Siemerling et al., 1922): a central gold-brown copper deposit in the frontal lens capsule with radial emanation into the posterior capsule areas. (321) Visual acuity is not compromised by these changes. Successful treatment leads to the regression of both ocular findings, making it possible to evaluate the course of disease (as well as to monitor inadequate treatment).

Kidneys: Dysfunction of the proximal tubule may occur as a late manifestation of Wilson's disease. Epithelial flattening, a loss of the brush-border membrane, mitochondrial anomalies and fatty cellular changes can be observed. These findings are, in turn, responsible for *proteinuria* with a predominance of *hyperaminoaciduria* (L. Uzman et al., 1948). Enhanced *calciuria* and *phosphaturia* may cause osteomalacia as well as hypoparathyroidism. (322, 336) Glucosuria and uricosuria, if present, are without clinical relevance. Due to decreased bicarbonate resorption, *tubular acidosis* may occur, with a tendency towards osteomalacia as well as the development of nephrocalcinosis and renal stones (in some 15% of cases). (336, 348, 381) The intensity of the copper deposits in the kidneys correlates closely with the cellular changes and functional disorders. • The glomerular function is not compromised, with the result that substances normally excreted in the urine are not retained.

Skeleton: Renal phosphate diabetes associated with hypercalciuria may lead to osteomalacia or osteoporosis. Likewise, inflammatory or degenerative arthrosis is thought to be a late manifestation of Wilson's disease. (345) Often, these developments are combined with intra-articular calcium deposits and chondromalacia, in particular *patellar chondromalacia*. Bone fractures are frequently observed even with minor traumas. Calcification may occur in articular cartilage, capsule and tendinous insertions, and deposits of calcium pyrophosphate dihydrate can appear in the intervertebral disks.

Myocardium: Copper deposits in the myocardium causes interstitial fibrosis, the sclerosing of small vessels and

focal inflammatory or degenerative lesions. This results in cardiac arrhythmia and cardiomyopathy.

Bone marrow: Leucopenia and thrombopenia may be a result of bone-marrow damage caused by copper deposits, although this can also be due to splenomegaly.

Skin and muscles: Acute *rhabdomyolysis* (355) and *dermatomyositis* are rare manifestations of Wilson's disease. • There may be evidence of *hyperpigmentation* and *acanthosis nigricans*. Bluish, lunular discolorations of the nails, so-called *azure lunulae,* are seldom. (315) (s. p. 88)

16.6 Clinical picture

The **symptoms** of Wilson's disease can hardly ever be recognized before the age of six. In most cases, the patients show symptoms between the 6th and 20th year of life – occasionally, however, as late as the 4th decade. About half the patients develop the disease before the age of 15. Sometimes, above-average growth in height is observed. Based on the prevailing symptoms, **three clinical forms** can be differentiated: (*1.*) *hepatic*, (*2.*) *neurological*, and (*3.*) *mixed courses*. The hepatic form is observed almost exclusively in children between the ages of 6 and 15, while the neurological form predominates in adults. • **Asymptomatic courses** are usually diagnosed only by chance. (319, 323, 332, 333, 357, 363, 370, 372)

Early diagnosis is essential. This is true both for the recognition of *heterozygous carriers* and for the diagnosis of asymptomatic *homozygous carriers* – often, the two groups can only be differentiated by using complicated methods (e. g. specific DNA markers, gene linkage analysis, ^{64}Cu kinetics). (337, 342)

Once the diagnosis of Wilson's disease has been confirmed beyond doubt, all other **family members** have to be examined as well. During this process, occasional cases of a presymptomatic course of disease are detected. A reduction of ceruloplasmin in serum may even be present in carriers of heterozygous features. Genetic analysis can provide a diagnosis in some 95% of cases, even prenatally.

Liver diseases

Hepatic disorders are a prominent feature of Wilson's disease in childhood and adolescence. In the early stages, a **fatty liver** is often observed, the aetiology of which is at first unclear. • A slight increase in the transaminases may be the first biochemical sign, before any other symptoms appear. • In **acute hepatitis** of varying degrees of severity, Wilson's disease must always be ruled out. • In the natural course of disease, **chronic active hepatitis** may be found in the initial phase. In terms of clinical and laboratory examination, it can only be distinguished from other forms of chronic active hepatitis by way of aetiology-specific findings. (s. fig. 31.22) • **Fulminant hepatitis,** which is observed quite often, shows rapid progression with the development of pronounced jaundice; there is evidence of increased copper values in the serum and urine, while ceruloplasmin in the blood is decreased or normal. Ascites and growing liver insufficiency are evident. (328) The transaminases are only slightly elevated. A marked reduction in alkaline phosphatase is considered to be a characteristic feature. (367) At the same time, haemolysis is often present due to an abrupt discharge of cytosolic copper into the bloodstream. Fulminant hepatitis in Wilson's disease is usually fatal if there is no chance of liver transplantation. (312, 326, 327, 332, 344, 351, 361, 368, 371) • **Liver cirrhosis** develops slowly. (s. figs. 31.23–31.25) Non-specific general complaints and skin stigmata of liver disease (s. fig. 4.21), which are typical in chronic forms, as well as the symptoms and sequelae of portal hypertension are evident. In association with thrombopenia, cutaneous and mucosal bleeding may occur due to synthesis disorders of the coagulation factors. Occasionally, recurrent bouts of jaundice (generally due to haemolysis) are observed. An acute necrotizing episode may have a fatal outcome. • Hepatic injury is seen as a consequence of augmented oxidative stress. (349)

Neurological and psychiatric disorders

Neurological symptoms are not observed before an advanced stage of disease is reached, particularly in older juveniles or adults. In their case history, half of these patients have not shown any signs of haemolytic anaemia or hepatic symptoms which could have pointed to Wilson's disease. However, a very discrete symptomatology at the onset progresses continuously and ultimately characterizes the clinical picture of untreated Wilson's disease. (311, 364, 372) • *Psychiatric symptoms* (personality changes, behavioural disorders, neuroses, psychoses) are detected in 55–65% of cases. • A drop in *performance at school* may be one of the first signs of Wilson's disease. • The following symptoms are found in varying degrees of intensity and in different combinations (although some of the late features are no longer observed nowadays due to effective treatment techniques which are initiated at an early stage):

- unsteady gait, balance disturbance, uncoordinated movements, grimacing
- dysarthria, scanning speech, palilalia
- dysphagia, salivation, raising and retracting of the upper lip
- dysgraphia, micrographia
- seizures
- tremor, athetosis, resting and intention tremor, nystagmus, rigor, flexion contractures, spasticity
- personality changes, behavioural disorders, neurotic or psychotic symptoms (including forgetfulness, irritability, emotional lability, inability to keep a distance)
- hypomimia and amimia, mask-like face

Haemolysis

In about 15% of patients suffering from Wilson's disease, haemolysis with corresponding jaundice can be observed, sometimes as a relapse and occasionally as a haemolytic crisis. (s. tab. 12.3) The release of large amounts of copper from necrotized liver cells causes copper-induced damage to erythrocytes with subsequent haemolysis (due to enzyme deficiency). Haemolysis may even constitute the first manifestation of Wilson's disease (326, 327, 358) and precede the hepatic findings by several years. Usually, it is transitory and self-limiting. In severe cases, this non-spherocytic, Coombs-negative, intravascular haemoloysis may be combined with haemoglobinuria. • Chronic haemolysis can lead to the formation of *pigment gallstones*.

16.7 Laboratory findings

When there are clinical signs suggesting the presence of Wilson's disease or corresponding differential diagnostic considerations, diagnosis (or diagnosis by exclusion) is established by laboratory parameters. AP is normal or slightly increased. The GPT/GOT quotient is generally > 4. (323, 332, 333, 346, 350, 377) (s. tab. 31.15)

Ceruloplasmin in the serum	↓	(< 20 mg/dl)
Copper content of the liver	↑	(> 250 µg/g)
Copper in the urine	↑	(> 70 µg/day)
Free copper in the serum	↑	(> 25 µg/dl)
Penicillamine test (600 mg)	+	(> 300 µg/6h)
Total copper in the serum	↓	(< 80 µg/dl)

Tab. 31.15: Decisive laboratory criteria for the diagnosis and follow-up of Wilson's disease

Ceruloplasmin: Usually, the serum value of ceruloplasmin is below 20 mg/dl in homozygous Wilson's disease; however, in about 15% of such symptomatic patients, the values are found in the lower normal range (20−30 mg/dl). In about 15% of heterozygous patients without manifestation of the disease, a slight decrease in the ceruloplasmin value is also observed. When the value is >30 mg/dl, Wilson's disease can usually be ruled out. Nevertheless, in strongly compromised liver function, the ceruloplasmin value increases due to hyperoestrogenism, which develops subsequently and stimulates ceruloplasmin synthesis in the liver. A decrease to <20 mg/dl is not confirmation of Wilson's disease. **Decreased values** are also found in nephrosis, Menkes' disease, aceruloplasminaemia (J.D. Gitlin, 1998), malabsorption syndrome, etc. **Increased values** are observed in cholestasis, during pregnancy, with oral contraceptives, in malignant and inflammatory processes, etc.

Cupruria: In pronounced liver cell decay, there is not only a rise in the copper value in serum, but more particularly in the amount of copper excretion in the urine. Cupruria of <50 µg/day rules out the presence of Wilson's disease (differential diagnosis: e.g. kidney disease). • The **penicillamine test** has proved successful: after administration of 600 mg penicillamine, copper excretion increases to >300 µg/6 hr (>600 µg/24 hr). However, it should be noted that this test may show similar positive results in cholestatic liver diseases.

Copper content of the liver: The diagnosis of Wilson's disease is confirmed by determining the liver copper content (normal: 20−50 µg/g dry weight) with atomic absorption spectrometry. Firstly, the puncture instruments and glass vessels have to be free of copper (cleaned with a 0.1 EDTA solution). An inhomogenous distribution of copper in the liver, particularly in cirrhosis, has to be taken into account. • In addition, an increased hepatic copper content is also found in other liver diseases, e.g. bile-duct atresia, primary biliary or primary sclerosing cholangitis, bile-duct obstruction, chronic hepatitis, neoplasm, α_1-antitrypsin deficiency, Gilbert's disease, Dubin-Johnson syndrome.

16.8 Diagnostic measures

Suspicion: Detection of acute or chronic liver disease of unclear aetiology (above all in a fatty liver) and/or haemolysis, and/or neurological or psychological peculiarities in children above the age of 6, in juveniles and in adults up to the age of 40 suggest the presence of Wilson's disease *("give it thought!")*.

Hints: Detection of a Kayser-Fleischer corneal ring (in the early phase by slit-lamp examination) is considered to be the most important clinical finding of manifest Wilson's disease. (s. fig. 4.17)

Confirmation: The diagnosis is verified by laboratory parameters (determination of the serum values of copper and ceruloplasmin as well as of copper excretion in the urine, if necessary using the penicillamine test) and by demonstrating the copper content of the liver, with simultaneous differentiation of existing liver damage.

Organ involvement: The type and extent of organ involvement can be determined effectively by a broad spectrum of examinations. To a certain degree, these examinations are also important for monitoring progress and therapy. (s. tab. 31.16)

Sonography: At an advanced stage, ultrasound yields a metastasis-like picture: fatty degeneration together with areas of fibrosis (= echogenic) and normal parenchyma (= hypoechoic).

MRI: An MRI brain scan may demonstrate typical changes such as atrophy and densification in the basal ganglia and the lenticular nucleus (i.e. in the putamen and the globus pallidus). The cause of the particular sensitivity of these regions is unknown. (313)

1. **Liver**
   laboratory parameters,
   liver biopsy/laparoscopy,
   copper content of the liver
2. **CNS**
   EEG, ENG, EMG (CT), MRI
3. **Eyes**
   slit-lamp examination
4. **Kidneys**
   calciuria, phosphaturia, glucosuria,
   aminoaciduria, tubular acidosis
5. **Sonography**
   liver status, portal hypertension,
   kidney stones, nephrocalcinosis,
   gallstones
6. **Skeleton**
   osteomalacia, osteoporosis,
   chondrocalcinosis, arthropathy
7. **Blood**
   thrombocytes, leucocytes,
   coagulation factors,
   signs of haemolysis (s. tab. 12.3)
8. **Heart**
   ECG, echocardiography

Tab. 31.16: Diagnostic measures for detecting the type and extent of organ involvement in Wilson's disease

16.9 Prognosis

The prognosis depends essentially on **early diagnosis** and consistent treatment. (350, 363) If untreated, Wilson's disease is fatal. When treatment is initiated too late, irreversible, chronic damage is inevitable and the prognosis is poor. However, when **early treatment** is applied at an initial stage, the prognosis for Wilson's disease is relatively good; remissions over a period of seven years have even been reported. *(One of our own patients has now been in remission for more than 20 years.)* The prognosis is more favourable if copper excretion therapy is started in the preclinical phase. The life expectancy of the patients is not compromised if therapy is successful. After recovery, the patient's general condition and physical performance are usually unimpaired. (346)

16.10 Treatment

Early diagnosis and thus **early treatment** form the basis for (*1.*) establishing copper homoeostasis which is as stable as possible, (*2.*) avoiding chronic or irreversible organ damage, (*3.*) supporting the regression of still reversible lesions, and (*4.*) improving (perhaps even normalizing) functional disorders. Therapy must be continued lifelong, since copper reaccumulates, with the result that Wilson's disease becomes manifest, and there is a danger of acute liver failure. (312, 319, 324, 332, 364, 372)

16.10.1 Dietary measures

Nutrition should be *low in copper*. Patients must avoid foodstuffs and beverages containing copper, e. g. edible offal, nuts, cocoa products, mushrooms, potato crisps, rye flour, oat flakes, beans, dried figs, certain types of cheese, meat and fish, pineapple, mineral water (see relevant *lists* as to the composition of foodstuffs and copper content in food). Vegetarian food, from which copper cannot be easily mobilized, is therefore recommended. • Cooking utensils containing copper should not be used. • *Alcohol* is strictly forbidden.

16.10.2 Drug therapy

D-penicillamine: The first-choice medication is D-penicillamine (J. M. Walshe, 1956). By forming copper chelate, it not only causes a reversal of copper deposition and cupruria (see penicillamine test), but also induces metallothionein synthesis, whereby the toxicity of the remaining copper is reduced, even though the copper content in the liver does not sink. Penicillamine reduces the activity of lysil oxidase, thus diminishing the deposition of collagen. As a result, however, the skin unfortunately becomes fragile and wounds heal more slowly. • The *initial dose* is adapted to the individual and ranges from 900−1,200 (−1,800) mg/day. The medication is given in three to four single doses per day, which have to be taken half an hour before meals. Therapeutic success is expected after six months at the earliest: there is continuous improvement with regard to abdominal complaints, neurological and psychological disorders, opthalmic findings and laboratory parameters. Occasionally, an initial worsening of the neurological symptoms is observed (most likely due to excessive copper mobilization), which, however, ultimately results in a constant improvement in the neurological status if treatment is carried on consistently. Whether or not the treatment has actually been successful can only be determined after about two years. • The removal of copper deposits is reflected in a normalization of copper excretion in the urine (after two days without medication) and serum parameters regarding copper metabolism. At this stage, a *maintenance dose* of 600−900 mg/day can be administered. Any interruption of treatment should not exceed a period of several weeks, since this would definitely result in a renewed overload of the organism with copper and possibly lead to acute and dangerous relapses. Treatment with penicillamine is continued even during pregnancy; breastfeeding is, however, not recommended. (312, 313)

In 20−25% of cases, **side effects** are observed, depending mainly on the dose (hypersensitivity reactions, aphthous lesions, arthralgia, nausea, fever). All in all, treatment of Wilson's disease with penicillamine is considered to be successful and safe. If penicillamine is not well tolerated or if serious side effects are observed (e. g. kidney or bone-marrow damage, polyneuropathy, pemphigus), treatment must be discontinued. • Penicillamine usually causes **pyridoxin deficiency**, so that substitution (25−40 mg/day) is recommended, particularly as

chronic liver damage leads to vitamin B_6 deficiency. • If necessary, **electrolytes** and **trace elements** also have to be substituted.

Zinc: Treatment with zinc is seen as an alternative therapy for mobilizing copper deposits (T. U. Hoogenraad et al., 1978). (335) *Zinc inhibits intestinal copper resorption and stimulates the synthesis of metallothionein in the liver and intestinal mucosa.* The recommended dosage is 3 (−4) × 50 mg/day, one hour before meals. The duration of administration and the dose are adjusted in line with therapeutic success. Zinc can also be used in long-term treatment (i. e. maintenance of copper homoeostasis) following the initial release of copper deposits via penicillamine. Side effects have been reported in the form of gastrointestinal complaints. There is an increase in AP as well as amylase and lipase. (360, 378)

Potassium sulphide: A decrease in intestinal copper resorption can also be achieved by the administration of potassium sulphide (3 × 20 mg/day) (M. M. Wintrobe et al., 1954). However, this therapy is not readily accepted by patients because the substance has a bad taste and very unpleasant smell.

Triethylene tetramine: The copper-chelating agent triethylene tetramine (trientine) can be considered as an alternative therapy to penicillamine (J. M. Walshe, 1982). (379) The initial dose is 3−4 × 600 mg/day; the recommended maintenance dose is 2 × 600 mg/day, administered about one hour before meals. The side effects are similar to those observed with penicillamine, but efficacy is lower. Iron-deficiency anaemia can occur. The substance is commercially available in the USA. (362) • *Further chelating agents being tested include 2,3,2-tetramine, APD and tetrathiomolybdate.*

16.10.3 Peritoneal dialysis

In acute copper toxicosis, peritoneal dialysis has been successfully implemented with simultaneous administration of penicillamine. In order to bridge the time until liver transplantation can be carried out, plasmapheresis and haemofiltrations are recommended.

16.10.4 Liver transplantation

In a fulminant course of Wilson's disease, MARS is a possible therapy. (366) In an advanced stage of cirrhosis with complications or where medication is not feasible, the only remaining alternative is liver transplantation. After successful transplantation, all clinical findings and laboratory parameters improve, and even neurological disorders become reversible. Transplantation is a causal therapy, which confirms that the primary metabolic defect of Wilson's disease is located in the liver. This means that no copper-releasing medication is required following transplantation. (317, 343, 354, 356, 365, 375)

16.11 Indian childhood cirrhosis

This condition, which is confined to India, leads to the development of childhood cirrhosis; it is fatal in almost all cases. Its aetiology is unknown. The condition affects children of both sexes between the ages of one and three years. Its familial occurrence points to genetic factors. There is increased copper ingestion through food and milk as well as from household utensils. Alkaloids may also be involved in aetiopathogenesis. • Initially, the symptoms include increased appetite, restlessness, sleep disturbances, bright and sticky stools as well as hepatomegaly. Kayser-Fleischer rings are not present. Serum ceruloplasmin is normal. The further course is characterized by jaundice, oedema and ascites, bouts of fever and liver atrophy. • The histological picture is similar to acute alcoholic hepatitis, but without fatty degeneration. Mallory's hyaline, which is surrounded by polymorphonuclear leucocytes, is detectable in 80−90% of hepatocytes. The liver shows the highest content of copper so far verified in humans; it is located in the cytoplasm. As a result of low-grade liver cell regeneration, only small nodules are formed in the extensive, dense connective tissue, so that micronodular cirrhosis results. • *Therapy* is based on a strict reduction in copper intake and the administration of D-penicillamine. (387)

Non-Indian childhood cirrhosis: This similar condition, found in other countries, is indistinguishable from Indian childhood cirrhosis. Therefore, it is also called *copper-related liver disease.* A genetic defect is probably involved. Any correlation with increased copper ingestion is unlikely. (386)

17 Haemochromatosis

► The first observation of haemochromatosis was made by Th. Bonnet (1679). The first clinical case was described by A. Trousseau (1865) with the *three main symptoms* of iron storage diseases: diabetes mellitus, bronze pigmentation of the skin and cirrhosis. In 1871 C. E. Troisier termed this condition "la cirrhose pigmentaire dans le diabète sucré". In 1877 H. Quincke detected iron deposits in the liver parenchyma of these patients and called this clinical picture "siderosis". For the same condition, V. Hanot and A. M. Chauffard (1892) coined the term "cirrhose hypertrophique pigmentaire dans le diabète sucré". In 1895 P. Marie also described this illness as "diabète bronzé". The term "haemochromatosis" was suggested by F. D. v. Recklinghausen in 1889. (453) Since 1935, haemochromatosis has been regarded as a genetically determined entity in itself and hypogonadism was added as a fourth cardinal symptom by J. H. Sheldon.

17.1 Definition

Haemochromatosis (HC) is a hereditary disease (autosomal recessive) affecting iron metabolism. It refers to pronounced iron deposition, mainly in the liver (> 50% of the total iron in the body), but also in other organs, e. g. pancreas, spleen, heart, endocrinium,

bone marrow, lymph nodes, salivary glands, basal skin layers and gastrointestinal epithelia. • Besides these *hereditary* (HFE-related) or idiopathic (non-HFE-related) primary forms, there are numerous *acquired* secondary forms of HC. At first, the cells of the RES become laden with iron. Only when the capacity of the RES is exceeded is there iron deposition in the parenchymal cells; this leads to damage of the respective organs. (s. tab 31.17)

Haemosiderosis (HS) denotes iron storage in the organism, whereby iron deposition in the liver is < 0.5 g/100 g liver WW. Iron deposition occurs almost exclusively in the RES; parenchymal cells are rarely affected. Haemosiderosis exists in two forms: (*1.*) *absolute HS* resulting in generalized iron deposition in the body as a whole, or (*2.*) *relative HS* localized in a certain organ with the rest of the body showing normal iron distribution. Haemosiderosis does not cause haemochromatosis. (s. tab. 31.17)

17.2 Classification

Hereditary haemochromatosis (HC) can be differentiated in one HFE-related and three non-HFE-related types as well as in a few other forms. (s. tab. 31.17)

17.2.1 HFE-related haemochromatosis

Type 1 is the classic **HFE-related form** of HC. It has an autosomal recessive inheritance pattern. The gene mutation is localized on chromosome 6, directly next to the A-locus of the HLA system. (412, 413) It was originally termed HLA-H gene (M. Simon et al., 1976) and later called HFE gene (J. N. Feder et al., 1996). The exact interpretation of the letters HFE is unknown (human ferritin?). In about 90% of patients with haemochromatosis, a homozygote point mutation (Cys 282 Tyr) in the HFE gene is evident (= replacing cysteine with tyrosine causes the disulfide bridge to fall apart). A further point mutation (H 63 Asp) is found in 5–10% of HC patients. Its role in iron metabolism is still not clarified (= asparaginic acid is replaced by histidine). This mutation is often observed in HC together with a heterozygote status for Cys 282 Tyr mutation (= *compound heterozygosity*). For these two point mutations the terms C 282 Y and H 63 D have been used; the combined mutation of C 282 Y/H 63 D is often evident. • The phenotype of HC in northern Europe and the USA comprises the genotypes: (*1.*) C 282 Y/C 282 Y (85–95%), (*2.*) wildtype / wildtype (5–8%), (*3.*) C 282 Y/H 63 D (= *compound heterozygosity*) (< 5%), (*4.*) C 282 Y/wildtype (< 2%), (*5.*) H 63 D/wildtype (< 2%), and (*6.*) H 63 D / H 63 D (< 1%). In southern European populations, the frequency of C 282 Y homozygosity is lower (ca. 65%). • The localization of the HFE gene on chromosome 6 has no close association to the receptor protein synthesis of ferritin or the iron-regulating factor. (391, 415, 439, 441, 443, 452, 468) (s. tab. 31.17)

Hereditary (primary) haemochromatosis

HFE-related haemochromatosis
type 1 Hereditary haemochromatosis

Non-HFE-related haemochromatosis
type 2 Juvenile haemochromatosis
type 3 Transferrin-receptor-associated HC
type 4 Ferroportin-associated HC

Aceruloplasminaemia
Atransferrinaemia
Neonatal haemochromatosis

Acquired (secondary) haemochromatosis
(iron content of the liver > 0.5 g/100 g liver WW)
- extreme iron intake due to dietary habits
  e. g. Bantu disease or African iron overload (together with genetic basis), dietary iron overload
- extreme iron intake due to therapy
  e. g. frequent blood transfusions, chronic haemodialysis
- haemolytically induced
  e. g. thalassaemia
- metabolically induced
  e. g. tyrosinaemia, porphyria cutanea tarda, Zellweger's syndrome, glycogenoses, lipidoses, paraneoplastic ferritin production (such as in bronchiolar carcinoma)

Haemosiderosis
- liver siderosis
  (iron content of the liver < 0.5 g/100 g liver WW)
  e. g. chronic alcohol damage, portocaval anastomoses, hepatitis C, cirrhosis
- pulmonary haemosiderosis
- renal siderosis
  e. g. paroxysmal haemoglobinuria
- cerebral siderosis
  e. g. Alzheimer's disease, Pick's atrophy, Huntington's chorea

Tab. 31.17: Classification of haemochromatosis and haemosiderosis (WW = wet weight)

17.2.2 Non-HFE-related haemochromatosis

Type 2 is **juvenile haemochromatosis**. This rare form of iron storage differs from type 1 through an earlier onset of clinical symptoms. It becomes manifest prior to the age of 30. Both sexes are affected equally. The gene defect is localized on chromosome 1 (= *hemojuvelin*) or 19 (= *hepcidin*). Compared to type 1, cardiomyopathy

and hypogonadism are more frequent, the course of disease is more severe and cardiac-induced death is more common. HFE mutations are absent, and there is no association with the HLA system. This form of HC has an autosomal recessive inheritance. (s. tab. 31.17)

Type 3 is the **transferrin-receptor-associated** HE. The gene defect is localized on chromosome 7q22 and affects transferrin receptor 2. This form of HC probably has an autosomal recessive inheritance. The histomorphological and clinical findings are similar to those of type 1. (450) (s. tab 31.17)

Type 4 is the **ferroportin-associated** HC, an autosomal dominant variant. It has only been observed in Italy. The gene defect is located on chromosome 2q32 and affects iron export protein ferroportin 1. In contrast to types 1 and 3, there is early iron storage in the macrophages. A characteristic feature are the markedly increased values of ferritin together with a slight elevation of transferrin saturation. (s. tab. 31.17)

17.2.3 Aceruloplasminaemia

Aceruloplasminaemia is a very rare, autosomal recessive disease with diffuse iron overload. It is caused by a mutation of the ceruloplasmin gene. This leads to excessive iron storage, mainly in the brain, liver and pancreas. The principal symptoms are increased serum ferritin, decreased serum iron and transferrin saturation as well as extrapyramidal disturbances, retinal degeneration, cerebellar ataxia and diabetes mellitus. (469–471) (s. tab. 31.17)

17.2.4 Atransferrinaemia

Atransferrinaemia is an extremely rare inherited syndrome. The first case involving a seven-year-old girl was described in 1961 (472). Clinically, there is a serious hypochromic iron-deficiency anaemia with a reduction in iron-binding capacity together with marked iron storage in the tissue. (472, 473) (s. tab. 31.17)

17.2.5 Neonatal haemochromatosis

Neonatal haemochromatosis (NH) was first described by H. Cottier in 1957. This fatal form of HC starts in utero and must be differentiated from the genetically induced types; it does not represent a variant of HC. Genetic markers are unknown, and the basic underlying defect is unclarified (inherited/acquired?). "Sporadic" cases have also been observed. Both sexes are affected with the same frequency. Even at birth, newborns have pronounced ferritin values, jaundice, hypalbuminaemia, hypoglycaemia, decreased transferrin, cholestasis, coagulopathy, oedemas and ascites as well as cirrhosis with iron storage. The babies affected die generally within the first few days or weeks. Pathogenetically, irregular transport of iron through the placenta or dysregulated iron metabolism in the foetal liver cells is assumed to be responsible. • The following *therapy* is recommended: acetylcysteine (100 mg/kg BW/day i.v. for 7 days), α-tocopherol (25 U/kg BW/day orally), desferrioxamine (30 mg/kg BW/day i.v. infusion over 8 hours until the serum ferritin is < 500 µg/l), selenium (3 µg/kg BW i.v. by continuous infusion) and prostaglandin E_1 (0.4 µg/kg BW i.v.). Liver transplantation, if successful, is curative. (388, 399, 423, 425, 432, 440, 460) (s. tab. 31.17)

17.3 Hereditary haemochromatosis

HFE-related haemochromatosis, which is synonymous with type 1, is the most frequent and most important form of HC.

17.3.1 Frequency

Hereditary haemochromatosis (HC) has an incidence of 0.25–0.5% in the Caucasian population. After Gilbert's disease, HC is the most common hereditary liver disease. Men are affected five to ten times more frequently than women. The gene mutation on chromosome 6 occurs with a frequency of 1:10 to 1:20 for the heterozygous type and of 1:200 up to 1:400 for the homozygous type, as far as this can be detected by current methods. The number of those affected with manifest HC is influenced by racial and exogenous factors (e.g. diet, alcohol, personal habits), which is an explanation for the variations in frequency between 1:500 to 1:4,000. Prevalence of the HC gene in the normal population is estimated at 6–7%. If both parents are heterozygous carriers, the probability of the children being homozygous is 1:4 and heterozygous 1:2. (391, 395, 398, 414, 437, 466, 468)

About 1:500 persons in Germany are carriers of homozygous HC. However, the number of clinically detected cases of HC only corresponds to a ratio of 1:5,000. A considerable number of homozygous carriers are diagnosed and treated far too late. • In 1% of patients with newly detected diabetes mellitus and in 3–15% of cirrhotic patients, the respective disease is aetiologically attributable to HC.

17.3.2 Pathogenesis

Iron storage is about 0.07 g per year in heterozygous carriers. In homozygous men, however, it is about ten times higher (0.6 g), whereas the rate in homozygous women is only 0.16 g due to loss of iron during menstruation, pregnancy and breastfeeding. Generally, men have stored a total of 20–30 g iron by the age of 50. As from 5–8 g, progredient organ damage begins. (420, 441, 447, 452, 468) (s. fig. 31.26)

A defect in the HFE gene leads to overenhanced (undesired) intestinal iron absorption. Thus the enteral absorption of iron is at a much higher level in HC (two-

to fourfold) because of this dysregulation, although the iron content of the organism is increased. After being above average for several years, iron uptake returns to normal, even in HC patients. However, following venesection, the iron absorption in HC increases again. This disorder is primarily caused by the *HFE mutation* present in the crypts of the small intestine cells, where iron uptake usually occurs. • Iron absorption is regulated by the iron-responsive-elements-binding-protein. This *IREBP* shows a normal function in HC, since its gene is not localized on chromosome 6. In contrast, there is a disturbance of the association between C 282 Y mutation of HFE and the *β-microglobulin*, so that the normal function of HFE is reduced. In addition, the defect HFE gene does not interact with the transferrin receptor 1, with the result that iron is more easily absorbed in the cell. Both the transmembranous protein *Nramp 2* and the iron reductase (Dcyt = duodenal cytochrome B) are considered as apical iron transporters. • Intestinal uptake of iron is still influenced by the basolateral iron transporter *ferroportin 1*, whose gene defect causes type 4 HC. Iron is also transported by the multi-copper-iron-oxidase *hephaistin*, which creates the connection between iron and copper transport. • Transferrin receptor 2 is also involved in the regulation of intestinal iron transport; in the case of a gene defect, it is responsible for the development of type 3 HC. The modes of action behind iron absorption and iron transport based on the above-mentioned (and other?) proteins have only been partially clarified. (394, 397, 405, 438, 448)

Iron metabolism: Iron is the sixth most abundant element in the universe; it has two oxidation states: Fe(II) and Fe(III). Normal **diet** accounts for 10−30 mg iron/day, with about 1.5 mg/day of this amount being stored mainly in the bone marrow, skeletal musculature and liver (hepatocytes, Kupffer cells, endothelial cells); in HC, however, the amount is 3−5 mg/day. Thus HC produces an annual iron surplus of 500−1,000 mg. • **Resorption** of bivalent iron takes place in the mucosal cells of the duodenum and proximal jejunum. There, it is oxidized by ferroxidases into trivalent iron and bound (by means of apoferritin) reversibly to water-soluble **ferritin** (molecular weight 460,000, 50 Å in diameter, iron content about 20%). A single molecule of ferritin may contain up to 4,500 atoms of iron. The synthesis of ferritin, which enters the cell via ferritin receptors, is stimulated by iron; the site of synthesis is the RES. Ferritin is also formed on the polysomes of the hepatocytes and broken down in the lysosomes. The plasma ferritin value correlates with the total amount of iron stored in the body. Enteral iron resorption is controlled by the intracellular ferritin concentration: there is a negative correlation between these two quantities. In HC, ferritin is considerably decreased in or absent from the mucosal cells; there may be disturbed regulation of ferritin gene expression, i. e. ferritin-determined dysregulation is observed. • A second (but labile) intracellular storage form of iron is **haemosiderin,** a water-insoluble pigment of Fe(III) hydroxide (ca. 37%), protein, carbohydrate, lipids, copper and calcium. Haemosiderin is formed during the lysosomal breakdown of ferritin, a process by which released iron can be stored in the lysosomes. It is mainly stored in the peripheral area of the lobules and in the portal fields. In contrast to ferritin, haemosiderin shows a *positive Berlin-blue reaction*. (s. figs. 31.28, 31.29) • Intracellular iron is, to a certain extent, bound to **transferrin,** which is mainly produced in the hepatocytes. It is considered to be a carrier molecule for extracellular iron. Only one third of the total *iron-binding capacity* of transferrin (normal 250−370 µg/dl) is iron-saturated. The saturation capacity beyond this normal degree of transferrin saturation is called *free (latent) iron-binding capacity*. There is a negative correlation between the amount of iron in the organism and the transferrin synthesis rate. The absorption of transferrin into the target cells occurs by means of a specific receptor. (394, 405, 420, 433, 438, 450) (s. pp 54, 104)

An **additional pathogenetic factor** (apart from excessive iron supply or alcohol abuse) is *vitamin C*, which has different effects: on the one hand, it enhances intestinal iron resorption as well as iron excretion from the RES, but on the other hand, it is broken down due to an iron overload, which results in subsequent vitamin C deficiency. • *Alcoholic beverages* may support the development of haemochromatosis when the respective iron content is considerably increased (depending on the iron content of the soil or of the drinking water used in their production). Furthermore, alcohol-induced folate deficiency may cause ineffective erythropoiesis with enhanced endogenous iron storage. • A genetic association also exists between HC and the *α_1-antitrypsin deficiency syndrome* as well as *porphyria cutanea tarda*, which leads to a more rapid development of cirrhosis. • Any rise in iron storage is due to a **disturbed iron balance:** if the daily supply continues to be higher than the daily loss, the individual organs will absorb iron in line with their respective storage capacity.

17.3.3 Iron toxicity

It was O. Warburg (1928) who first observed the toxic effects of "free" (ionized) iron on cell metabolism. Increased acid phosphatase, decreased glucose-6-phosphatase, disturbances in oxidative cell metabolism as well as augmented glycolytic breakdown processes in the liver and cardiac muscle cells have since been reported. • There are **three molecular mechanisms** which are important for the toxic effects of free iron on cell metabolism: (*1.*) increased formation of *reactive oxygen intermediates* (with release of lysosomal enzymes and indirect stimulation of fibrogenesis) (s. p. 71), (*2.*) direct *stimulation of collagen synthesis* (with enhanced fibrogenesis) (s. p. 410), and (*3.*) changes in or damage to *DNA* (with the possible induction of carcinogenesis). (s. fig. 31.26)

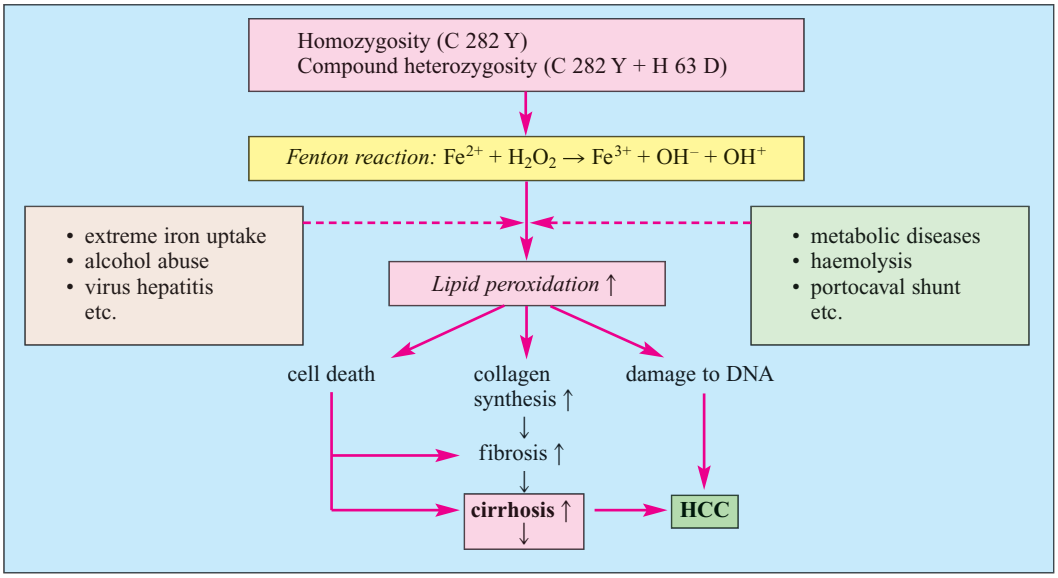

Fig. 31.26: Postulated pathogenetic and morphological cascade of damage in HC

17.3.4 Morphology

After being transported in increased quantities to the liver, iron is mostly stored as haemosiderin and ferritin in the hepatocytes of the lobular periphery. Initially, this *liver cell siderosis* does not impair the function of hepatocytes, as iron is absorbed by lysosomes. Iron storage commences in lobular zone 1 and progresses to zone 3. Gradually, the cells of the whole lobule become involved in iron deposition, mainly in the form of centroaxial pigment pathways, with enhanced iron deposition in the periportal area (haemosiderin granules). (409, 427)

When the storage capacity of the lysosomes is exhausted (usually with a liver iron content of >4,000 µg/g wet weight), there are unspecific inflammatory reactions in the portal field and lobular periphery, including single-cell necrosis as well as activation of fibroblasts and macrophages. Siderin, released from necrotized hepatocytes, is absorbed by Kupffer cells, which are usually increased in number and arranged in a nodular form. Coarse, grained siderosis of stellate cells likewise points to the extent of liver cell necrosis (= sideronecrosis). This phase may be called *acute siderophile hepatitis*. (408) Occasionally, there is formation of giant siderosomes and *glycogen vacuolations of the nuclei* in the hepatocytes of the peripheral lobules. (s. fig. 31.1) • Some excess iron is transported into the *bile ducts* and stored in their epithelial cells. The fainter and more finely granulated pigmentation (compared to hepatocytes) is important for the morphological diagnosis of HC.

The inflammatory mesenchymal reactions in the portal field and lobular periphery induce extremely low-cell perilobular and tylotic fibrosis. (427) Slowly, star-shaped **portal fibrosis,** so-called *holly-leaf fibrosis*, develops. This morphological picture, similar to that of chronic hepatitis, was termed *chronic siderophile hepatitis* by H. Kalk (1962). • The hepatic lobules are gradually grouped into islet-like structures. Within the tylotic connective tissue, very fine epithelial tubuli, also known as pseudo bile ducts, are found; they are more strongly loaded with iron pigments than are the preformed bile ducts. These pseudo bile ducts are seen as minimal and ineffective attempts to regenerate the parenchyma, although the constant iron supply prevents epithelial substitution. This leads to the development of almost uniform, micronodular (later on mixed-nodular) and very coarse **pigment cirrhosis,** which is rich in fibres and poor in regeneration. (s. figs. 31.27–31.29)

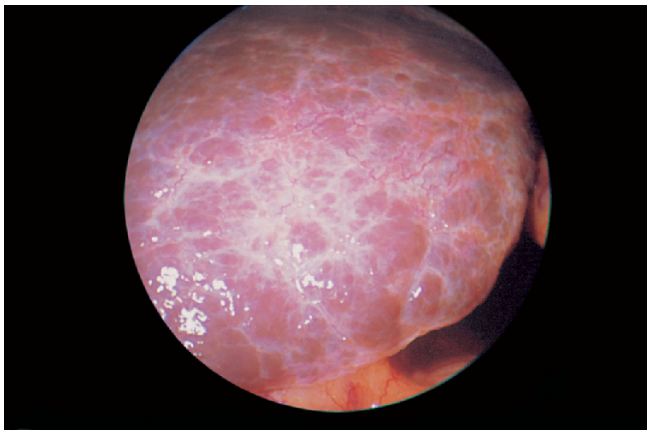

Fig. 31.27: Haemochromatotic cirrhosis: micronodular, slate-grey to brownish discolouration, rich in septate fibrosis, marked neovascularization

The morphological damage in other **iron-storing organs** (particularly the pancreas, heart and endocrinium) follows pathological mechanisms similar to those in the liver. These organs also show brown tissue changes with increasing fibrosis. HFE is expressed in all tissues involved in HC. • In HC, zinc is also stored in the liver at up to eight times the normal levels.

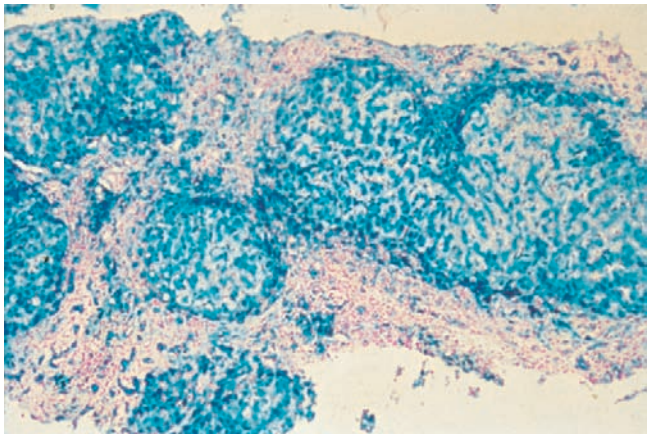

Fig. 31.28: Micronodular cirrhosis due to hereditary (HFE-related) haemochromatosis (Berlin blue)

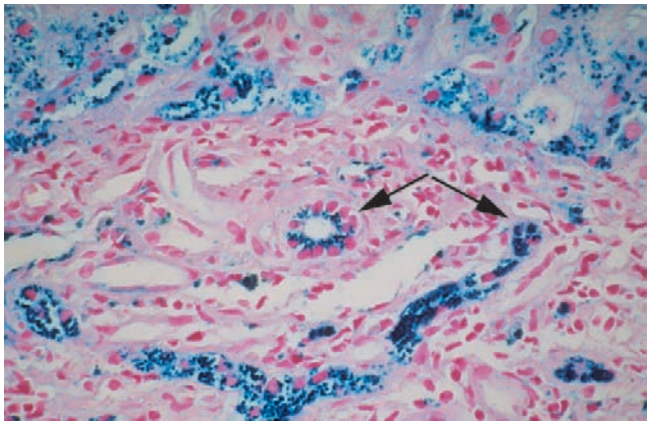

Fig. 31.29: Cirrhosis in hereditary haemochromatosis. Massive siderosis in hepatocytes and in epithelia of preformed bile ducts (arrows) (Berlin blue)

17.3.5 Early diagnosis

For successful treatment and thus also for prognosis, it is of the utmost importance to detect HC in its early phase. At this stage, however, neither subjective complaints nor clinical findings are apparent. The development of symptoms is slow and gradual – in men, not before the age of 30; in women, usually not before the menopause. • It is only by chance that an *increase in serum iron* (>160 µg/dl), *ferritin* (>200 µg/dl) or *transferrin saturation* (>45%) occasionally becomes evident. The combined determination of ferritin and transferrin saturation has a sensitivity of 90–95%, although specificity is lower. (s. tab. 31.18) These purely accidental findings, which initially appear as moderate deviations from normal values, are by no means conclusive, but they are nevertheless suggestive of HC. • However, *hepatic siderosis* can likewise be detected incidentally in liver biopsy, sometimes with an iron content of >1.7 mg/g dry weight, allowing the additional option of determining the hepatic iron index. (396, 398, 404, 441)

For the early diagnosis of HC, it has proved very useful to determine the **hepatic iron index:** iron content of the liver (in µmol/g dry weight) divided by age (given in years) (M. L. Bassett et al., 1984). A ratio of >2.0 suggests HC, while a ratio of <1.5 points to heterozygous HC or chronic alcoholic liver disease. (456, 461) Depending on the results obtained, subsequent determination of the *phenotype* or even of the *genotype*, if necessary, will follow (analogous to preventive diagnosis).

In the future, non-invasive **liver-iron quantification** (measuring the magnetic susceptibility as in "magnetic biopsy") by way of SQUID biosusceptimeter (BTi ferritometer) as a quick and sensitive method will open up new possibilities of early detection and therapy control.

17.3.6 Preventive diagnosis

The detection of HC requires targeted preventive diagnosis of family members (including siblings) and close relatives. Firstly, the **HFE genotype**, based on the determination of the *C282 Y mutation*, shows a similarly high diagnostic sensitivity to that of a liver biopsy; HFE genotyping is therefore important for family screening. In heterozygosity, however, liver biopsy should be performed as a diagnostic marker instead of HFE genotyping. (391, 395–398, 404, 415, 420, 436, 441, 451, 452, 455, 457) • The **phenotype** varies considerably and is influenced by a number of factors (alcohol abuse, oestrogens, dietary iron intake, loss of iron, etc.). Elucidation of the phenotype focuses on the determination of *transferrin saturation* and *ferritin* concentration in the blood. With increased values, liver biopsy is indicated: an iron content of >1.7 mg/g dry weight points to HC, while a value of >4.5 mg/g or an iron index of >2.0 confirms the presence of HC and is also an indication for therapy.

HLA system: This system may have one of *three constellations*: (*1.*) when two alleles (A3 and B14 or B7) of the relative are identical to those of the patient, there is a high risk of HC (= annual check-ups are required); (*2.*) with only one HLA allele of the relative being identical to that of the diseased person, there is merely a minor risk (= check-ups every 4–5 years are sufficient); (*3.*) if neither allele of the relative is identical to those of the patient, there is no risk at all (= no further check-ups are required). (413, 428) • It should be noted, however, that the formerly recommended determination of the HLA types (A3, B7, B14) is no longer common.

17.3.7 Manifestation

Subjective complaints

When the organism is constantly overloaded with iron, subjective complaints gradually develop (in 5–40% of cases). These present a variety of uncharacteristic features, varying from individual to individual.

Fatigue	**(40−60%)**	**Impaired potency**	**(10−20%)**
Abdominal pain	**(30−40%)**	**Nausea**	**(10−20%)**
Stress dyspnoea	**(20−30%)**	**Diarrhoea**	**(5−10%)**
Weight loss	**(15−25%)**	**Meteorism**	**(5−10%)**
Heart problems	**(15−25%)**	**Dysmenorrhoea**	**(5−10%)**
Arthralgia	**(15−25%)**	**Proneness to infection**	**(5−10%)**
Thirst	**(10−20%)**		

Clinical findings

During the development of the disease, clinical findings appear which can be attributed to those organs primarily involved in iron storage and to their respective damaging mechanisms. (390, 395, 404, 415, 437, 441, 451, 468)

1. Liver involvement	**(>90%)**
2. Cutaneous pigmentation	**(60−80%)**
3. Diabetes mellitus	**(50−70%)**
4. Cardiomyopathy	**(20−50%)**
5. Arthropathy	**(30−50%)**
6. Endocrinopathy	**(20−50%)**

1. Liver involvement: The liver is enlarged and of firm consistency. Skin stigmata of liver disease occur with varying degrees of intensity. (s. p. 83) Portal hypertension with splenomegaly (30−50%), oesophageal varices and ascites develop. • Laboratory findings may include a slight increase in transaminases, P-III-P and IgG values as well as borderline pathological ICG and galactose tests. (s. p. 114) Occasionally, CA 19-9 levels are moderately increased. Vitamin E is reduced. A striking feature is the detection of normal parameters despite the presence of manifest cirrhosis. The existence of cirrhosis with a ferritin value of <1,000 µg/l is unlikely. • Determination of α_1-foetoprotein is recommended at long intervals.

2. Cutaneous pigmentation: "Bronze diabetes" (s. p. 635) is rare. The colour of the skin is usually grey-brown, particularly in areas exposed to light, but also in axillae and conjunctivae as well as in the genital area and oral cavity. The dirty grey discolouration is due to increased melanin and enhanced iron storage, particularly in the sweat glands. Pigmentation is most striking along the lines of the palms. (s. fig. 4.12!) (s. p. 88)

3. Diabetes mellitus: A glucose tolerance disorder is detectable at an early stage. Subsequently, 60−70% of the patients develop the distinct clinical picture of diabetes, including diabetic complications (retinopathy, polyneuropathy, nephropathy, arteriopathy). The β-cells show iron deposition and fibrosis, in contrast to the α-cells (so that glucagon secretion is still normal). It remains unclear whether an additional genetic defect is present in the β-cells. Usually, there is peripheral insulin resistance, which often becomes reversible as a result of successful treatment. With progressive destruction of the β-cells, insulin-dependent diabetes mellitus develops.

4. Cardiomyopathy: Iron deposition in the myocardium and/or the conduction system causes the walls of the heart, particularly of the ventricles, to thicken; apart from that, necrosis of the cardiac muscle cells with subsequent fibrosis can be observed. Dilatative cardiomyopathy is accompanied by tachyarrhythmias. Cases of sudden death have been described. Considerable improvement is generally achieved by the release of iron deposits. The aetiology of cardiomyopathy can be clarified by myocardial biopsy. (403, 446, 459, 465)

5. Arthropathy: Initially, the small joints (finger, toe and wrist) are affected. Subsequently, the large joints (knee, shoulder and hip) also become involved in the clinical picture of arthrosis. The patients complain at an early stage (in 30−50% of cases) of arthralgia located in the areas described above, but there seems to be no correlation with the degree of iron overload. X-ray scanning shows subchondral cysts and sclerotic changes, narrowing of the articular space, hypertrophic bone proliferations, chondrocalcinosis, and osteoporosis (particularly when hypogonadism is present). This kind of damage in the joints, cartilaginous parts and adjacent bones is caused by the formation of calcium phosphate crystals within the synovial cells, mainly due to iron-induced pyrophosphatase inhibition. Venesection treatment has little effect on arthropathy. (392, 448)

6. Endocrinopathy: Pronounced iron deposition can be found in the thyroid and parathyroid glands, the anterior lobe of the hypophysis and the adrenal cortex. Testicular atrophy is caused by insufficiency of the anterior lobe of the hypophysis, particularly since only a small (perivascular) amount of iron, or no iron at all, is deposited in the testes. Hypogonadism (loss of libido, menstrual disorders, impotency) may regress after the removal of iron deposits. In addition, signs of hypothyroidism, hypoparathyroidism and slight insufficiency of the adrenal cortex are found. Biochemical evidence showing decreased serum values of the relevant hormones (gonadotropins, testosterone, oestrogens, aldosterone, thyroid hormones) may provide an overview of the underlying endocrinopathy. Gynaecomastia has not been observed. (406, 430, 468)

Laboratory findings

There are many reasons why laboratory parameters of iron metabolism may deviate from normal values; they should always be checked to facilitate the differential diagnosis of HC or of acquired haemochromatosis and haemosiderosis. • In cases of HC, there are various deviations from normal values. (398, 404, 441, 451) (s. tab. 31.18)

The determination of **serum iron concentration** often shows normal values over a long period of manifest HC. In suspected HC, repeated examinations may be necessary due to daily fluctuations and circadian rhythms in the serum iron concentration as well as in factors influencing laboratory procedures. It is only in the later course of HC that serum iron is constantly and signifi-

cantly increased. In 1953 H. KALK described **hypersiderinaemia** as the typical fifth cardinal symptom of HC. • When serum iron continues to fall, liver carcinoma can be suspected.

Determination of **transferrin saturation** (after 12 hours of fasting) supports a strong suspicion of manifest HC when the value is > 55%, while a saturation of > 75% is almost diagnostic proof of homozygosity. This method is also seen as a reliable screening test. A value of > 55% shows a specificity and sensitivity of 92% for HC. With a value of > 50% in women and > 60% in men, the ferritin level should be checked as well. An increased saturation value calls for liver biopsy, whereas a normal value requires a follow-up after one to two years.

The **total iron-binding capacity** (TIBC) of transferrin can be calculated from the transferrin concentration in µg/dl or µmol/l: transferrin x 1.41 or transferrin x 25.2. This gives a normal value for men of 268−436 µg/dl or 48−78 µmol/l and for women of 257−402 µg/dl or 46−72 µmol/l. • The difference between TIBC and the amount of iron found before Fe^{3+} and magnesium carbonate are added is called **free iron-binding capacity.** This is the FIBC which is possible beyond the normal saturation capacity of about one third of the transferrin. • The **transferrin saturation** (in %) can be calculated as follows: serum iron (µg/dl) x 100 divided by transferrin (mg/dl) x 1.25. • *A free IBC of ≤ 50 µg/dl, i. e. transferrin saturation of > 62%, suggests the presence of HC.*

The **ferritin value** corresponds well to the total value of body iron, whereby 1 µg/l ferritin is equivalent to about 7.5 mg body iron. The sensitivity of the ferritin value in HC is 85% and the specificity is 95%. A value of > 700 µg/l points both to the presence of HC and to the development of liver fibrosis or cirrhosis. In contrast, a normal ferritin value and normal transferrin saturation rule out HC in about 95% of cases. (s. tab. 31.18)

Normal values	Haemochromatosis
1. **Serum iron**	
Men 59−158 µg/dl 10.6−28.3 mol/l	> 180 µg/dl
Women 37−145 µg/dl 6.6−26.0 µmol/l	> 32 µmol/l
2. **Ferritin**	
Men 35−217 µg/l Women 23−110 µg/l	> 300 µg/l
3. **Transferrin**	
Men 210−340 mg/dl Women 200−310 mg/dl	> 500 mg/dl
Saturation 16−45%	
Heterozygotes	> 55%
Homozygotes	> 70%
4. **Hepatic iron index** $\frac{\text{iron content of the liver (µmol/g)}}{\text{age (years)}} =$	> 2.0
5. **Deferoxamine test** 500 mg i.m./iron excretion rate	> 4 mg/6 hr

Tab. 31.18: Biochemical findings in hereditary haemochromatosis

Hypochromic anaemia with *poikilocytosis* may point to disturbed erythropoiesis as a result of iron overload. • Transferrin deficiency may, however, also be causally related to this disease.

While the **deferoxamine test** (s. p. 105) is often considered obsolete, it can nevertheless serve as another stone in the mosaic and prove helpful in the diagnostics or follow-up of HC, since it is an iron chelation test that is easy to apply, free of side effects and inexpensive. In healthy persons, this test reveals a urinary iron excretion of < 1−2 mg; in haemosiderosis, the excreted amount is < 4 mg, and in HC > 4 mg. (450)

A **gastric mucosa biopsy** may facilitate differentiation between HC and secondary haemochromatosis, yet this examination is also regarded as obsolete today. (Iron in macrophages with HC = ∅, with secondary HC = ↑↑ .)

Imaging techniques

CT determination of increased liver density due to iron overload in haemochromatosis yields values of 80−140 Hounsfield units (= white liver). The deposition of 1 g iron causes an increase in density of 1 HU. CT densitometry makes it possible to monitor the course of the blood-letting therapy. (447) (s. p. 181) • In **MRI,** a haemochromatotic liver appears dark grey to black (= *dark liver*) due to signal loss depending on the iron content. (s. fig. 8.8) With an iron concentration of 1 mg/g liver tissue, accuracy is 95−100%. (393, 402, 422, 424, 435) (s. p. 185) • Both examination techniques are of great importance in detecting liver cell carcinoma. • By using the newly developed **SQUID biosusceptimeter** (BTi ferritometer), even more reliable quantification of liver iron is expected.

Liver biopsy and laparoscopy

Laparoscopically, a dark brown liver surface with augmented fibrosis can be seen. (s. fig. 31.26) • Quantitative measuring of iron in the liver bioptate is the most reliable technique for diagnosing haemochromatosis. If possible, this kind of biopsy should be carried out under laparoscopic guidance, and *two biopsy punches* should be taken. The first sample is designed to serve as a histological specimen as well as to detect iron and iron localization, while the second sample is used for quantitative iron determination. • At the same time, the *hepatic iron index* is calculated arithmetically. This index also facilitates the differential diagnosis of chronic alcoholic disease, which is not always an easy task, particularly at the cirrhotic stage (index < 1.4, in HC > 2.0). (434, 464)

17.3.8 Prognosis

The following *three factors* are of decisive importance for course and prognosis:

1. early diagnosis
2. consistent long-term treatment
3. close (lifelong) cooperation between patient and physician

► Therapeutic results will be better if the morphological damage is less severe and the other organs have not been seriously affected. • It is necessary to regard HC treatment as a lifelong procedure requiring the joint therapeutic involvement of the patient and the general practitioner or specialist. (441)

Untreated HC has a slow course and follows certain patterns in its progression; spontaneous regressions are not to be expected. The 5-year survival rate after establishing the diagnosis is only 18%. The mean survival rate was about 1.5 years in 1935, about 4.4 years after the introduction of insulin treatment, and 8.2 years with consistent venesection therapy. (467)

However, with early diagnosis and consistent treatment, **HC** has a good prognosis. In a comparison of treated versus untreated patients with HC, 5-year survival rates of 89% (vs. 33%) or 92% and an overall 10-year survival rate of 76% were observed. When cirrhosis was already manifest, the survival rate was only 62% after 10 years, whereas it was 93% in non-cirrhotic patients. • We believe that normal life expectancy is indeed a realistic perspective. *One of our patients died after 19 years of ongoing treatment of causes apparently unrelated to HC.*

The greater the effect of iron depletion, the more likely it is that morphological liver changes or even fibrosis and hepatic transformation processes will be reversed. • Portal hypertension and oesophageal varices may also regress, and a decisive improvement in diabetic metabolism is often observed. • *One of our patients with diabetes mellitus showed a normal diurnal blood sugar profile after eight years of consistent treatment, while the glucose tolerance test (monitored over longer periods of time) produced a moderately pathological result.* • In contrast, arthropathy appears to be unresponsive to treatment and may cause considerable suffering.

The main **causes of death** in HC are related to the following developments which aggravate the clinical picture (particularly in those cases where alcohol consumption is continued):

1. complicative diabetes mellitus
2. cardiac insufficiency
3. primary liver cell carcinoma
4. hepatic coma, liver failure
5. bleeding oesophageal varices
6. intercurrent infections
7. acute abdomen

The correlation between **hepatocellular carcinoma** and haemochromatosis was described by M.J. Stewart in 1922. Primary liver cell carcinoma is seen as the most common cause of death in haemochromatosis. Its frequency ranges between 6–42% or 7.3–18.9% (mean value approx. 14%), i.e. the risk of developing liver cell carcinoma in HC is about 200 times higher than in the normal population. Nevertheless, carcinogenicity is not necessarily related to the presence of cirrhosis, nor to appropriate therapy or non-treatment (cf. potential cocarcinogens, alcohol or hepatitis viruses). HCC development is mainly multilocular. Manifestation of a sarcoma has only rarely been observed. The latency period is reported to be 20–30 years. Follow-up with sonography and AFP determination at six-month intervals is indicated. HCC can also develop some years after complete iron depletion. Long-term iron overload acts as a cocarcinogen, apparently by way of DNA impairment. (400, 401, 410, 416, 418, 421, 426, 431, 439, 462)

In HC, the frequency of **extrahepatic carcinoma** may also be increased: in one Italian study, the figure was 3.7%. This correlation, which has also been assumed by other authors, raises the question as to whether there are additional cocarcinogenic factors, such as alcohol or hepatitis viruses. (459) • It is a striking feature that **alcoholism** is much more frequently observed in patients with HC than in people suffering from liver cirrhosis or from hepatic carcinoma of different genesis. • It is also remarkable that prevalence of **HBsAg** and **anti-HCV** is two and three times higher respectively in HC patients than in the normal population. Presumably, the function of the immune system is blocked by iron. This would help to explain the high carcinoma rate in patients with HC, due to the procarcinogenicity of HBV and HCV on the one hand and of iron on the other hand. • In cases of iron overload, **yersiniosis** should also be considered as a typical concomitant infection, possibly with the development of liver abscesses: iron is of exceptional importance for the metabolism of yersinia and to a considerable extent conducive to their growth. (389, 467) (s. p. 486)

17.3.9 Treatment

The paramount aim of treatment is to reach a negative iron balance. This must be achieved before complications due to the chronic iron overload have their effect on the predisposed organs. • *The extreme uncontrolled increase in iron absorption is treatable by adjuvant measures, albeit with limited success.*

Venesection treatment

Venesection treatment (W.D. Davis et al., 1950) is the method of choice; it is the most effective therapy. Bloodletting of about 500 ml blood frees the body of some 250 mg iron. With an average accumulation of >25 g

iron, *weekly venesection* should be considered over a period of at least two years. • In patients with haemochromatosis (without simultaneous anaemia), blood-letting of 500 ml is carried out weekly until the serum ferritin value has normalized. Experience shows that the deironing of the body using this technique takes about 18 months. Blood-letting is well-tolerated, and the patient's physical capacity is usually not affected. In general, the haemoglobin value does not fall below 12g/dl. • By the time the respective *blood values* (ferritin level and transferrin saturation) normalize, a large quantity of iron has been discharged. At this stage, blood-letting can be reduced to two or three times per three months, provided the ferritin value and transferrin saturation remain within the normal range. Subsequently, three to four venesections per year usually suffice to maintain the iron balance. *Proton pump inhibitors* suppress the absorption of dietary, non-haeme bound iron. This can reduce the frequency of phlebotomies. • *Plasmapheresis* is not required, since the plasmaproteins removed by blood-letting are easily replaced by the organism. However, in isolated cases, it may be necessary due to a cirrhosis-induced decrease in protein synthesis. The haemoglobin content should not (or only for a short period) fall below 11.5−12.0 g/dl, and the protein concentration should not fall below 6.0 g/dl. (404, 407, 415, 417, 431, 437, 441, 451) • *The number of venesections per year which are required to maintain the iron balance should be adhered to lifelong. This therapy must never be discontinued!*

Deferoxamine

This specific iron-chelating agent is obtained from actinomyces cultures. In a dose of 1.5 g, it is capable of mobilizing about 25 mg iron, i.e. the same amount as during a 500 ml venesection. • An *indication* may be given, even if only temporarily, when blood-letting cannot be carried out (e.g. anaemia, cardiac insufficiency). Deferoxamine binds iron in the tissue and in the serum; excretion occurs via the urine and faeces. However, it only has a biological half-life of about ten minutes, i.e. after this time merely half the pharmacon is still effective. Oral administration is ineffective. For this reason, subcutaneous injection is required as a slow infusion of 12 hours (using a portable infusion pump). With a daily dose of 25−50 mg/kg BW, removal of iron deposits will probably be successful. • Local irritations have been observed as *side effects*. Oculotoxicity (initial visual loss) may be evident, depending on the dose. Neurotoxic disturbances usually only occur when the dose is higher than 2−3 g/day (>90 mg/kg BW). This type of treatment is more complicated, less effective and much more expensive − it is intended as an alternative only when there is no possibility of venesection treatment. • In the foreseeable future, we can expect the development of other **iron-chelating agents** which will meet the ideal standards: oral administration, higher efficiency, lower costs and fewer side effects.

Adjuvant treatment measures

(*1*.) **Low-iron nutrition** is a basic requirement (there is practically no iron-free form of nutrition). However, intake of foodstuffs rich in iron is contraindicated when the iron balance has to be maintained. The iron balance is not greatly influenced by this measure, but it nevertheless has a certain effect: low-iron nutrition corresponds to two or three venesections per year! • *Every kind of adjuvant therapy should be used as a matter of principle − nothing can be said against such measures.*

(*2*.) This is also true of the daily consumption of **black tea** (1 cup with 5 g tea in the morning and at lunchtime). Tea is deemed to be an iron-chelating agent (iron tannate), which significantly reduces the resorption of iron, particularly in a low-iron diet − with or without ascorbic acid or milk added. (411, 429) • *We have always made sure that these adjuvant measures are strictly adhered to.*

(*3*.) **Deferoxamine** can also be used as an adjuvant agent in the initial treatment phase to reinforce venesection therapy. This is administered as an i.m. injection of 500 mg/week, usually by the family doctor, about two to three days before blood-letting is carried out.

(*4*.) **Abstention from alcohol** is absolutely essential and must be strictly adhered to. This is not only because of the minor amounts of iron usually found in alcoholic beverages, but also in order to avoid additional alcohol-induced lipid peroxidation and further stimulation of fibrosis already caused by iron.

(*5*.) The use of **antioxidants** is plausible from a pharmacological point of view, since lipid peroxidation due to iron intake may indeed lead to further tissue damage. (460) Silymarin (e.g. 2 x 140−170 mg), vitamin E (e.g. 100−200 mg) or β-carotene are good choices due to their lack of side effects and plausibility of efficacy.

(*6*.) HC patients who are **HBV-negative** should be actively immunized against hepatitis B. • As far as possible, patients should avoid any risk of HCV infection.

Normalization of the respective serum parameters, stabilization of the body's iron content at 3−5 g and improvement in the organ damage are to be expected in quite a short period of time when blood-letting therapy (if necessary, with deferoxamine as an option in long-term treatment) and adjuvant treatment measures (which may serve to support or speed up the successful outcome) are carried out consistently. The fact that better results can be achieved if the above-mentioned measures are applied has been shown by several impressive and carefully conducted studies. Therapeutic removal of iron overload is in any case not bound to a time factor! Generally, the faster and more constant the iron removal process is, the better. • Hypogonadism and arthropathies are less susceptible to treatment. Given the basic possibility of fibrosis regression, a certain optimism in this respect is definitely justified.

Simultaneous treatment

At the same time, consideration has to be given to the treatment of complaints or sequelae of organic manifestations, such as careful regulation of diabetes, management of cardiac insufficiency or dysrhythmia, hormone substitution, pain therapy in arthropathy, etc.

Liver transplantation

In principle, the indication for liver transplantation is limited to individual cases suitable for surgery (i.e. only in cases of early diagnosis and appropriate treatment). Unlike in Wilson's disease, the genetic defect is *not* removed by transplantation, and the transplanted liver may store iron again. • Consequently, a genuine indication is only given in acute liver failure and in an operable carcinoma restricted to the liver. The final stages of cirrhosis certainly continue to justify a transplantation, although the indication in this case is not based on HC, but on the fact that the diagnosis was established too late and/or that treatment was inadequate or not carried out at all. In young men with severe iron overload, a swift liver transplantation is always indicated. (419, 449)

A number of grave **mistakes** may occur in the treatment of HC. These include:

1. delay in reaching a precise diagnosis (including quantitative determination of iron in the liver bioptate)
2. blood-letting therapy carried out too hesitantly or inadequately
3. failure to carry out a family examination (preventive diagnosis)
4. merely monitoring family members who (still) show no signs of manifest disease instead of treating them properly

18 Haemosiderosis

Acquired ("secondary") haemochromatosis is caused by other diseases/conditions characterized by iron overload resulting from excessive iron storage in the organism (e.g. due to diet, medical treatment or haemolysis). The term acquired haemochromatosis should not be used unless the iron content of the liver is >0.5 g/100g wet weight. The causes are manifold. • In these patients, there is occasionally an HLA constellation which is identical to that of HC or a family history of iron storage. Therefore, it is postulated that these patients might be heterozygous HC carriers or that there might be another hitherto undetected triggering factor or a genetic defect in addition. (s. tab. 31.17)

18.1 Alcohol abuse

The most common cause (about 30% of cases) of hepatic siderosis is alcohol-induced liver disease. Its aetiology is unknown. With this form of acquired iron deposition, the more pronounced courses are characterized by stronger brownish cutaneous pigmentation, hypogonadism, glucose intolerance and hepatomegaly together with increased iron and ferritin values in the serum. This results in a similar symptomatology to that found in HC, only with a hepatic iron index clearly below 2.0. Blood-letting therapy is successful and reliable within a relatively short period of time. Iron deposition occurs mainly in the Kupffer cells, while the hepatocytes and the septa are largely free of iron.

18.2 Porphyria cutanea tarda

In porphyria cutanea tarda, iron deposition in the liver is generally low to moderate, predominantly in the hepatocytes of the acinar periphery. The cause is thought to be reduced activity of uroporphyrinogen decarboxylase resulting in decreased synthesis of porphyrin and haem. However, this variant of hepatic siderosis only becomes manifest as a result of various triggering factors. Treatment is also based on venesection.

18.3 Blood transfusion

Transfusional haemosiderosis (R.M. Kark, 1937) can occur following numerous blood transfusions. There are 200–250 mg iron in 500 ml (= 1 unit) blood. Thus numerous units (more than 50–60) must be transfused before siderin is clinically recognizable. It is initially deposited in the RES of the bone marrow, spleen and liver, ultimately in the hepatocytes as well.

18.4 Haemolytic anaemia

In all haemolytic anaemias, especially the genetically related forms, siderin is firstly deposited in Kupffer cells.

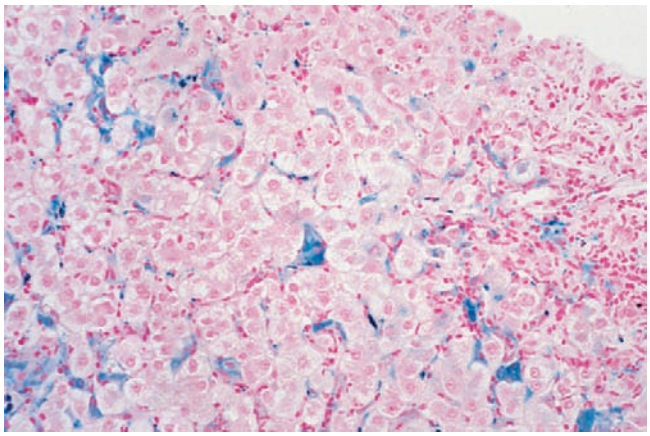

Fig. 31.30: Siderosis of Kupffer cells in haemolytic anaemia (Berlin blue)

The extent of siderosis depends on the duration and severity of the haemolysis. (s. fig. 31.30) In long-term haemolysis, iron is also stored increasingly in the hepatocytes. In such cases, the morphological and clinical picture of haemochromatosis can develop.

18.5 Chronic liver disease

Haemosiderosis can be found in several chronic liver diseases, such as chronic hepatitis (especially HVC leading to a reduced response rate to α-interferon), late-stage cirrhosis, spontaneous or surgical portal-systemic shunts, and non-alcoholic steatohepatitis.

18.6 African iron overload

In rural sub-Saharan Africa, there is a beer which is traditionally brewed in iron vats. The daily iron overload can be as much as 200 mg, with markedly increased iron absorption (T.H. Bothwell et al., 1965). • Such a condition is also observed in South Africa among the black population. Their diet consists of porridge fermented in iron pots with an acid pH value (V.R. Gordeuk et al., 1986). • In both cases, absorption of iron is facilitated by various factors, e.g. protein or vitamin C deficiency, alcohol abuse, acidic diet. It has been suggested that such iron overload is triggered by genetic factors.

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