Important Clinical Syndromes Associated with Liver Disease

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KEYWORDS

- Icterus • Cholestasis • Bilirubin • Bile acids
- Hepatic encephalopathy • Portal hypertension
- Portosystemic shunting • Ascites • Coagulopathy
- Liver disease

Several clinical syndromes can develop in many different liver diseases. They are important to understand the clinical manifestations of hepatobiliary diseases. The signs, diagnostic procedures, and specific diseases associated with these syndromes are discussed.

ICTERUS AND CHOLESTASIS
Bile Production and Flow

Cholestasis is a reduced bile flow. Normally, the flow in the biliary tree results from bile production in the proximal part and concentration of bile in the distal part. Only the flow of bile in the common bile duct is influenced by active transport due to peristalsis (gall bladder contractions and closure or relaxation of the sphincter of Oddi).[1,2] More than 50% of the bile is immediately released into the duodenum,[3] the rest being stored and concentrated in the gallbladder. The major trigger for gallbladder contraction is the hormone cholecystokinine,[4–7] which is secreted by the duodenal mucosa under the influence of fat or protein (amino acids). The gallbladder does not contract suddenly, like the urinary bladder, but gradually over 1 to 2 hours. Furthermore, the gallbladder is emptied incompletely and variably following a meal (5%–65%).[3] Bile is concentrated about tenfold in the gallbladder and in the larger bile ducts by active absorption of Na^+, HCO_3^-, and water.[8–11]

Some 50% of bile production depends on active excretion of bile salts by the hepatocytes into the canaliculi. Bile acids are produced by the liver from cholesterol, which is a major route of cholesterol excretion. The 2 acids formed are cholic acid and chenodeoxycholic acid (the so-called primary bile acids). Bile acids are made hydrophilic before excretion into bile by conjugation with glycine and taurine. A small fraction of

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the membrane (about 15%) of hepatocytes surrounds the smallest bile ducts (canaliculi) and contains active transporters. Active bile acid excretion creates a huge concentration gradient between the cells and the canaliculi with a factor of 2,000. The osmotic gradient induced causes excretion of water into the canaliculi, which is a major driving force of the bile flow.

When bile reaches the intestinal tract, conjugated bile acids are partly transformed by enteral bacteria in 2 ways. The secondary bile acids, deoxycholate and lithocholate, are produced by hydroxylation from cholic acid and chenodeoxycholic acid, respectively. Lithocholic acid is poorly absorbed, but it is hepatotoxic and may induce severe cholestasis. The small reabsorbed fraction is sulfated (tertiary sulfolithocholic acid) in the liver; in this form it is not reabsorbable in the next enteric cycle. Conjugated bile acids are actively reabsorbed in the ileum. The second bacterial transformation of bile acids is deconjugation. Unconjugated bile acids are absorbed in the entire intestinal tract by passive diffusion. All reabsorbed bile acids are transported to the liver by the portal blood flow, efficiently (90% in each passage) cleared by the liver, and if necessary reconjugated and then re-excreted into the canaliculi. Only a small fraction of the bile acid pool is lost in this enterohepatic circulation which cycles 10 to 15 times per day. The lost fraction is replenished by de novo synthesis in the liver.

Some bile production occurs by secretion by hepatocytes of Na^+ into the canaliculi, passively followed by water. The remaining 30% of the bile is produced by the epithelium of the intrahepatic bile ducts by excretion of water in combination with bicarbonate and chloride.

Cholestasis

Cholestasis is a reduced bile flow in the biliary tract. The cause of cholestasis may be inside (intrahepatic) or outside the liver in the common bile duct (extrahepatic). Intrahepatic cholestasis occurs in most clinical cases.[12,13] Due to the large reserve capacity of the hepatobiliary system, clinical signs of cholestasis (eg, icterus) only develop when the entire liver is affected diffusely. Obstruction of the bile flow by focal lesions is easily compensated for by the remaining liver. Intrahepatic cholestasis occurs predominantly at the level of hepatocytes and canaliculi or in bile ductuli in zone 1 of the liver lobules (the periportal zone). Extrahepatic cholestasis occurs by obstruction of the common bile duct. Again, due to the reserve of the liver, clinical signs occur only when there is nearly complete blockage of the passage.

Intrahepatic cholestasis

Intrahepatic cholestasis may occur due to leakage of the tight junctions that separate bile canaliculi from blood sinusoids. This situation occurs in endotoxemia and sepsis, and in cases of adverse reaction to drugs. Leptospira produce enzymes that destroy the tight junctions, leading to severe intrahepatic cholestasis in leptospirosis without severe reduction of other liver functions. Another reason for intrahepatic cholestasis is swelling of hepatocytes, which occlude the canaliculi and bile ductules (feline liver lipidosis). Necrosis of liver cells may occur in almost all liver diseases and gives a direct connection between canaliculi and the sinusoidal/perisinusoidal lymph and blood flow. Because active excretion of bile components with water causes pressure in the biliary system, bile leaks easily back into the low pressure blood and lymphatic system. In many liver diseases there are portal or periportal processes that block the bile flow out of the liver lobules. Examples are infiltration of inflammatory cells (hepatitis), tumor cells (malignant lymphoma and other forms), and deposition of collagen (chronic hepatitis, other fibrotic diseases, cirrhosis). Diffuse swelling of hepatocytes (lipidosis), diffusely spread space-occupying lesions (tumor metastases), and

disruption of the normal acinar architecture (cirrhosis) affect the bile flow at different levels in the liver lobules. The most severe form of intrahepatic cholestasis is seen in dogs with destructive cholangitis, whereby many or all peripheral intrahepatic branches of the biliary tree become necrotic (eg, due to idiosyncratic reaction to sulfonamides/trimethoprim-sulfamethoxazole).[14] Such dogs may have a completely disrupted bile flow, which may be detectable by an empty gall bladder at ultrasonography, and severe icterus. In all cases of cholestasis (also extrahepatic) the hepatocytes may become overloaded with substances that cannot be adequately excreted. Due to diffusion through the sinusoidal membrane, they may enter the perisinusoidal space of Disse. With the hepatic lymph flow, all such compounds will then enter the blood circulation.

Extrahepatic cholestasis, extrahepatic bile duct obstruction

Extrahepatic causes of cholestasis are rare. In dogs and cats, clinical cases of extrahepatic cholestasis have common bile duct obstruction. In most cases, tumors of the pancreas or the duodenum underlie the obstruction. Gallstones may also block the common bile duct at the level of Vater's papilla. Hyperplasia of the biliary epithelium due to long-term high doses of progestins may also occlude the extrahepatic bile ducts. Cholangiocarcinomas may spread through the biliary tree and cause severe extrahepatic (and intrahepatic) cholestasis. Nematodes ascending into the biliary system have been reported to cause common bile duct obstruction, but this is a rare event, if it occurs at all in vivo. The common bile duct or lobular ducts may become obstructed if (part of) the liver is dislocated in a diaphragmatic herniation. In such cases cholestasis and icterus may occur intermittently. Cholangitis may cause diffuse intra- and extrahepatic cholestasis, which is rare in dogs, but the most common cause in cats. Chronic EHBDO causes dilatation of the extrahepatic bile ducts, which become wide and tortuous. These changes are easily detectable with ultrasonography. The gallbladder is not always distended, and in chronic cases it may even be abnormally small, containing highly concentrated mucinous bile from which the pigment has been resorbed (white bile).[12] Therefore, due to the physiologic variability of gallbladder filling, the size of the gallbladder is not an indicator of EHBDO. Morphine derivatives induce complete closure of the sphincter of Oddi,[15] so that a full gallbladder during surgery may be normal. Complete EHBDO causes absence of bile components in the feces, so that the normal color (due to the black-brown degradation products of bilirubin) may change. Bile acid-driven fat resorption is then also disturbed, resulting in soft grey feces with a high fat content (acholic feces). This finding, always in association with severe icterus, is diagnostic for EHBDO.[16] Hepatobiliary scintigraphy may be used to quantify the degree of cholestasis.[15,17]

Histology of cholestasis

Cholestasis may be visible in the canaliculi, the hepatocytes. and in the macrophages and Kupffer cells. In the canaliculi, cellular debris and bile may produce bile thrombi, visible as brown casts in the canaliculi. However, these casts are easily washed out of the liver tissue on the slide during staining procedures. Cellular debris and bile plugs containing bile pigment (bilirubin) are phagocytosed by Kupffer cells and are seen as intracellular brown-yellow material. Accumulation of bile pigment in hepatocytes may also be visible as brown-yellow pigmentation. In animals with EHBDO high levels of toxic bile acids cause hepatocellular degeneration and necrosis in the periportal zone with a secondary inflammatory reaction of polymorphonuclear cells. In acute cases periportal edema also occurs. In cases with chronic (several weeks) EHBDO, the edema disappears and concentric periportal fibrosis develops, which may give

a unionlike aspect. In chronic cases bile ducts proliferate and become tortuous, which is visible as multiple bile ducts instead of just one in the portal areas. In severe chronic cholestasis of any origin the biliary excretion of copper may be decreased, leading to increased concentration in the liver. With histochemical staining slight accumulation of copper may be detectable in the periportal zone (primary copper storage diseases give more severe accumulation in the centrilobular area).

Biochemistry of cholestasis

Biochemically, cholestasis leads to increased concentration of all bile constituents, such as cholesterol, bile acids, and bilirubin, and also of enzymes that are highly active in biliary epithelial cells or the specialized biliary part of the membrane of hepatocytes: It is not possible to differentiate between extra- and intrahepatic cholestasis with biochemistry.

Bilirubin Metabolism and Icterus

Bilirubin is the pigment that gives bile its yellow-brown color. It is the normal end-product of the catabolism of heme. Heme resides in red cell hemoglobin and in many enzyme systems, which are preferentially localized in the liver (cytochromes, catalase, and peroxidase). Although the pool size of hemoproteins in the liver is small compared with the hemoglobin pool, the production of bilirubin from hepatic heme accounts for 30% of the total production, because the hepatic heme turnover rate is much higher (2 hours to 4 days versus 98 days for hemoglobin). Bilirubin is cleared from the plasma by the liver, and has to be conjugated by the hepatocytes preceding biliary excretion. The unconjugated form is stringently hydrophobic and bound to albumin in the circulation. On conjugation, bilirubin is excreted into bile and the conjugate is not reabsorbed from the intestines. Rarely, in cases of bacterial overgrowth, bilirubin is deconjugated by bacterial enzymes and the unconjugated pigment is reabsorbed in the small intestines into an enterohepatic cycle. Bacterial degradation of bilirubin in the colon produces stercobilins, black and brown pigments that give feces its normal color.

Healthy animals have only unconjugated bilirubin in their circulation. Cholestasis causes accumulation of conjugated bilirubin in plasma, which is not only re-excreted by the liver but may also be excreted by the kidneys in the urine. Bilirubin in feline urine is abnormal and indicates liver disease. However, the kidney in dogs, particularly males, has all the enzymes to produce bilirubin out of heme and to conjugate it, so that it can be excreted into urine. Therefore, the urine of healthy male dogs may contain detectable concentrations of bilirubin.

Urobilinogen is a colorless product, a small fraction of which is absorbed into the portal blood. Most of it is cleared by the liver, but a minor part reaches the systemic circulation and can be excreted by the kidneys. In cases of EHBDO much less bilirubin reaches the intestinal tract so that the amount of urobilinogen in the urine is even lower than normally. Measurement of urobilinogen in urine has been used to differentiate between different forms of icterus and cholestasis. However, due to many physiologic variations and technical errors, this parameter has no clinical value.

Bilirubin is cleared from the blood, conjugated, and excreted into bile by the liver. The clearance is not an efficient process[18,19] in contrast to the hepatic clearance of bile acids. Whereas bile acids are nearly completely cleared during the first passage, bilirubin requires many passages to become cleared completely (**Fig. 1**). As a consequence, bilirubin is equally distributed over the entire circulation, but bile acids are highly concentrated in the portal blood and have a low concentration in the systemic circulation. This explains the differences in the reaction pattern of bilirubin and bile
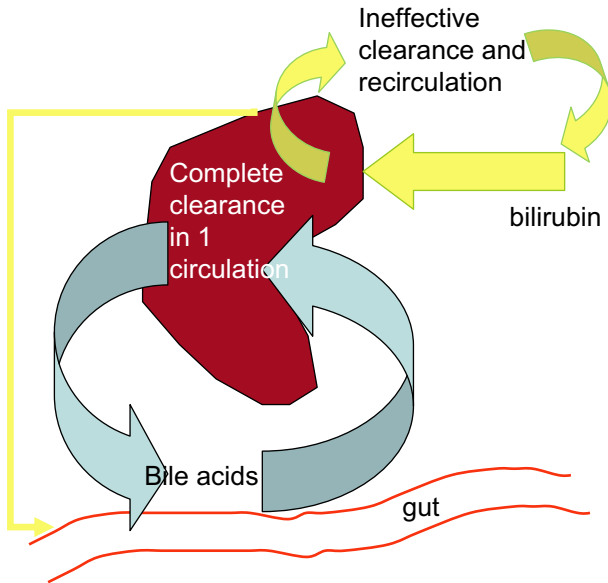
Fig. 1. The differences between bilirubin and bile acid metabolism. Both metabolites are produced in the liver. Bile acids are reabsorbed and undergo an enterohepatic circulation, which is maintained by an efficient clearance of bile acids from the portal vein. Bilirubin is not absorbed from the small intestines and its hepatic clearance from the blood has low efficiency. Consequently, there is a high gradient between the portal and systemic concentrations of bile acids, but not of bilirubin. Furthermore, systemic bile acids are increased due to portosystemic shunting and cholestasis; bilirubin is only increased due to cholestasis (or increased production in case of severe hemolysis).

acids in different liver diseases. In diseases with cholestasis, all bile components including bilirubin and bile acids gain entry to the systemic circulation with the hepatic lymph. This process is not related to hepatic clearance or portal perfusion of the liver. Conversely, in diseases characterized by portosystemic shunting (congenital portosystemic shunts, portal hypertension, acquired collateral circulation, and so forth), the high portal bile acid concentration reaches the systemic circulation giving a high plasma bile acid concentration. However, the bilirubin concentration is not influenced by abnormal liver perfusion. Animals with congenital portosystemic shunts will never have icterus.

The main processes by which plasma bilirubin may increase are increased production and cholestasis. The bilirubin concentration in health is low (<3.5 μmol/L). An increased level becomes clinically visible only as icterus (yellow discoloration of sclerae, mucous membranes and skin) when the concentration exceeds 15 μmol/L. Due to the huge liver reserve capacity, most patients remain in the subclinical region and do not become icteric, despite the fact that nearly all nonvascular liver diseases lead to some degree of cholestasis. Some 10% of all liver patients have clinical jaundice.

Given the 2 main reasons for hyperbilirubinemia, increased production and cholestasis, measurement of unconjugated and conjugated bilirubin has been used as an expression of these 2 processes. However, with sensitive techniques, it has been shown that hemolytic (increased production) and hepatobiliary diseases (cholestasis) are not different with respect to the fraction of unconjugated bilirubin, which always

varies between 15% and 40%. In liver diseases, there is considerable hemolysis (eg, due to portal hypertension causing reduced splanchnic blood flow with prolonged trapping and degradation of red blood cells in the spleen, and altered erythrocyte membrane fluidity caused by high plasma bile acid concentrations). Furthermore, animals with liver disease may have increased bilirubin production from hepatocyte hemoproteins. Hepatic and hemolytic diseases also have comparable reductions of the bile flow as an expression of cholestasis.[20] Cholestasis in hemolytic disease is caused by liver cell necrosis in the centrolobular region of the liver lobules due to hypoxia with a secondary inflammatory reaction. Such liver damage occurs only in sudden, severe types of hemolysis. With mild anemia, the liver is not damaged and the reserve capacity of the liver prevents such patients from becoming icteric. As hepatic and hemolytic jaundice always consist of a mixed type of hyperbilirubinemia, the measurement of unconjugated and conjugated bilirubin is clinically useless. Furthermore, if only severe hemolysis leads to jaundice, such animals should have pale mucous membranes (and hematocrit <20%). Moderately pale or normally colored mucous membranes in the presence of icterus immediately indicate the presence of a primary disease of the liver or biliary tract.[20,21]

A final remark with respect to bilirubin concerns its binding to albumin. Conjugated bilirubin in plasma binds covalently (irreversibly) to protein albumin. This bilirubin can only escape the circulation when albumin becomes catabolized; its half-life is about 2 weeks. Therefore, after complete recovery from the underlying cholestatic disease, icterus may remain for several weeks and does not necessarily reflect the actual situation, which may be important when evaluating the effect of therapy.[22]

In summary: (1) jaundice is the result of cholestasis; the underlying cause (hemolysis or hepatobiliary disease) is visible by clinical examination of the mucous membranes; (2) cholestasis occurs in most hepatobiliary diseases but in most cases there is no icterus; (3) animals with congenital portosystemic shunts or portal vein hypoplasia do not have icterus; (4) increased (basal or postprandial) bile acid concentrations are not specific for cholestasis or portosystemic shunting; (5) acholic feces is diagnostic for EHBDO, but is not present in all cases; (6) icterus due to intrahepatic cholestasis indicates a disease that affects the entire liver diffusely.

PORTAL HYPERTENSION, ASCITES AND ACQUIRED PORTOSYSTEMIC COLLATERAL CIRCULATION
Portal Hypertension

Portal hypertension is an abnormally high pressure in the portal circulation. The normal blood pressure in the portal vein is low, 0 to 5 mmHg. Portal hypertension can be caused by an increased delivery of blood to the portal system, or by an increased resistance to the passage of portal blood. An increased delivery of blood occurs animals with arteriovenous shunts in the splanchnic circulation, usually in the liver, causing the direct connection of the arterial blood pressure with the portal system.[23] This is a rare condition, usually visible with ultrasonography as a pulsating bunch of vessels within 1 liver lobe. Usually, however, portal hypertension is caused by an increased resistance to the portal blood stream. The cause can be prehepatic (in the portal vein itself), intrahepatic, or posthepatic (hepatic veins, caudal vena cava, heart). Posthepatic causes have little influence on the liver functions, but increased hydrostatic portal blood pressure may cause ascites.

Most cases of clinically relevant portal hypertension have a cause inside the liver.[23] As applies for most liver dysfunctions, clinical problems develop only when the entire liver is affected. Liver diseases causing portal hypertension give rise to different liver

dysfunctions, such as reduced protein and albumin production. However, even in severe liver dysfunction, the capacity of the liver to produce proteins is only moderately affected due to the large plasticity of the liver. Therefore, albumin levels usually do not fall below 18 to 20 g/L, which is more than the concentration that, by itself, may cause edema and ascites (<15 g/L). However, the combination of portal hypertension and moderate hypoalbuminemia often produces ascites in such animals. The hindrance to the portal circulation develops by way of compression of the portal veins in the portal and periportal area of the liver lobules. Because the cause lies at the site of entry of blood into the liver lobules, the liver itself is not congested. Due to loss of functional tissue, most of these diseases are associated with an abnormally small liver. The most frequent cause of portal vein compression is deposition of collagen (fibrosis)[23,24] and infiltration of inflammatory cells (chronic hepatitis). In advanced cases, cirrhosis, defined as disruption of the normal lobular architecture of the liver by fibrous tissue, occurs. Then, resistance to the portal blood flow occurs at different levels of the lobule and is most severe. The other most frequent cause of portal hypertension is portal vein hypoplasia,[25–27] a congenital disease in which the peripheral portal vein branches have not been formed or are incomplete, making the portal system a dead end. Portal vein hypoplasia (formerly called microvascular dysplasia) is associated with variable degrees of liver fibrosis, which may increase the resistance to normal liver perfusion.

The main prehepatic cause of portal hypertension is portal vein thrombosis. Portal vein thrombosis is a rare condition, which may result from an abnormal portal vein intima (eg, hemangiosarcoma), from hypercoagulability due to decreased antithrombin III activity (usually caused by severe proteinuria). Other predisposing conditions are Cushing's disease, pancreatitis, and liver cirrhosis.

Posthepatic causes of portal hypertension may be localized in the inferior vena cava and the heart. Obstruction of the hepatic veins either intra- or extrahepatic (Budd-Chiari syndrome and veno-occlusive disease, respectively) occur in other species, but not in cats or dogs. Heart failure is the most common posthepatic cause of portal hypertension. Thrombosis of the inferior vena cava is rare, and is often caused by an adrenal tumor giving local thrombophlebitis. Such a thrombus grows out in the direction of the blood stream and may occlude the lumen over a long distance. In posthepatic causes of portal hypertension the liver is congested and enlarged. Liver functions, however, remain adequate and biochemical examination usually reveals no or only slight liver cell damage and dysfunction.

If disorders affecting the afferent portal system cause reduced perfusion of the liver, there is secondary hypoplasia of the portal veins and increased growth of tortuous hepatic arteries (arterialization) in the portal areas.[23] These distinct histologic features occur with portal vein thrombosis, congenital portosystemic shunts, portal vein hypoplasia, and arteriovenous fistulas. With the exception of congenital shunts, all of these diseases cause increased resistance for the portal blood flow through the liver, and therefore portal hypertension.

In posthepatic causes of portal hypertension, the central vein branches may be distended and the liver cells in zone 3 degenerated. In chronic cases, fibrous tissue develops around the terminal veins and hepatocyte hyperplasia may occur in zone 1 (periportally).

In portal hypertension there is reduced portal blood flow to the liver.[28–30] With intrahepatic causes, if the hindrance of the portal flow is at the sinusoidal or postsinusoidal level of the acini, the inflowing arterial blood may cause a reversion of the portal blood flow from the liver back into the portal vein; the normal hepatopetal (into the liver) flow then becomes hepatofugal (back out of the liver). The stasis or reversion of the portal

flow may be visualized with Doppler ultrasonography. Reversed portal flow is only possible if there are acquired portosystemic collateral vessels, and thus is there is chronic severe portal hypertension (see later discussion). Such abnormal flow patterns may occur in the case of portal vein hypoplasia (microvascular dysplasia), arteriovenous fistula, and advanced cirrhosis.

The clinically recognizable effects of portal hypertension may be ascites and the occurrence of hepatic encephalopathy (HE) due to acquired portosystemic shunting.

Ascites

Accumulation of free abdominal fluid may result from severe portal hypertension, or from the combination of moderately increased portal blood pressure and hypoalbuminemia. Because of the high reserve capacity of the liver, hypoalbuminemia occurs only when liver function is chronically and severely impaired. Examples are chronic hepatitis/cirrhosis, congenital portosystemic shunts, and severe forms of portal vein hypoplasia.[23,26–28,31] However, even in the most severe cases, the synthesis of albumin is only moderately reduced, typically resulting in plasma albumin concentrations of 18 to 22 g/L. Reduced oncotic pressure may be the only cause of edema/ascites when albumin concentrations are $\leq$15 g/L. Therefore portal hypertension must be present in liver diseases in order to cause ascites.

In posthepatic causes of portal hypertension (eg, heart failure), the liver functions are not or only slightly impaired; protein production remains adequate. In such cases, the hydrostatic blood pressure is the only factor causing ascites, which occurs only if the blood pressure is high. This situation occurs only in cases of near-complete obstruction of the inferior vena cava or severe cardiac failure.

Prehepatic portal hypertension (portal vein thrombosis), if located in the stem of the portal vein, may cause near-complete obstruction. The severely increased hydrostatic blood pressure may then cause ascites. This situation does not occur if only a branch of the portal vein is occluded.

In dogs with severe cholestasis, associated with high systemic plasma levels of bile acids, a specific mechanism of ascites formation may occur. In man, rats, cats, and dogs, different bile acids have been shown to inhibit the activity of 11β-hydroxysteroid dehydrogenase (OHSD). the function of OHSD is to prevent cortisol from binding to the aldosterone receptor. Cortisol is present in about tenfold excess to aldosterone, and has the same affinity for the aldosterone receptor. On binding, cortisol activates the intracellular aldosterone pathways and then acts as aldosterone. To keep the aldosterone receptor free to bind its specific ligand, the membranes of cells that express the aldosterone receptor also express OHSD. OHSD converts cortisol into cortisone, which does not bind to the receptor.[32–38] Inhibition of the enzyme by bile acids may cause unexpected hyperaldosteronism, exerted by cortisol. The author has seen this only in cases with severe cholestasis, such as in dogs with destructive cholangitis. Such cases may develop ascites, hydrothorax, or edema as a result, which is refractory to most diuretics but responds well to spironolactone, an aldosterone receptor antagonist.

The cause of ascites formation is reflected in the type of ascitic fluid.[28,39] With pre- and posthepatic causes there is a high portal blood pressure and congestion of the splanchnic vascular bed. The abdominal free fluid then contains lymph, plasma, and erythrocytes. The fluid is more or less turbid and pink-colored. If the cause is intrahepatic, there is only moderate portal hypertension and hypoalbuminemia. In these cases there is increased hepatic and splanchnic lymph production, which exceeds the capacity of the lymphatic system, causing a clear, nonhemorrhagic, colorless transudate (or yellowish in cases of icterus).

The diagnosis of the underlying cause of ascites starts by examining a few milliliters of ascitic fluid. If cardiac failure can be excluded with physical examination, pink-colored fluid indicates either portal vein or thoracic vena cava obstruction. Clear colorless transudate indicates an intrahepatic disease requiring liver biopsy for final diagnosis. Clear colorless transudate may also occur in nonhepatic diseases associated with severe albumin loss (nephrotic syndrome and protein-losing enteropathy); in these conditions the plasma albumin concentration is ≤ 15 g/L. Pink-colored fluid may be seen with some abdominal tumors, although this is rare.

The ascitic fluid may contain a large amount of albumin due to diffusion of this low molecular weight protein out of the circulation. Complete removal of the abdominal fluid in animals with portal hypertension is useless (the cause remains and the ascites recurs quickly) and undesirable. Some of the body's albumin stores is removed with the fluid, leading to accelerated ascites formation. During this process, hypovolemia occurs, which stimulates compensatory aldosterone production (Na^+ retention, K^+ excretion). The loss of K^+ may cause a marked worsening of the clinical or subclinical HE. The ascites can be treated more effectively by a low-sodium diet and potassium-sparing diuretics.

Portosystemic Collaterals

There are small, nonfunctional blood vessels in the omentum and mesentery, which expand and become functional as a result of a high portal pressure. Acquired portosystemic shunting develops only in the case of a high pressure gradient between the portal vein and the vena cava, and is seen only if the cause is pre- or intrahepatic. High pressure in the caval and the portal vein induces no portosystemic shunting. Functional portosystemic shunting develops gradually over time; it usually takes 6 to 8 weeks before measurable dysfunction occurs. The degree of shunting may vary from slight to 100%, depending on the cause and the stage of the process. Acquired portosystemic collaterals are always multiple and they are typically localized in the mesorectum, in the omentum just caudal to the left kidney, and along the gastric cardia and in the esophageal wall. Intraluminal bleeding into the esophagus from these tortuous vessels is a feared complication of portal hypertension in humans, but it does not occur in dogs or cats, due to the submucosal location in man, in contrast to the subserosal collaterals in dogs and cats.

The collateral circulation may cause HE. In addition, toxins from the gastrointestinal tract are inadequately cleared by the liver, causing excitation of the vomiting center and nausea, inappetence, and vomiting.

The presence of portosystemic shunting resulting from formed collaterals can be determined with an ammonia tolerance test; the postprandial bile acid test is not specific to distinguish portosystemic shunting from cholestasis, which are both present in such cases.[39,40]

HEPATIC ENCEPHALOPATHY
Forms of Hepatic Encephalopathy and Diseases Involved

HE is a dysfunction of the brain secondary to liver dysfunction.[41] HE is a frequent liver-associated syndrome in dogs and is less frequent in cats. Like portal hypertension and cholestasis, HE is not a disease but a manifestation of clinical symptoms that may develop in different liver diseases.

There are 2 different forms of HE: a rare acute type and a common chronic type. The acute form of HE, caused by fulminant hepatic failure, results in a fatal failure of all liver functions. Such animals will die within a few days and the encephalopathy is severe.

Therefore, they are comatose, and total liver failure causes severe icterus and coagulopathy due to disseminated intravascular coagulation (DIC). All liver enzymes in plasma will be raised dramatically. This form cannot be treated but is easily diagnosed.

The chronic form of HE is more common by far.[41,42] In dogs and cats (and humans), the underlying lesion is portosystemic collateral circulation, which may be due to acquired multiple collaterals due to portal hypertension or a single congenital portosystemic shunt. This form of HE is often called portosystemic encephalopathy (PSE). The reserve capacity of the liver prevents the development of HE even in severe parenchymal liver diseases (with the exception of fulminant failure) that are not accompanied by portosystemic shunting. Therefore, PSE occurs only if significantly reduced parenchymal liver function occurs in combination with portosystemic shunting. Either one of the components will not lead to HE.

The combination of shunting and impaired liver function occurs in all pre- and intrahepatic diseases causing portal hypertension, and also in animals with congenital portosystemic shunt. In the latter condition, the liver has been deprived of growth factors from birth and is therefore abnormally small with a too small, hypofunctional parenchymal mass.

The strict association of chronic HE with portosystemic shunting has 2 exceptions. In cats, chronic HE may occur in liver steatosis (lipidosis) due to prolonged fasting. Because cats cannot synthesize arginine in their liver, depletion of this essential amino acid occurs during fasting. Arginine is an important intermediate in the urea cycle. Therefore anorexia in cats may cause the combination of liver lipidosis and impaired ammonia detoxification. HE is a frequent phenomenon in such cats. In contrast to all other forms of HE, this form should not be treated by dietary protein reduction, but instead by forced feeding of amino acid (protein)-rich nutrients. In all species there may be rare congenital errors of metabolism in which one of the enzymes involved in ammonia metabolism fails. These animals may have severe hyperammonemia and HE in the absence of portosystemic shunting and or liver pathology. In contrast to all other forms of HE, these cases are characterized by high plasma ammonia and low bile acid concentrations.

Symptoms

The clinical symptoms of HE are variable. The neurologic signs in the first stage are aspecific and are often only recognized retrospectively, when more specific signs have developed. The subtle first signs are apathy, listlessness, and decreased mental alertness. In more advanced cases, signs include ataxia, circling, head pressing against obstacles, salivation, stupor, and coma. Epilepsy is uncommon but may occur occasionally in association with and subordinate to other signs of HE. Epilepsia alone in the absence of other signs of HE is never caused by HE. the episodic nature of HE is characteristic, with fluctuations between grade 1 and the more advanced stages in the same animal. Usually 1 or a few days of severe signs of HE alternate with more or less normal periods lasting 1 or several weeks. In addition to the neurologic signs of HE, nonneurologic signs related to the underlying disease may be seen. These signs are associated with the underlying chronic liver diseases and may include polyuria, vomiting, diarrhea, weight loss, decreased endurance, and, in case of congenital portosystemic shunt, retarded or insufficient growth and dysuria due to ammonium biurate crystalluria.

The signs of HE are classified according to the schedule in **Table 1**.

Pathogenesis

HE is a biochemical disorder of the metabolism of the brain as result of hepatic dysfunction. Because the integrity of the brain is not involved unless in advanced stages, the

Table 1
Classification of hepatic encephalopathy signs

Stage	Signs
Stage 1	Apathy, decreased mental alertness, staring glance, unawareness of surroundings
Stage 2	Ataxia, circling, head pressing against obstacles, blindness, salivation
Stage 3	Stupor, severe salivation, completely inactive but can be aroused
Stage 4	Coma, total irresponsiveness
Nonneurologic signs associated with liver diseases causing HE	
All stages	Nonneurologic signs associated with the underlying disease: polyuria/polydipsia, vomiting, dysuria due to ammonium biurate crystals, and so forth
General	Periodic occurrence is typical

neurologic signs are completely reversible if the underlying liver disease can be treated. Essentially, HE is a neurotransmitter dysfunction involving several transmitter systems in the brain. The most important neurotransmitter systems involved are the glutamate, dopamine/noradrenaline, and gamma-aminobutyric acid and benzodiazepine (GABA/BZ) pathways. Only the most important features directly relevant for understanding the clinical findings and treatment of HE are discussed here.

Glutamate neurotransmission and ammonia metabolism

Glutamate is one of the most important excitatory neurotransmitters. It is regulated by the hepatic metabolism of ammonia and deranged in cases of hyperammonemia.[41–43] Ammonia is mainly produced in the intestinal tract, in the colonic lumen by bacterial degradation of nitrogenous compounds (proteins, amines, urea) and by the intermediary metabolism in the small and large intestinal mucosa, which liberates ammonia from glutamine. Much of the intestinal ammonia is resorbed and enters the portal vein. The healthy liver is extremely efficient and has a huge reserve capacity for removing ammonia from the blood. Ammonia is nearly completely removed from the portal blood during one passage through the liver. Therefore, even severe liver dysfunction does not critically affect the liver's capacity to detoxify ammonia, which explains why HE occurs only if the ammonia-rich portal blood bypasses the liver due to (congenital or acquired) portosystemic shunting. One-way for the liver to handle ammonia is conversion into urea by the urea cycle of hepatocytes. Urea formation is concentrated in periportal zone 1 of the liver lobules and occurs exclusively in the liver. Urea is released into the blood and most of it is excreted permanently by the kidneys in the urine. Some urea enters the saliva and recirculates in an enterohepatic cycle. Normally, only a small amount of ammonia escapes this pathway and plasma ammonia concentrations in peripheral blood in healthy animals are low (<45 μmol/L). In most tissues of the body (eg, muscle, brain, and liver) ammonia is further metabolized by enzymatic incorporation into glutamate and glutamine. The end product, glutamine, enters the circulation and becomes metabolized in the intestinal mucosa and the kidneys to liberate ammonia again. Intestinal ammonia enters the cycle again; the kidneys can excrete ammonia produced in the tubular cells into the urine. The definite fate of ammonia in the kidneys depends on the pH of the urine. Normally most of the ammonia is permanently lost in the urine. However, if the kidneys produce alkaline urine, ammonia is reabsorbed and released into the renal veins. The liver itself is

one of the most important tissues for glutamine formation, which is concentrated around the central veins. The dual mechanism for ammonia metabolism in the liver is important in pH regulation; in acidosis zone 1 urea synthesis decreases to spare bicarbonate and ammonia detoxification is taken over by zone 3 hepatocytes, which produce glutamine.

In the case of portosystemic shunting, ammonia-containing blood bypasses the liver and systemic concentrations increase. High plasma ammonia levels become toxic to neurons if the defense of the protecting astrocytes becomes overwhelmed. Neuronal cells are separated from the blood by a layer of astrocytes, and substances from the circulation have to pass the astrocytes before they can reach the neurons. Blood ammonia enters the astrocytes, and these cells incorporate it into glutamine by a 2-step reaction catalyzed by glutamine synthetase. Under normal conditions, glutamine diffuses into the adjacent neurons where it is converted into glutamate by glutaminase, and glutamate in the neurons is partly converted into GABA (**Fig. 2**). These 2 compounds are important neurotransmitters. Excitatory glutamate and inhibitory GABA form a finely tuned equilibrium determining the excitability of postsynaptic neurons. In hyperammonemia, the capacity of astrocytic glutamine synthetase becomes exhausted and free ammonia diffuses into neurons. High neuronal ammonia concentrations inhibit glutaminase activity leading to accumulation of glutamine and depletion of the neurotransmitter glutamate. The disturbed glutamate-glutamine ammonia shuttle between astrocytes and neurons is one of the most important factors in the pathogenesis of HE.

To understand the clinical implications of hyperammonemia, it is important to know that only the molecular, nonionized form (NH_3) passes cell membranes, whereas NH_4^+ does not. Intracellularly, both forms have equal metabolic effects. In extra- and intracellular fluid, there is an equilibrium between NH_4^+ and $NH_3 + H^+$ ($pK_a = 9.15$). The ionic form of ammonia is thus pH dependent, with more NH_3 in the case of alkalosis. Alkalosis aggravates the neurotoxic effect of hyperammonemia. Alkalosis is compensated by the formation of alkaline urine from which the nonionized ammonia is readily
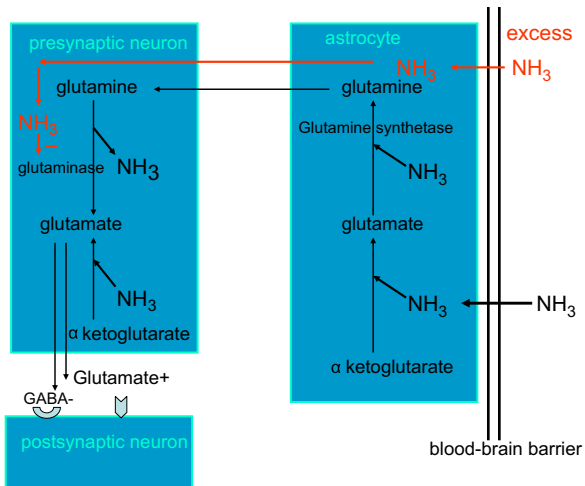

Fig. 2. A major cause of HE is increased plasma ammonia concentration. Excess ammonia is not detoxified by the astrocytes, and reaches the neurons in the brain. High neuronal ammonia inhibits glutaminase, so that the production of the neurotransmitter, glutamate, is decreased and the equilibrium between glutamate and GABA is disturbed causing neural dysfunction.

reabsorbed. This process may change the kidney from an ammonia-excreting organ into an ammonia-generating organ. The most severe form of alkalosis is associated with hypokalemia (**Fig. 3**). Low plasma potassium is replenished by exchange of intracellular potassium against sodium and hydrogen; the hydrogen shift induces extracellular alkalosis and intracellular acidosis. In the case of hypokalemic alkalosis, ammonia penetrates the cells easily but becomes ionized intracellularly and is trapped in the cell. Cells (also neurons) then act as one-way scavengers of this toxic compound.

Conditions of alkalosis and hypokalemia may well occur in chronic liver diseases. Hypokalemia may be caused by diarrhea, insufficient intake due to anorexia or vomiting, and loss due to salivation (a sign of HE). Portal hypertension causing ascites results in hypovolemia, which activates the renine angiotensine aldosterone system and causes renal loss of potassium. Therefore, one should never remove much ascitic fluid from a liver patient; the induced hypokalemic alkalosis may aggravate even subclinical HE to a severe degree within a few hours. Animals with ascites should only be treated with potassium-sparing diuretics (spironolactone).

Catabolic conditions are common in advanced liver disease and may contribute to the onset of HE. Increased breakdown of peripheral proteins liberates glutamine, which has to be metabolized in zone 3 hepatocytes, in the renal tubules, or in the intestinal mucosa, always leading to ammonia formation. The neurotoxic action of ammonia is further enhanced by methane- and ethanethiol formed by the intestinal flora from methionine. Oral administration of methionine should therefore be prevented in HE-related liver disease.

The GABA/BZ receptor complex

Neurotransmission by way of the GABA/BZ receptor system is one of the most important inhibitory systems in the brain. In HE, the GABA tone is abnormally high inducing suppression of normal brain functions.[44,45] The pathophysiology of this mechanism is complex and not fully understood. BZ-related ligands may be formed in the intestinal tract or locally in the brain. The GABA/BZ receptor complex binds many different drugs that are related to GABA, BZ, or barbiturates. Reversal of hepatic coma by injection of benzodiazepine antagonists such as flumazenil has been reported in humans and experimental animals, but there are no long-acting antagonists available for practical use. The increased GABA/BZ tone in animals with HE causes an enhanced effect
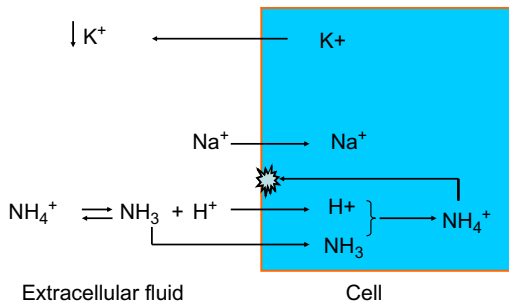

Fig. 3. Hypokalemic alkalosis. Hypokalemia causes a shift of K^+ from the cells to plasma, in exchange with H^+. The resulting extracellular alkalosis and intracellular acidosis cause free entrance of ammonia into the cells, whereby the ammonia is transformed into the ionized form (NH_4^+) which cannot escape, making (neural and other) cells a one-way trap for ammonia.

of sedative and anesthetic drugs. Such drugs should be avoided or at least used with much caution in such animals.

Catecholaminergic neurotransmission

Dopamine and norepinephrine also play a role in the pathogenesis of HE, related to the role of the liver in the metabolism of amino acids. Normally, the liver removes the aromatic amino acids (AAA; tryptophan, tyrosine, and phenylalanine) efficiently from the portal circulation, resulting in low systemic concentrations. The brain needs low concentrations of AAA, which are the precursors of dopamine and norepinephrine. In this catecholamine pathway, the capacity of the enzyme, tyrosine 3-hydroxylase, is rate limiting. The high systemic concentrations of AAA that are associated with portosystemic shunting are being processed by way of alternative metabolic routes, giving rise to alternative products such as octopamine and tyramine. These compounds bind to the catecholamine receptors, but have only weak intrinsic activity. Such false neurotransmitters block the normal catecholamine neurotransmission, which contributes to the pathogenesis of HE (**Fig. 4**). The branched-chain amino acids (BCAA), valine, leucine, and isoleucine, are passively involved in this process. Together with the AAA they form the neutral amino acids that use the same carrier system to enter the brain through the blood-brain barrier. Plasma BCAA levels are reduced in catabolic chronic liver disease because they are used as an alternative energy source in muscles and other tissues. The low BCAA/AAA ratio in plasma gives AAA even easier access to the brain because they experience less competition for the common carrier.

Blockage of central catecholamine receptors may partially explain the polyuria[46,47] that occurs in many dogs with chronic liver disease. Catecholamines inhibit the release of ACTH from the intermediate lobe of the pituitary. In health, the anterior rather than the intermediate pituitary lobe is involved in the pituitary-adrenocortical feedback system. However, in animals with HE the intermediate lobe may change from a silent organ to a secreting organ leading to hyperadrenocorticism. Hyperadrenocorticism is a complicating factor in HE by the induction of catabolism. Like all manifestations of HE, it disappears with the successful treatment of the underlying cause.

Diagnosis of hepatic encephalopathy

Ammonia measurement in plasma is the only practical way to diagnose HE.[41–43,48,49] Due to the incorporation of ammonia into glutamine in many tissues, the arterial
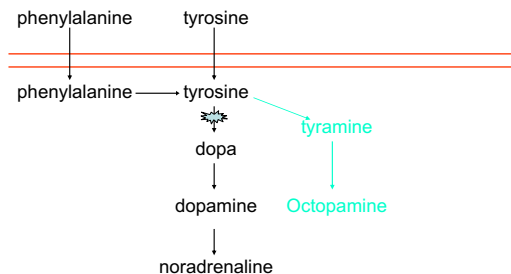

Fig. 4. Dopaminergic blockade. Dopaminergic dysfunction is one factor underlying HE. Excess aromatic amino acids (eg, tyrosine), which are not cleared from the portal blood by the liver, enter the brain. The low capacity for conversion of tyrosine into catecholamines (dopamine, norepinephrine) is overwhelmed, causing production of alternative transmitters, which block the receptor and prevent normal neurotransmission.

concentration may be much higher than that in venous blood. Therefore moderate increments of plasma ammonia may be missed in venous samples. In case of doubt an ammonia tolerance test (ATT, using venous samples) always gives a clear result. This test is sensitive and specific to detect all forms of portosystemic shunting, and is not abnormal in liver diseases without shunting (except fulminant liver failure).

The ATT can best be performed by administering 2 mL/kg of a 5% NH_4Cl solution deep (10–20 cm) rectally by way of a soft catheter.[40] Sampling should be done before and at 20 and 40 min after administration. With this protocol the test gives a semi-quantitative estimate of the degree of shunting. The ATT should not be done in animals in which the basal ammonia value is already high (>150 µmol/L). In other cases, the additional ammonia load is safe and does not increase the signs of HE. In animals without portosystemic shunting, the basal values are within normal limits and they show no increase of ammonia with this test. In cases with high basal values, the ATT is pointless because this already proves HE and portosytemic shunting.

Ammonia should be measured in freshly sampled blood collected in an EDTA coated tube. Samples may be stored in melting ice for 30 minutes. These restrictions are necessary because ammonia is spontaneously liberated from nitrogenic sources such as amino groups in proteins and urea, if kept at room temperature. Ammonia measurement is easy and reliable with sophisticated desktop equipment.[49]

In about half of the dogs with HE, ammonium biurate crystals may be detectable in the urine sediment. If present, this is an indication for conditions causing HE. The dysfunctioning liver not only fails to process ammonia but also converts uric acid into allantoin. Ammonia and uric acid flocculate easily in acidic urine to form crystals and sometimes larger calculi in the renal pelvis or urine bladder. Such animals sometimes present with major symptoms of hematuria or dysuria. The urinary crystals are not diagnostic because they also occur in dogs with congenital defects of uric acid metabolism.

Treatment of hepatic encephalopathy

HE is a complex of symptoms that may occur in a variety of liver diseases. Therefore, it is of importance to diagnose and, if possible, treat the underlying disease. HE disappears immediately on recovery of the causal disease. It is important to give symptomatic support for HE and gain time for treatment of the underlying disease, prepare a patient for surgery, or for life-long support in cases in which liver functions are permanently insufficient.

HE can be treated by feeding a low-level high-quality protein diet.[41–43] The commercial hepatic support diets are more effective than kidney diets. Low protein of high quality reduces intestinal ammonia and aromatic amino acid production. The animal's caloric requirement should always be fulfilled to prevent catabolism. The degree of protein restriction depends on the given indication. In cases of chronic active hepatitis and cirrhosis, the hepatitis can be cured but the fibrosis and often the portosystemic collaterals remain. Such animals need life-long protein restriction at a level just enough to prevent HE, in order not to compromise hepatic protein synthesis, which could induce ascites formation. Cats require about twice as much protein as dogs, but, taking this into account, the recommendations for dogs can be followed.

Oral administration of disaccharides such as lactulose (1–3 ml/kg/day divided into 2 to 3 doses) may be helpful. Lactulose is not absorbed in the small intestines and is degraded into volatile free fatty acids by colonic bacteria.[43] The resulting acidification gives a shift to nonabsorbable ionized ammonia, increased colon motility, and an altered and less ammoniagenic flora. The ammonia formation from glutamine metabolism in the intestinal mucosa may also be reduced by lactulose. In animals with

advanced HE, forced intravenous diuresis may be helpful to excrete as much ammonia as possible (100 ml/kg/day). Here too, lactulose and correction of catabolism are essential.

In all cases, conditions that aggravate HE, such as hypovolemia, hypokalemia, and alkalosis, should be corrected and drugs like benzodiazepines, barbiturates, and methionine should be avoided. Glucocorticoid medication, which induces catabolism, should be avoided if possible.

BLOOD COAGULOPATHY IN LIVER DISEASE

Normal blood coagulation occurs by way of the intrinsic and extrinsic pathways; the activity can be measured by the activated partial thromboplastin (cephalin) time and the prothrombin time, respectively. Both pathways unite in the formation of thrombin from prothrombin. All of the clotting factors except the Von Willebrand subtype of factor VIII are synthesized in the liver. The activation of factors II, VII, IX, and X depends on the availability of vitamin K. The equilibrium between activation and inactivation of the coagulation cascade also depends on the activity of several clotting inhibiting proteins, primarily antithrombin III, a low molecular weight protein synthesized in the liver. In addition, the clearance of activated clotting factors and of antithrombin depends on the reticuloendothelial system, which is largely localized in the liver. Some of the clotting factors such as fibrinogen behave as acute phase reactants, and are produced in excess by the hepatocytes in cases of inflammatory or neoplastic disease. The liver may also affect the primary hemostasis by splanchnic pooling of blood and hence prolonged capturing of thrombocytes at their site of degradation, the spleen. This may induce abnormally low thrombocyte counts.

This brief outline of hepatic involvement in hemostasis indicates that the liver may affect blood coagulation in many ways.[50–53] In practice, however, most of them never give rise to clinical manifestations of coagulopathy. Biochemically, the extrinsic and intrinsic clotting times may be slightly prolonged due to insufficient protein synthesis. In chronic complete EBDO, inadequate fat resorption may lead to lack of vitamin K, with the relevant clotting factors not being activated. This does not lead to clinical coagulopathy, but biochemically the prothrombin time may be prolonged.[54,55] Administration of vitamin K normalizes blood clotting in such cases, but is pointless in other liver diseases.

A frequent mechanism behind coagulopathy in hepatobiliary diseases is DIC. This is especially the case if there is diffuse liver cell necrosis, as in several forms of hepatitis, lymphosarcoma, or metastatic tumors. Depending on the activity of the process (the amount of thromboplastin released from necrotic cells per unit of time), coagulopathy may then be subclinical or clinically apparent. Biochemically, low fibrinogen and thrombocyte levels and the presence of fibrin degradation products indicate DIC. For this reason, it is essential to measure blood coagulation, especially plasma fibrinogen, before taking a liver biopsy. Fibrinogen levels less than 50% of the lower reference value are a contraindication for taking a liver biopsy. Once the disease causing the hepatocellular necrosis is being treated, DIC disappears spontaneously. In dogs with chronic hepatitis and DIC, the coagulation usually normalizes within 1 to 2 weeks after starting prednisone medication.

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