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Interpretation of Liver Enzymes

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Biochemical screening tests facilitated by convenient automated chemical analyses are commonly used for routine health assessments. The presence of liver disease is often first recognized on the basis of liver enzymes. Although liver enzyme measurements are sometimes referred to as "liver function tests," they reflect hepatocyte membrane integrity, hepatocyte or biliary epithelial necrosis, cholestasis, or induction phenomenon rather than liver functional capacity. Interpretation of liver enzymes must be integrated with consideration of the patient's database, including the medical history, physical examination findings, other routine laboratory test results, specific assessments of liver function, and imaging studies. Confirmation of a specific liver disease usually requires acquisition of a liver biopsy. This article provides a clinical review of the most commonly used liver enzymes in small animal practice.

INITIAL PATTERN RECOGNITION

In a general patient population, abnormally increased liver enzyme activity is considerably more common than the prevalence of liver disease. This relates to the influence of systemic disorders on the liver. Occupying a sentinel position between the alimentary canal and systemic circulatory system, the liver has wide exposure to toxins and drug metabolites, endotoxins, and infectious agents. Consequently, a wide spectrum of nonhepatic disorders may influence liver enzyme activity.

The pattern of liver enzyme abnormalities in relation to the signalment, history, total bilirubin concentration, serum bile acid values, and comorbid conditions or medications provides the first indication of a liver-specific disorder. The full assessment of the liver enzyme aberration takes into consideration (1) the predominant pattern of enzyme change (hepatocellular leakage enzymes versus cholestatic enzymes), (2) the fold increase of enzyme activity greater than the normal reference range (using arbitrary cutoffs, the magnitudes of increased enzyme activity are considered as mild, <5 times the upper reference range; moderate, 5–10 times the upper reference range; or marked, >10 times

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the upper reference range), (3) the rate of change (increase or resolution), and (4) the nature of the course of change (fluctuation versus progressive increase or decrement). The reference range reflects the mean value within 2 standard deviations observed in a "normal" population. Thus, up to 2.5% of normal individuals can have borderline abnormal enzyme values. The specificities (number of negative tests in individuals lacking the disease of interest) of the most commonly used serum enzymes in dogs and cats are summarized in Fig. 1, as taken from a population of dogs (n = 915) and cats (n = 534) with biopsy-confirmed liver status. These animals (100 dogs and 66 cats) were initially suspected of having liver disease but were proven not to have liver disease on the basis of liver biopsy.

Recognizing whether enzyme abnormalities are persistent or cyclic helps to categorize different hepatobiliary disorders. For example, dogs and cats with nonsuppurative necroinflammatory hepatitis or cholangitis may have widely fluctuating liver enzymes in the absence of overt illness in the early stages of the syndrome. Animals exposed to toxins causing hepatic necrosis may have astounding transaminase activity that dissipates over time. Investigating liver function with paired fasting and postprandial serum bile acid determinations or urine bile acid or creatinine measurements (urine collected 4–8 hours after meal ingestion) may expedite pursuit of a liver biopsy when clinical signs remain vague and serum liver enzymes are only mildly increased. Finding high bile acid values corroborates the need for histologic investigations. Imaging studies, including thoracic and abdominal radiographs, assist in detecting primary underlying disorders that have secondarily influenced the liver (causing increased release of liver enzymes). Ultrasonographic interrogation of the

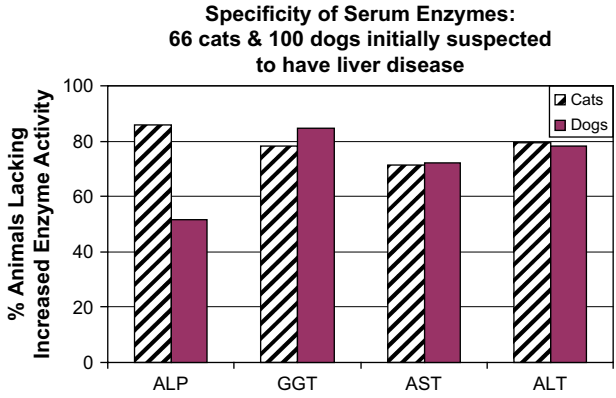

Fig. 1. Specificity of routinely used serum enzymes applied as screening tests in health surveillance of dogs and cats. Data represent the percentage of animals lacking liver disease (liver biopsy completed) having a negative test result. Additional data from this large clinical population are provided in other figures in this article. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase. (*Data from* the New York State College of Veterinary Medicine, Cornell University, Ithaca, NY, 2006.)

hepatobiliary system helps to identify focal abnormalities, involvement of biliary structures, perfusion abnormalities, and general changes in hepatic parenchymal echogenicity. Thoracic radiographs assist in the recognition of metastatic lesions, primary cardiopulmonary disease, and presence of an enlarged sternal lymph node reflecting abdominal disease (eg, inflammation, neoplasia).

Diagnostic enzymology involves the interpretation of serum enzymes originally located within the hepatocyte or attached to its plasma membrane. The process of enzyme release may be as simple as altered membrane integrity (direct efflux to the sinusoidal compartment through leaky gap junctions), cell necrosis, release of membrane-bound enzymes with membrane fragments (in necrotizing, metastatic, infiltrative, and cholestatic liver disorders), or release of membrane-bound enzymes from their phosphatidylinositol anchor as soluble fractions [1]. A general overview of the enzymes discussed in this article is provided in Table 1.

AMINOTRANSFERASES: ALANINE AMINOTRANSFERASE AND ASPARTATE AMINOTRANSFERASE

The serum aminotransferases (aspartate aminotransferase [AST], previously called serum glutamate-oxaloacetate aminotransferase [SGOT], and alanine aminotransferase [ALT], previously called serum glutamate-pyruvate aminotransferase [SGPT]), are commonly measured as a means of detecting liver injury. These enzymes catalyze the transfer of the α-amino groups of aspartate and alanine to the α-keto group of α-ketoglutaric acid (α-KG), which are reactions essential for gluconeogenesis and urea formation (Fig. 2).

ALT facilitates the mobilization of carbon and nitrogen from muscle (in the form of alanine) to the liver, where it can be used for protein synthesis, energy production, and nitrogen elimination in the urea cycle. In the liver, ALT transfers ammonia to α-KG, regenerating pyruvate that can be diverted for gluconeogenesis. Overall, this process is referred to as the glucose-alanine cycle (Fig. 3).

ALT and AST are present in high concentrations in liver but also exist in other tissues (Figs. 4 and 5) [2,3]. AST is present not only in the liver but in higher concentrations in the kidney, heart, and skeletal muscle and in measurable amounts in the brain, small intestine, and spleen. Comparatively, ALT is primarily located in the liver, with concentrations 4-fold higher than in the next most abundant site (cardiac muscle) and 10-fold higher than in the kidney. In health, the hepatocellular ALT activity is 10,000-fold greater than in plasma. Distribution of ALT and AST within the hepatocyte is variable (Fig. 6). Although most transaminases reside within the soluble fraction of the cytosol, an important component of AST resides within mitochondria (20%) [4]. The distribution of transaminases within the acinar zones also differs. ALT achieves higher concentrations in periportal hepatocytes, and AST achieves higher concentrations in periacinar (zone 3) hepatocytes. Consequently, the relative activity of ALT or AST in serum may reflect the acinar zone of liver injury [5].

Table 1
Liver enzymes

Cytosolic enzymes	ALT	Primarily located in hepatocyte cytosol, with higher values in periportal cells (zone 1) Rapidly leaks with altered membrane integrity and persists for days $t_{½}$ controversial, ranging from hours to many days, removed by sinusoidal hepatocytes, removal may be impaired in severe liver disease augmenting high enzymes
	LDH	Wide tissue distribution, with highest concentrations (in descending order) in skeletal muscle, heart, and kidney, with lesser amounts in intestine, liver, lung, and pancreas Multiple isozymes: LDH_5 predominates in liver and contributes to serum LDH Poor specificity because biochemistry profiles report total LDH High LDH activity in diffuse severe hepatic necrosis or inflammation, myositis, muscle trauma, and lymphosarcoma external to liver Rapid $t_{½}$, transiently increases only during active necrosis
	SDH	Released during hepatic degeneration or necrosis or secondarily to altered membrane permeability Highest tissue concentrations in liver Reflects ongoing hepatocellular injury but offers no advantage over ALT In vitro lability during transport complicates interpretation
Cytosolic or mitochondrial	AST	Present in multiple tissues, including skeletal muscle, cardiac muscle, kidney, brain, and liver Located in hepatocyte cytosol (80%) and mitochondria (20%) Rapidly leaks with altered membrane integrity Prominence in zone 3 Mitochondrial enzyme leaks in necrosis AST may have higher sensitivity for liver injury in some animals compared with ALT $t_{½}$ controversial, ranging from minutes to hours in dog, 77 minutes in cat
	Arginase	Exclusive to liver, located in hepatocyte cytosol and mitochondria Rapidly leaks with substantial membrane injury Modest increases with glucocorticoid induction in dogs $t_{½}$ short, such that it only marks acute severe tissue damage

(*continued on next page*)

Table 1
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Membrane-bound enzymes	ALP	Multiple isoenzymes, isozymes, or isoforms Liver-, bone-, intestinal-, placental-, and glucocorticoid-induced (latter in dogs only) Liver-induced ALP in biliary membranes, glucocorticoid-induced ALP in sinusoidal hepatocyte membranes Isoenzyme characterization has limited clinical value Bone-induced ALP is increased in juvenile animals with bone growth, hyperthyroid cats, and bone inflammation or neoplasia Liver-induced ALP and glucocorticoid-induced ALP undergo induction phenomenon in dogs glucocorticoid-induced ALP associated with acquired glycogen vacuolar hepatopathy (in dogs) Canine ALP $t_{1/2}$ liver-induced ALP = 70 hours, glucocorticoid-induced ALP = 70 hours, intestinal-induced ALP = 6 minutes Feline ALP $t_{1/2}$ liver-induced ALP = 6 hours, intestinal-induced ALP <2 minutes
	γ-GT	Present in multiple tissues, kidney, pancreas, intestine, and liver Liver γ-GT is a major source of serum enzyme Biliary membrane localization: highest values in cholestatic disorders Glucocorticoid γ-GT induction in dogs $t_{1/2}$ not determined in dogs or cats

Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; $t_{1/2}$, half-life.

The location of transaminases in the soluble cytosolic fraction of the hepatocyte allows immediate release with even minor changes in hepatocellular membrane permeability. This indiscriminant transaminase leakage limits the diagnostic value of these enzymes for differentiating reversible or irreversible membrane changes as well as the extent of tissue involvement. Nevertheless, the magnitude of transaminase activity does seem to correlate with the number of involved cells. Transaminases leak into the perisinusoidal space from the sinusoidal borders of hepatocytes or through leaky gap junctions into the ultrafiltrate in the space of Disse. From here, they diffuse through the dynamic fenestrae in the sinusoidal endothelium and mingle with the systemic circulation.

Hepatic transaminases are known to increase with muscle injury as well as after vigorous physical activity in dogs [6]. Regarding exercise, it remains unclear whether these enzymes "escape" from hepatocytes or originate from well-perfused active muscle [7,8]. A 1.4- to 2-fold increase in plasma AST associated with increases in creatine kinase (CK) and lactate dehydrogenase (LDH) has been shown in dogs after moderate to severe short-term exercise

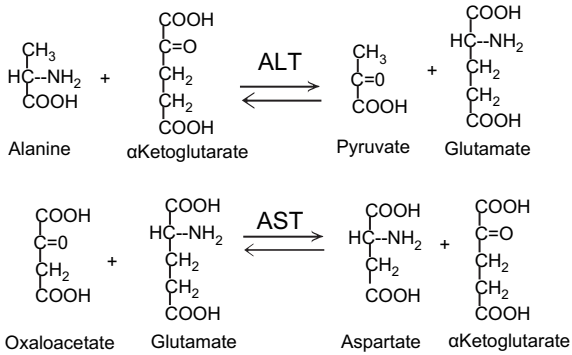

Fig. 2. Reactions catalyzed by the aminotransferases commonly measured as markers of hepatocellular injury.

(15-minute run at 16 km/h with a 10% incline). Similarly, a 1.4- to 2.9-fold increase in plasma AST and lactic dehydrogenase occurred in dogs after electrophysiologic stimulation of hind limb muscles (10 pulses per second for 30 minutes) [9].

The half-life ($t_{1/2}$) of transaminases remains controversial, with estimates ranging between 3 hours and 17 days made using intravenous injections of hepatic homogenates [3,10]. In one study, three dogs injected with a 20% liver tissue homogenate (sampled over 70 hours) demonstrated an average $t_{1/2}$ for AST of 263 minutes and for ALT of 149 minutes [3]. Another study (15,000-g liver homogenate supernatant [ALT = 254 U/g and AST = 382 U/g] given intravenously to seven dogs) demonstrated sustained plasma transaminase elevations for 13 to 17 days for ALT and for 3 to 5 days for AST [10]. A $t_{1/2}$ for ALT of 59 ± 9 hours and for AST of 22 ± 1.6 hours was calculated

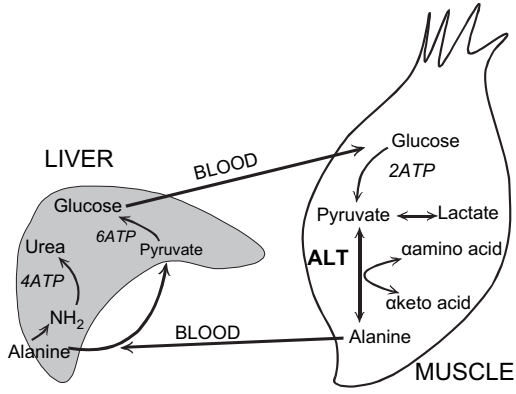

Fig. 3. Drawing depicts the function of ALT in the glucose-alanine cycle, as described in the text.

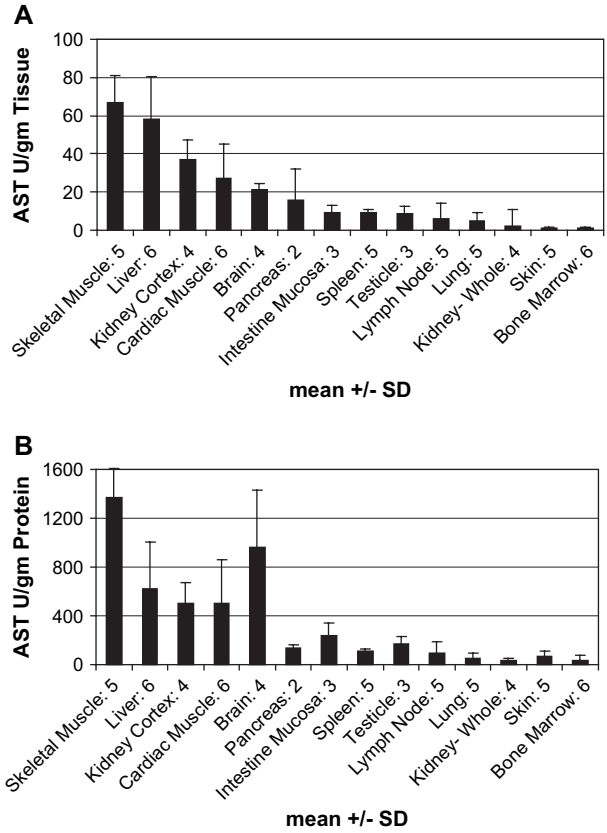

Fig. 4. Tissue distribution of AST in dogs on the basis of units of activity per wet tissue weight (*A*) and per tissue protein concentration (*B*). Numbers affiliated with the tissue label indicate the number of dogs sampled. (*Data from* Nagode LA, Frajola WJ, Loeb WF. Enzyme activities of canine tissues. Am J Vet Res 1966;27:1385–93.)

[10]. The plasma $t_{1/2}$ of AST in the cat has been estimated to be 77 minutes [11]. Considering that it requires five times the $t_{1/2}$ for plasma clearance, long-term persistence of transaminases may contribute to sustained high serum enzyme activities in some disorders. Because catabolism of transaminases occurs by absorptive endocytosis in the sinusoidal hepatocytes, slow enzyme clearance may augment high plasma enzyme activity in patients with substantial liver disease (eg, acquired portosystemic shunting, nodular regeneration, hepatic fibrosis) [12,13].

Alanine Aminotransferase

The largest increases in ALT develop with hepatocellular necrosis and inflammation. In this circumstance, gradual and sequential decreases in ALT activity can be a sign of recovery. In acute liver disease, a 50% or more decrease in serum ALT activity over several days is considered a good prognostic sign.

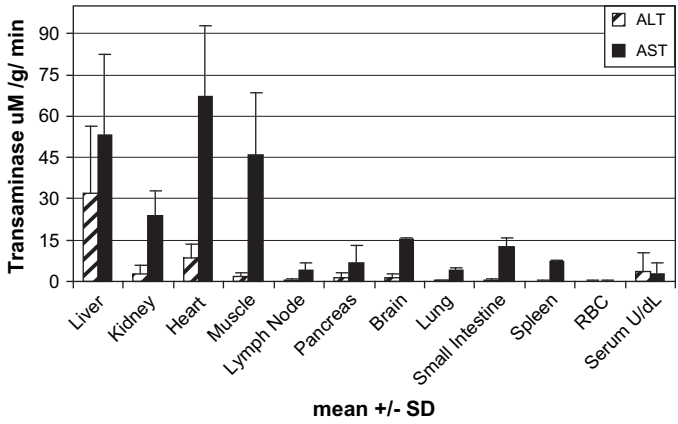

Fig. 5. Comparative tissue distribution of AST and ALT in dogs (n = 6) on the basis of micromoles per gram wet liver tissue weight per minute. (*Data from* Zinkl JG, Bush RM, Cornelius CE, et al. Comparative studies on plasma and tissue sorbitol, glutamic, lactic, and hydroxybutyric dehydrogenase and transaminase activities in the dog. Res Vet Sci 1971;12:211–14.)

Some animals with severe disease have normal serum ALT activity, however. It is also important to acknowledge that declining serum ALT activity may represent a paucity of viable hepatocytes in chronic liver disease or severe toxicity or even toxin-suppressed transaminase synthesis (eg, microcystin, aflatoxin).

After acute severe hepatocellular necrosis, serum ALT activity usually increases markedly and sharply within 24 to 48 hours to values greater than or equal to 100-fold normal, peaking during the first 5 postinjury days [14–20]. If the injurious event resolves, ALT activity gradually declines to normal over a 2- to 3-week interval. Although this pattern is considered "classic," some severe hepatotoxins are not associated with profound or protracted serum transaminase activity. This is encountered with toxins that inhibit transaminase

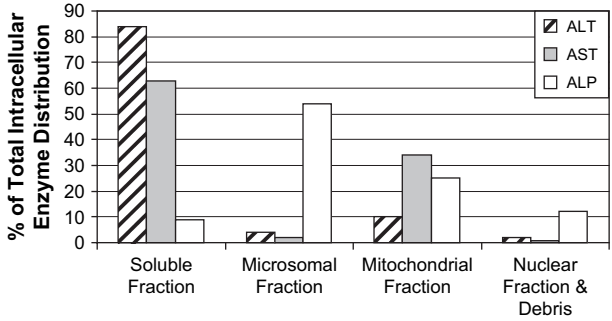

Fig. 6. Distribution of ALT, AST, and alkaline phosphatase (ALP) in the canine liver. (*Adapted from* Keller P. Enzyme activities in the dog: tissue analyses, plasma values, and intracellular distribution. Am J Vet Res 1981;41:575–82.)

gene transcription or that otherwise interfere with transaminase biosynthesis (eg, aflatoxin B_1 hepatotoxicity, microcystin hepatotoxicity) [21,22].

Classic toxins used to exemplify the clinical response to a necrotizing hepatotoxin are carbon tetrachloride (CCl_4^-), acetaminophen, and nitrosamine. Data from experimentally intoxicated patients were used to exemplify enzymatic response patterns. Hepatocellular necrosis induced by nitrosamines increases plasma ALT activity, but this increase was not significant until after 1 week of intermittent chronic exposure. The increase in transaminase activity persists for weeks until the necrosis resolves. Low-grade hepatocellular degeneration is also observed in some dogs with portosystemic shunts (PSSs). Released enzymes in these patients may have delayed sinusoidal clearance because histologic changes are minor. Changes in plasma ALT activity before and after exposure of dogs to nitrosamines, before and after surgical creation of PSSs, and in a clinical patient that survived food-borne aflatoxin hepatotoxicity are profiled in Fig. 7. This figure exemplifies the influence of different forms of liver injury on serum enzyme profiles [20,23]. Hepatotoxicity induced by acetaminophen is the classic example of hepatotoxicity induced by an electrophile adduct. Marked increases in plasma ALT and AST activities develop within 24 hours, yet these may decline within 72 hours to near-normal values. This toxin is highly dose dependent in dogs and cats. The ALT profiles in animals receiving nonlethal and lethal amounts of acetaminophen are illustrated in Fig. 8 [24–28]. Cats are exceedingly susceptible to acetaminophen toxicosis, with hematologic signs dominating their clinical presentation after as little as 125 mg. Although dogs are more resistant than cats, a dose of 200 mg/kg of body weight may be life endangering.

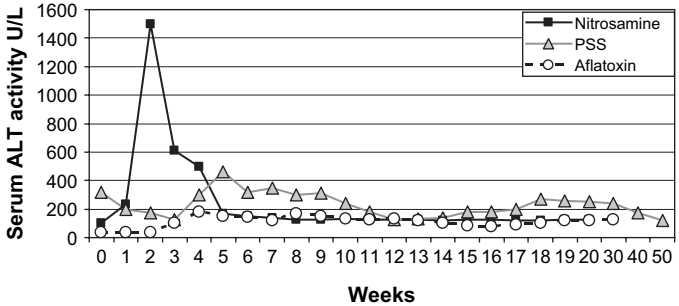

Fig. 7. Plasma ALT activity profiles before and after short-term exposure of dogs to nitrosamines, before and after surgical creation of PSSs that produced low-grade hepatic degeneration, and in a clinical patient that survived severe food-borne aflatoxin hepatotoxicity. (*Data from* the New York State College of Veterinary Medicine, Cornell University, Ithaca, NY, 2006; Strombeck DR, Harrold D, Rogers Q, et al. Plasma amino acids, glucagon, and insulin concentrations in dogs with nitrosamine-induced hepatic disease. Am J Vet Res 1983;44: 2028–2036; and Schaeffer MC, Rogers QR, Buffington CA, et al. Long-term biochemical and physiologic effects of surgically placed portacaval shunts in dogs. Am J Vet Res 1986;47:346–55.)

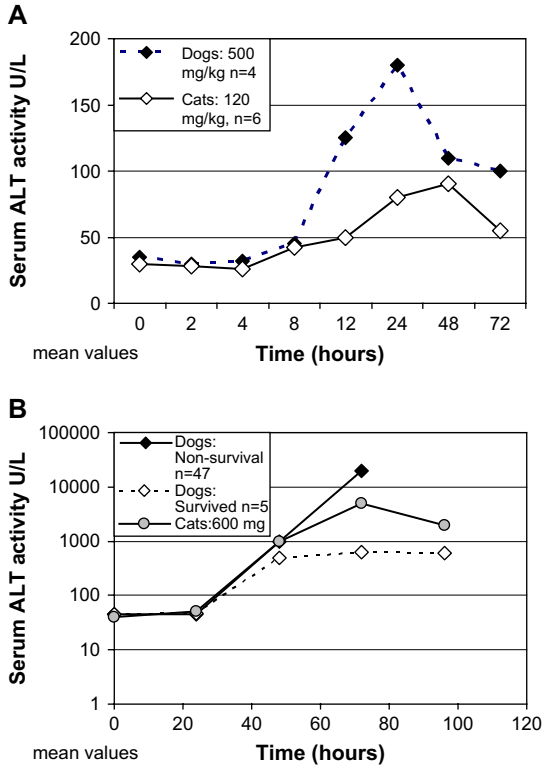

Fig. 8. Plasma ALT profiles in dogs and cats receiving nonlethal (*A*) and lethal (*B*) amounts of acetaminophen. (*Data from* references 24–27).

Acute hepatic necrosis caused by infectious canine hepatitis (adenovirus) increases plasma ALT activity by 30-fold, with enzyme activity peaking within 4 days [29]. Thereafter, a chronic sustained increase in ALT is common, and the patient may develop chronic hepatitis. This infectious disorder is now rarely encountered in companion dogs in North America. Hepatic injury induced by toxins usually causes plasma ALT activity to increase, peak, and normalize sooner than observed in infectious viral hepatitis. Chronic hepatitis, a persistent necroinflammatory disorder, is associated with varying severities of necrosis and fibrosis, cyclic disease activity, and plasma enzyme "flares." At times, plasma ALT activity achieves values 10-fold normal or greater. Enzyme fluctuations contrast with enzyme profiles associated with a single injurious event or toxin exposure. In these latter cases, serum ALT activity declines as injury resolves, but serum ALP activity may increase as a result of the regenerative proliferative process. The sensitivity of ALT in the detection of hepatobiliary syndromes in the dog and cat is shown in Fig. 9 using clinical data from 815

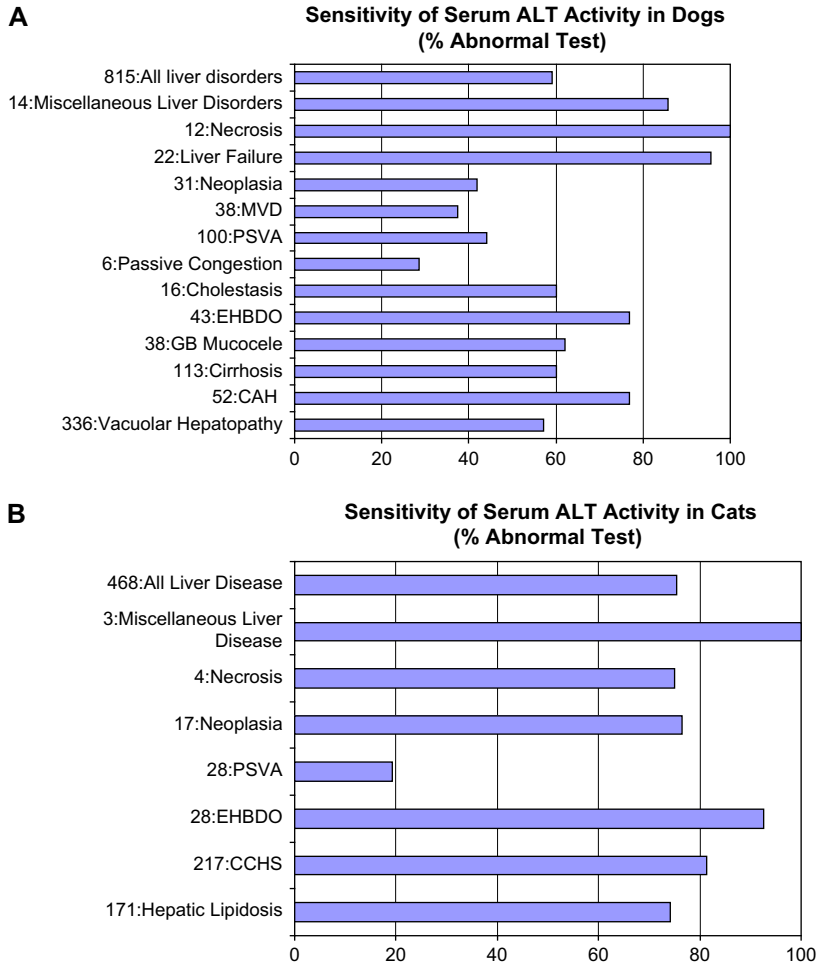

Fig. 9. Sensitivity of serum ALT activity for the detection of hepatobiliary syndromes in the dog (*A*) and cat (*B*). The number preceding the disease description indicates the number of cases included. Miscellaneous liver disorders included syndromes that could not be classified in other categories and for which there were fewer than five cases. CAH, chronic ''active'' hepatitis; CCHS, cholangitis or cholangiohepatitis syndrome of cats; EHBDO, extrahepatic bile duct occlusion; GB, gallbladder; MVD, microvascular dysplasia; PSVA, portosystemic vascular anomaly. All diagnoses were confirmed by liver biopsy or definitive imaging studies in dogs with a PSVA. (*Data from* the New York State College of Veterinary Medicine, Cornell University, Ithaca, NY, 2006.)

dogs and 468 cats with biopsy-confirmed disorders. Unfortunately, the high sensitivity of ALT in some disorders is not linked to high specificity for differentiating clinically significant liver disease or specific histologic abnormalities or for identifying dogs with hepatic dysfunction.

Aspartate Aminotransferase

As previously discussed (see Figs. 4 and 5), AST is present in substantial concentrations in a wide variety of tissues [2,3,11,14,30]. Distinct hepatocellular cytosolic and mitochondrial isozymes have been proven in several species. In people, most of the circulating AST is mitochondrial in origin, and the extremely short $t_{1/2}$ of this isozyme is useful for distinguishing severe ongoing hepatocellular insult [31].

Increased serum AST activity can reflect reversible or irreversible changes in hepatocellular membrane permeability, cell necrosis, hepatic inflammation, and, in the dog, microsomal enzyme induction. After acute diffuse severe hepatic necrosis, serum AST activity sharply increases during the first 3 days to values 10- to 30-fold normal in dogs and up to 50-fold normal in cats [14,16,32]. If necrosis resolves, the serum AST activity gradually declines over 2 to 3 weeks. In most cases, AST activity generally parallels changes in ALT activity. In some animals, however, AST becomes quiescent before ALT [14]. Although increased AST activity in the absence of abnormal ALT activity implicates an extrahepatic enzyme source (notably muscle injury), there are clinical exceptions that may relate to the severity and zonal location of hepatic damage. In some cats with liver disease, AST performs as a more sensitive marker of liver injury compared with ALT. This has been observed in cats with a variety of syndromes, including hepatic necrosis, cholangiohepatitis, myeloproliferative disease and lymphoma associated with hepatic infiltration, and chronic bile duct obstruction. This trend is evident by comparing sensitivities for ALT and AST, as displayed in Figs. 9 and 10 [32,33]. A similar behavior of AST noted in fewer dogs with naturally developing liver disease is corroborated by conclusions made in two retrospective studies of canine liver disease [34,35]. Contribution of AST from other tissues, particularly in animals with metastatic neoplasia, systemic inflammatory conditions, and congestive heart failure, may help to explain enzyme performance. Dogs treated with glucocorticoids may develop mildly increased serum AST activity that resolves within several weeks of glucocorticoid withdrawal [36].

ALKALINE PHOSPHATASE

Alkaline phosphatase (ALP) is a member of a family of zinc metalloprotein enzymes that split terminal phosphate groups from organic phosphate esters. These enzymes function at membranous interfaces and operate best at an alkaline pH. The exact functions of ALP in intermediary metabolism continue to be refined. Unlike transaminases, ALP is attached to cell membranes by glucosyl phosphatidylinositol linkages. These "anchors" must be cleaved by endogenous phospholipases before soluble enzyme can be distributed into the systemic circulation [37–39]. Release of ALP from its membrane linkage is facilitated in the presence of bile acids that exert a detergent-like influence on the membrane anchor; this action also augments ALP release in cholestatic disorders [37,38]. Increased serum ALP activity in the dog is the most common biochemical abnormality on routine biochemical profiles. It is also a biochemical test that

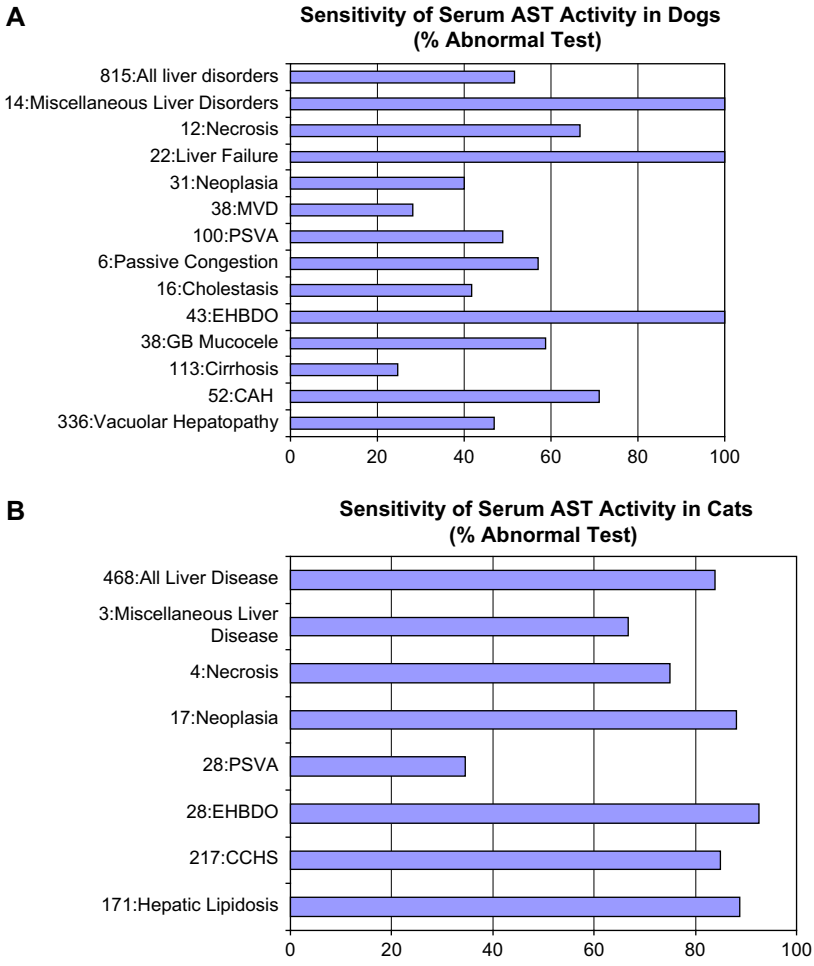

Fig. 10. Sensitivity of serum AST activity for the detection of hepatobiliary syndromes in the dog (*A*) and cat (*B*). The number preceding the disease description indicates the number of cases included. Miscellaneous liver disorders included syndromes that could not be classified in other categories and for which there were fewer than five cases. CAH, chronic ''active'' hepatitis; CCHS, cholangitis or cholangiohepatitis syndrome of cats; EHBDO, extrahepatic bile duct occlusion; GB, gallbladder; MVD, microvascular dysplasia; PSVA, portosystemic vascular anomaly. All diagnoses were confirmed by liver biopsy or definitive imaging studies in dogs with a PSVA. (*Data from* the New York State College of Veterinary Medicine, Cornell University, Ithaca, NY, 2006.)

can defy diagnostic scrutiny in the dog, with ALP having the lowest specificity of the routinely used liver enzymes (see Fig. 1). The diagnostic complexity involving this enzyme in the dog involves the regulation and induction phenomenon that influence ALP isozyme gene transcription.

Tissues containing the highest quantities of ALP in the dog, in descending order, are the intestinal mucosa, kidney (cortex), placenta, liver, and bone. Tissue concentrations of ALP in cats have been variably reported: Hoffmann and colleagues [40] found the highest tissue ALP activity in the intestine, followed by the renal cortex, liver, and bone. Everett and colleagues [41] found the highest ALP activity in the kidney, followed by the intestine, bone, and liver, and Foster and Thoday [42] found highest concentrations in the kidney, followed by the intestine, liver, and bone. Distinct serum ALP isozymes can be extracted from some of these tissues. The three major isozymes encountered in canine serum include bone-induced (B-ALP), liver-induced (L-ALP), and glucocorticoid-induced (G-ALP) enzymes [43–46]. There are two genes responsible for ALP production in the dog [37,38,47]. The first is the tissue-nonspecific ALP gene that transcribes L-ALP, B-ALP, and the kidney-induced ALP isoforms (isoforms are similar forms of an enzyme transcribed from the same gene but having different posttranslational processing) [37]. These ALP isoforms differ only in their degree of glycosylation. The second gene, the intestinal ALP gene, is specific for the intestinal-induced ALP isoenzyme product (I-ALP) produced in the intestinal mucosa [37]. The I-ALP and G-ALP forms differ only in carbohydrate composition, and recent work has confirmed that G-ALP is synthesized in the liver, where it is attached to perisinusoidal membranes of hepatocytes [37]. The tissue-nonspecific ALP and G-ALP can be induced in dogs but not in cats by endogenous or exogenous steroidogenic hormones and certain drugs.

In dogs, the $t_{1/2}$ of the placental-induced ALP, renal-induced ALP, and I-ALP are short (<6 minutes). In addition to their short $t_{1/2}$, intestinal and renal isozymes are excreted into the intestinal lumen and urine, respectively [43,48]. In the cat, the $t_{1/2}$ of the intestinal isoenzyme is less than 2 minutes. Because the placental and renal isoenzymes are structurally similar, they are also surmised to have a short $t_{1/2}$ in the systemic circulation [40,47,49]. The isozymes with an ultrashort $t_{1/2}$ are not routinely detected in canine or feline serum in patients having high ALP activity. The exception is the placental isoenzyme, which has been detected in late-term pregnant cats [49]. In dogs, L-ALP and G-ALP are primarily responsible for high serum ALP activity, whereas L-ALP is primarily responsible in the cat. Juvenile dogs and cats maintain higher serum ALP activity than mature adult animals, however, as a result of higher bone metabolism and B-ALP release associated with bone growth and remodeling [40,43,49,50]. The $t_{1/2}$ of L-ALP and G-ALP in the dog is approximately 70 hours [43,46,48], whereas the $t_{1/2}$ of L-ALP in the cat is remarkably shorter at approximately 6 hours [40,47,49]. Increased total serum ALP activity develops in 43% to 75% of hyperthyroid cats, depending on the chronicity of their endocrinopathy [51,52]. The B-ALP isoenzyme may substantially contribute to the total ALP activity in these cats, similar to hyperthyroid human beings (Fig. 11) [42,50,53–58]. Evidence of enhanced bone mobilization (increased osteocalcin), increased parathormone, and reduced ionized calcium has been demonstrated in hyperthyroid cats with high serum ALP activity. Further, in

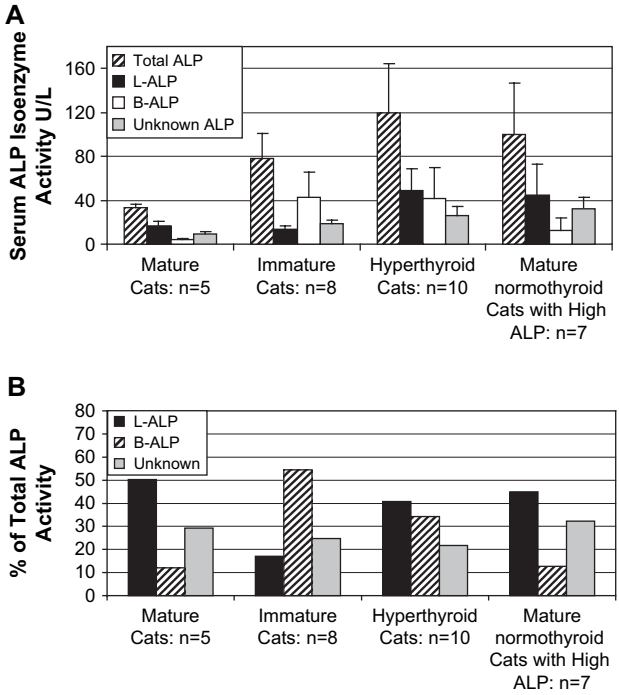

Fig. 11. Serum ALP isoenzyme activity in mature and immature healthy cats, hyperthyroid cats, and mature euthyroid cats with high serum ALP activity (*A*) and the percentage of total ALP activity in each group (*B*). (*Data from* Horney BS, Farmer AJ, Honor DJ, et al. Agarose gel electrophoresis of alkaline phosphatase isoenzymes in the serum of hyperthyroid cats. Vet Clin Pathol, 1994:23:98–102.)

one study, 88% of cats had measurable serum L-ALP and B-ALP isozymes whether or not they had high serum ALP activity [42].

The comparably small magnitudes of ALP activity in cats with liver disease (twofold to threefold normal) relative to the dog (often >fourfold to fivefold normal) reflect the lower specific activity of hepatic ALP in cats and the shorter L-ALP enzyme $t_{1/2}$ [11,49]. Nevertheless, this difference does not diminish the clinical utility of serum ALP in the diagnosis of feline liver disease when the species-appropriate perspective is maintained (see Fig. 1; Figs. 12 and 13).

The utility of serum ALP activity as a diagnostic indicator in the dog is complicated by the common accumulation of the L-ALP and G-ALP isozymes. Studies confirm that the canine liver is the common site of L-ALP and G-ALP synthesis in response to steroidogenic hormones [59,60]. The clinical utility of ALP in the dog is not improved by differentiating ALP isoenzymes, because the G-ALP isoenzyme is so easily induced by chronic stress and perhaps inflammatory mediators associated with systemic disease and spontaneous liver disorders. Glucocorticoid exposure imparts a rapid but transient increase in L-ALP

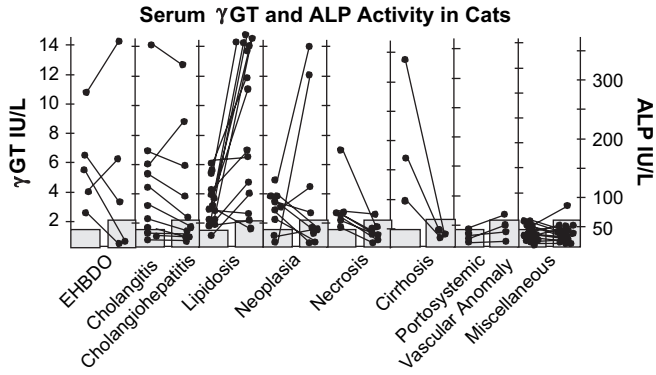

Fig. 12. Dot-plot shows serum ALP and γ-glutamyltransferase (γ-GT) activity in cats with biopsy-confirmed spontaneous hepatobiliary disorders. Miscellaneous represents cats with nonhepatobiliary disorders. Boxes in lanes represent the reference range. EHBDO, extrahepatic bile duct occlusion. (*Data from* the New York State College of Veterinary Medicine, Cornell University, Ithaca, NY.)

induction or production that plateaus within 7 to 10 days. In contrast, the G-ALP undergoes an initial 10-day transcription lag phase; this phenomenon is shown in Fig. 14 [59].

The B-ALP isozyme increases secondary to osteoblast activity. This isozyme is detected, as previously mentioned, in the serum of young growing animals and may also be detected in patients with bone tumors, secondary renal hyperparathyroidism, or osteomyelitis. The contribution of B-ALP to the total serum ALP activity usually does not lead to an erroneous diagnosis of cholestatic liver disease, however [43]. Bone remodeling secondary to neoplasia may not substantially affect serum ALP activity or may cause only a trivial two- to threefold increase in the dog. In the young growing cat, however, increased serum B-ALP activity may simulate enzyme activity realized with hepatobiliary disease.

The L-ALP isozyme is derived from membranes in the canalicular area of the hepatocyte and refluxes into plasma secondary to enhanced de novo hepatic synthesis, canalicular injury, cholestasis, and solubilization of membrane-bound protein by the detergent action of bile salts [43,61–66].

Although ALT is immediately released from the hepatocellular cytosol in acute hepatic necrosis, the small quantities of membrane-bound ALP cannot be readily dispatched. Rather, it takes several days for induction of membrane-associated enzyme to "gear up" and spill into the perisinusoidal ultrafiltrate in the space of Disse. The liver ramps up L-ALP production at a faster rate than G-ALP, even in the presence of glucocorticoids. The L-ALP rapidly plateaus, however; thereafter, the G-ALP assumes a dominant role in total serum ALP activity in patients with chronically increased enzyme activity. The largest increases in serum ALP activity (L-ALP or G-ALP 100-fold normal or greater) develop in dogs with diffuse or focal cholestatic disorders, massive

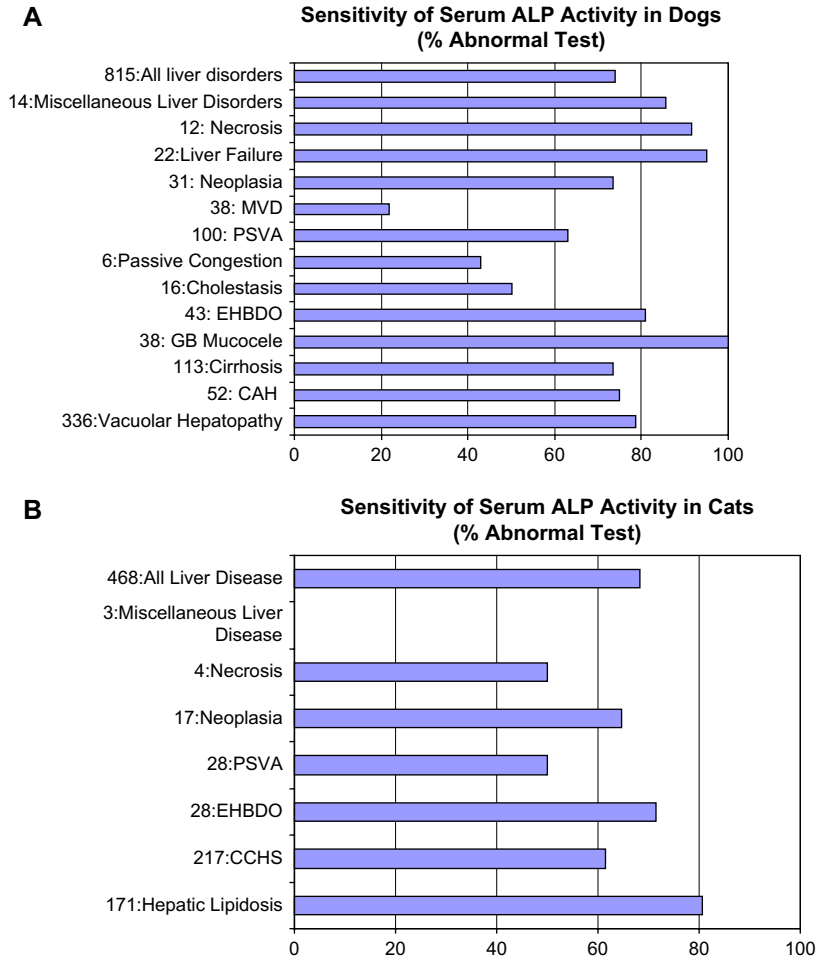

Fig. 13. Sensitivity of serum ALP activity for the detection of hepatobiliary syndromes in the dog (*A*) and cat (*B*). The number preceding the disease description indicates the number of cases included. Miscellaneous liver disorders included disorders that could not be classified in other categories and for which there were fewer than five cases. CAH, chronic ''active'' hepatitis; CCHS, cholangitis or cholangiohepatitis syndrome of cats; EHBDO, extrahepatic bile duct occlusion; GB, gallbladder; MVD, microvascular dysplasia; PSVA, portosystemic vascular anomaly. All diagnoses were confirmed by liver biopsy or definitive imaging studies in dogs with a PSVA. (*Data from* the New York State College of Veterinary Medicine, Cornell University, Ithaca, NY, 2006.)

hepatocellular carcinoma (HCCA), or bile duct carcinoma and in those treated with glucocorticoids.

Although serum activity of ALP may be normal or only modestly increased in dogs with metastatic neoplasia involving the liver, a dramatic increase in serum ALP may be realized in dogs with mammary neoplasia. Approximately

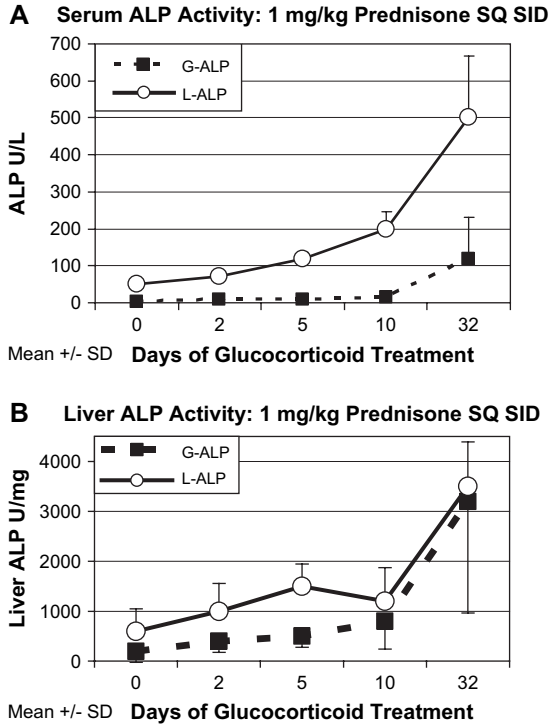

Fig. 14. Sequential measurements of L-ALP and G-ALP isoenzymes in dogs before and after initiation of prednisolone at a dose of 1 mg/kg administered subcutaneously (SQ) once daily (SID). Data depict the acute initial rise in serum L-ALP activity and the later increase in liver tissue L-ALP. (*Data from* Wiedmeyer CE, Solter PE, Hoffmann WE. Kinetics of mRNA expression of alkaline phosphatase isoenzymes in hepatic tissues from glucocorticoid-treated dogs. Am J Vet Res. 2002;63:1089–95.)

55% of dogs with malignant mammary tumors and 47% of dogs with benign mammary tumors develop high serum ALP activity [67,68]. Although there was no significant difference in total ALP activity between dogs with malignant and benign neoplasms (with and without osseous transformations), the highest serum ALP activity developed in dogs with malignant mixed tumors. This association has not been reconciled with osseous transformation or myoepithelial ALP production within tumor tissue. Approximately 11% of dogs with malignant tumors and 7% of dogs with benign tumors developed a fourfold increase in serum ALP activity. Nevertheless, serum ALP has no value as a diagnostic or prognostic marker in dogs with mammary neoplasia. It remains unclear whether disease remission (eg, treatment involving surgery or chemotherapy) is followed by a regression in serum ALP activity and whether serum ALP activity functions as a paraneoplastic marker of mammary neoplasia.

After acute severe hepatic necrosis, ALP activity increases two- to fivefold normal (in the dog and cat), stabilizes, and then gradually declines over 2 to

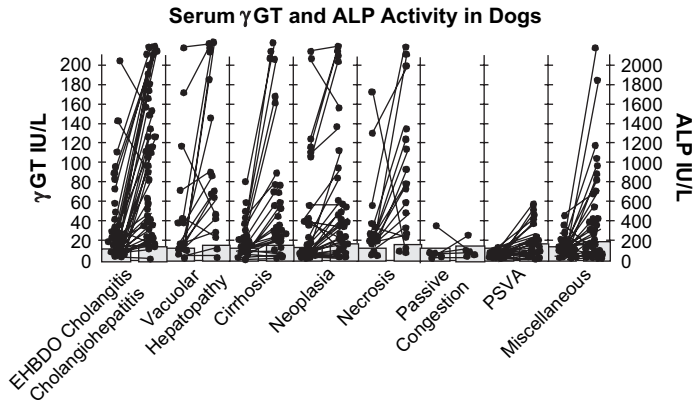

Fig. 15. Dot-plot shows serum ALP and γ-glutamyltransferase (γ-GT) activity in 270 dogs with biopsy-confirmed spontaneous hepatobiliary disorders. Miscellaneous represents dogs with nonhepatobiliary disorders. Boxes in lane represent the reference range. EHBDO, extrahepatic bile duct obstruction; PSVA, portosystemic vascular anoma. (*Data from* the New York State College of Veterinary Medicine, Cornell University, Ithaca, NY.)

3 weeks [18,69]. Sustained ALP activity often reconciles with biliary epithelial hyperplasia associated with the proliferative reparative process that follows panlobular necrosis. Thereafter, ALP activity stabilizes and gradually declines but not into the normal range for several weeks to months [70–72]. In the cat, extrahepatic bile duct obstruction results in a twofold increase within 2 days, as much as a fourfold increase within 1 week, and up to a ninefold increase in serum ALP activity within 2 to 3 weeks [33,41,69,73]. Thereafter, activity stabilizes and gradually declines but usually not into the normal range; the declining enzyme activity coordinates with developing biliary cirrhosis. Cats with experimentally induced incomplete biliary tree occlusion developed ALP values approximately 50% lower than those observed with complete common duct occlusion [41]. In contrast, even partial (experimental) occlusion of the biliary tree in the dog causes marked increases in total serum ALP activity [69–72,74–76]. Inflammatory disorders involving biliary or canalicular structures or disorders compromising bile flow increase serum ALP activity secondary to membrane inflammation or disruption and local bile acid accumulation. In the dog and cat, however, similar magnitudes of serum ALP activities develop in spontaneous intrahepatic cholestasis as compared with disease or obstruction involving the extrahepatic biliary structures; the reader is referred to Figs. 12 and 15. Consequently, ALP activity cannot differentiate between intra- and extrahepatic cholestatic disorders.

Many extrahepatic and primary hepatic conditions enhance production of L-ALP. In the cat, the syndrome of hepatic lipidosis is associated with profound increases in total ALP activity and marked jaundice. A considerable number of disorders leading to inappetence usually precede development of this

potentially lethal syndrome [77]. Although the underlying mechanisms provoking high serum ALP activity have not been proven, they likely involve canalicular dysfunction or compression.

In the dog, primary hepatic inflammation as well as systemic infection or inflammation and exposure to steroidogenic hormones may induce a vacuolar hepatopathy [78]. When severe, this disorder also may impose a cholestatic effect on the liver. Although initially well characterized as a glucocorticoid-initiated lesion, it is now well established that nearly 50% of dogs with this syndrome lack overt exposure to glucocorticoids or other steroidogenic substances [78]. Chronically ill dogs may produce the G-ALP isozyme secondary to stress-induced endogenous glucocorticoid release. Chronically ill dogs with this lesion (lacking exogenous glucocorticoid exposure) often demonstrate normal dexamethasone suppression and corticotropin responses. In some dogs, however, this lesion signals the presence of atypical adrenal hyperplasia associated with abnormal sex hormone production (especially 17-OH progesterone). Diagnostically, canine vacuolar hepatopathy is usually first recognized because of markedly increased serum ALP activity in a dog lacking signs of liver disease. This curious physiologic response to endogenous or exogenous steroidogenic hormones is characterized histologically [78–81]. Hepatocytes become markedly distended (up to a 10-fold cell expansion) with glycogen, and in severe cases (rare), cell swelling can impose intrahepatic sinusoidal hypertension and canalicular compression, leading to jaundice and even abdominal effusion. There is no consistent relation between the magnitude of serum ALP activity, the presence of high G-ALP activity, and the histologic lesion. Unfortunately, G-ALP is not useful for syndrome characterization, because this isozyme can become the predominant enzyme in dogs treated with glucocorticoids; dogs with spontaneous or iatrogenic hyperadrenocorticism; dogs with hepatic or nonhepatic neoplasia; and, most importantly, dogs with many different chronic illness, including primary liver disease [43,82,83].

In controlled studies of this syndrome, induction of ALP occurred as early as 1 week after initiation of daily administration of prednisone (2 mg/kg once daily) [83], as early as 2 days after daily administration of prednisone (4.4 mg/kg once daily) [79], and as early as 3 days after daily administration of dexamethasone (2.2 mg/kg once daily) [36]. The initial increase in ALP activity is attributable to the L-ALP isozyme; thereafter, however, the G-ALP becomes the dominant isozyme (Fig. 16) [62,63]. Different magnitudes of enzyme activity develop depending on the type of glucocorticoid administered, the dose given, and the individual patient response [81,82]. Increases in total serum ALP activity attributable primarily to G-ALP usually exceed those associated with liver or bone isoenzymes. In one study, after consecutive daily administration of prednisone at a dose of 4.4 mg/kg and treatment discontinuation, dogs reached a maximum ALP activity of 64-fold normal by day 20 (see Fig. 16) [79]. The ALP activity gradually decreased to 8-fold normal by day 56. This study is relevant to clinical practice, because a commonly prescribed immunosuppressive dose of prednisone was used. In conclusion, the

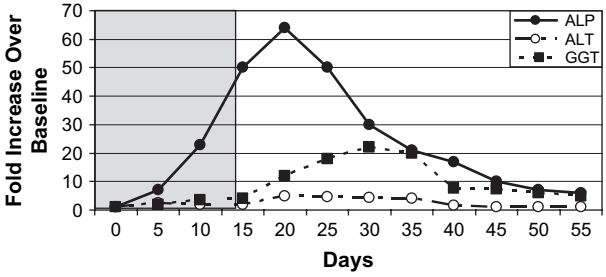

Fig. 16. Response depicting the fold increase from baseline of ALP, ALT, and γ-glutamyltransferase (GGT) activity in dogs given prednisone at a dose of 4.4 mg/kg/d (*gray shaded area*). Enzyme activity continued to increase after treatment was suspended. Data represent mean values. (*Data from* Badylak SF, Van Vleet JF. Sequential morphologic and clinicopathologic alterations in dogs with experimentally induced glucocorticoid hepatopathy. Am J Vet Res 1981;42;1310–18.)

production of G-ALP does not imply that a dog treated with cortisone has iatrogenic hyperadrenocorticism, a suppressed pituitary adrenal axis, or a clinically important vacuolar hepatopathy.

By comparison, the feline liver is relatively insensitive to glucocorticoids. Administration of prednisolone (5 mg twice daily) to normal cats for 30 days failed to elicit an increase in ALP activity in serum or liver tissue [40]. When cats received prednisolone at a dose of 2 mg/kg once daily for 16 days, changes in serum ALP also did not develop or were minor. Morphologic hepatocellular alterations are rare and minor in most studies but were suggested to reflect hepatocellular vacuolar glycogen retention in some cats in two investigations [84,85].

In dogs, serum total ALP activity and L-ALP isozyme also may be induced by administration of certain anticonvulsants (phenobarbital, primidone, and phenytoin) [86,87]. Induced ALP activity usually increases 2- to 6-fold normal activity. During a 30-day study of drug administration to normal dogs, phenytoin (22 mg/kg administered orally three times daily) produced a uniform small increase in serum ALP activity; phenobarbital (4.4 mg/kg administered orally three times daily) produced peak serum enzyme activity 30-fold normal by 24 days, which thereafter declined; and primidone (17.6 mg/kg administered orally three times daily) produced a 5-fold increase in serum ALP activity by day 28. Healthy dogs receiving combination therapy (eg, primidone, phenytoin) developed ALP increases ranging from 2- to 12-fold normal, with some receiving high-dose phenobarbital developing ALP activity 30- to 40-fold greater than the normal range. In contrast to the dog, the administration of phenobarbital (0.25 grain twice daily) for 30 days in cats failed to elicit an increase in serum or liver tissue ALP activity [40].

γ-GLUTAMYLTRANSFERASE

γ-Glutamyltransferase (γ-GT) is a membrane-bound glycoprotein that catalyzes the transpeptidation and hydrolysis of the γ-glutamyl group of glutathione

(GSH) and related compounds. Through this reaction, it plays a critical role in cellular detoxification and confers resistance against several toxins and drugs. Its reactions with GSH are essential for maintaining the balance of the intracellular redox status. Because GSH is the most abundant intracellular nonprotein thiol and is involved in a myriad of biologic processes (eg, regulation of the intracellular redox status, conjugation of electrophile toxins), γ-GT plays a formidable role in intermediary metabolism. In addition to ensuring cysteine availability, γ-GT hydrolyzes GSH-related compounds, including leukotriene-C, prostaglandins, and several γ-glutamyl amino acids, and catalyzes the transfer of the γ-glutamyl group from GSH to dipeptides and amino acids [88]. The latter transamidation process is essential for amino acid transport (recovery) in the renal tubules. Experimental work suggests that expression of γ-GT is regulated by glucocorticoids, and it is known that induction phenomena increase hepatic γ-GT production [89]. Because acute exposure to oxidative stress increases gene transcription for γ-GT synthesis, it seems that the regulation of γ-GT synthesis is an adaptive response protecting cells against oxidative injury. Although enhanced synthesis contributes to the serum γ-GT activity, cholestatic disorders promote the bile acid solubilization and release of γ-GT from its membrane anchor.

A connection between γ-GT and neoplastic transformation has been made repeatedly in the liver as well as in experimental carcinogenesis. It has been proposed that increased γ-GT expression may contribute to tumor progression and formation of aggressive and drug-resistant phenotypes. One theory suggests that increased γ-GT synthesis enhances the capacity for GSH-mediated drug detoxification, thereby limiting drug residence time. An alternative argument is that enhanced γ-GT activity increases availability of cysteinyl-glycine residues that complex extracellularly with drug metabolites, augmenting formation of reactive oxygen species [90].

The highest tissue concentrations of γ-GT in the dog and cat are located in the kidney and pancreas, with lesser amounts in the liver, gallbladder, intestine, spleen, heart, lungs, skeletal muscle, and erythrocytes [71,80,91]. Serum γ-GT activity is largely derived from the liver, although there is considerable species variation in its localization within this organ. Hepatic microsomal localization has been proven for γ-GT in the dog, where it is associated with canaliculi, bile ducts, and zone 1 (periportal) hepatocytes [72,76,91]. Increased serum γ-GT activity reflects enhanced synthesis in the liver and regurgitation of eluted enzyme from membrane surfaces. The diagnostic performance of γ-GT has been scrutinized in clinical patients with and without liver disease [71,92–94].

Experimental study of serum γ-GT activity in dogs and cats undergoing acute severe diffuse necrosis has shown no change or only mild increases (1- to 3-fold normal) that resolve over the ensuing 10 days. In the dog, extrahepatic bile duct obstruction causes serum γ-GT activity to increase from 1- to 4-fold normal within 4 days and from 10- to 50-fold normal within 1 to 2 weeks. Thereafter, values may plateau or continue to increase as high as 100-fold normal [17,71,76]. In the cat with extrahepatic bile duct obstruction,

serum γ-GT activity may increase up to 2-fold normal within 3 days, 2- to 6-fold normal within 5 days, 3- to 12-fold normal within a week, and 4- to 16-fold normal within 2 weeks [18,69].

Glucocorticoids and certain other microsomal enzyme inducers may stimulate γ-GT production in the dog similar to their influence on ALP. Administration of dexamethasone (3 mg/kg once daily) or prednisone (4.4 mg/kg intramuscularly once daily) increased γ-GT activity within 1 week to 4- to 7-fold normal and up to 10-fold normal within 2 weeks [75,79,80]. Increased synthesis of γ-GT secondary to glucocorticoid administration is surmised to involve the liver. In comparison to glucocorticoid induction, dogs treated with phenytoin or primidone (anticonvulsants) develop only a modest increase in serum γ-GT activity up to 2- or 3-fold normal, unless they also develop idiopathic anticonvulsant hepatotoxicosis [95].

Some cats with advanced necroinflammatory liver disease, major bile duct obstruction, or inflammatory intrahepatic cholestasis develop a larger fold increase in γ-GT activity relative to ALP (see Fig. 12) [93]. In other species, cholestasis is known to enhance enzyme synthesis as well as membrane release of γ-GT. It remains undetermined whether glucocorticoids or other enzyme inducers clinically influence serum γ-GT in the cat. It is noteworthy that the normal range for feline serum γ-GT activity is much narrower and lower than in the dog. Thus, interpreting feline γ-GT activity using the canine reference range leads to erroneous conclusions. Additionally, because of the comparatively low γ-GT activity in feline serum, assay sensitivity may be a problem if reagent solubility is less than optimum (ie, low γ-GT activity may be undetected).

Remarkably increased γ-GT values have been observed in dogs and cats with primary hepatic or pancreatic neoplasia. Although a unique γ-GT isozyme is associated with HCCA in human beings, it has not been determined that a similar paraneoplastic phenomenon occurs in dogs or cats. In people, γ-GT is also used in surveillance for hepatic metastasis; however, it does not seem to be suitable for this application in the dog or the cat.

The sensitivity of γ-GT for the detection of liver disease is summarized in Fig. 17. Like ALP, γ-GT lacks specificity in differentiating between pavenchymal hepatic disease and occlusive biliary disease. It is not as sensitive in the dog as ALP but does have higher specificity [34,93]. In cats with inflammatory liver disease, γ-GT is more sensitive but less specific than ALP, and these two enzymes perform best when interpreted simultaneously [93]. The prediction that hepatic lipidosis has developed secondary to necroinflammatory liver disease, biliary duct occlusion, or pancreatic disease can be made by examining the relative increase in γ-GT compared with ALP. Necroinflammatory disorders involving biliary structures, the portal triad, or the pancreas are associated with a fold increase in γ-GT exceeding that of ALP in the cat (see Fig. 12). With the exclusion of these underlying disorders, cats with hepatic lipidosis have higher fold increases in ALP relative to γ-GT (this is illustrated by data presented in Figs. 12, 13, and 17). The mechanism for this difference presumably is the involvement of duct epithelium in inflammatory processes (biliary ducts

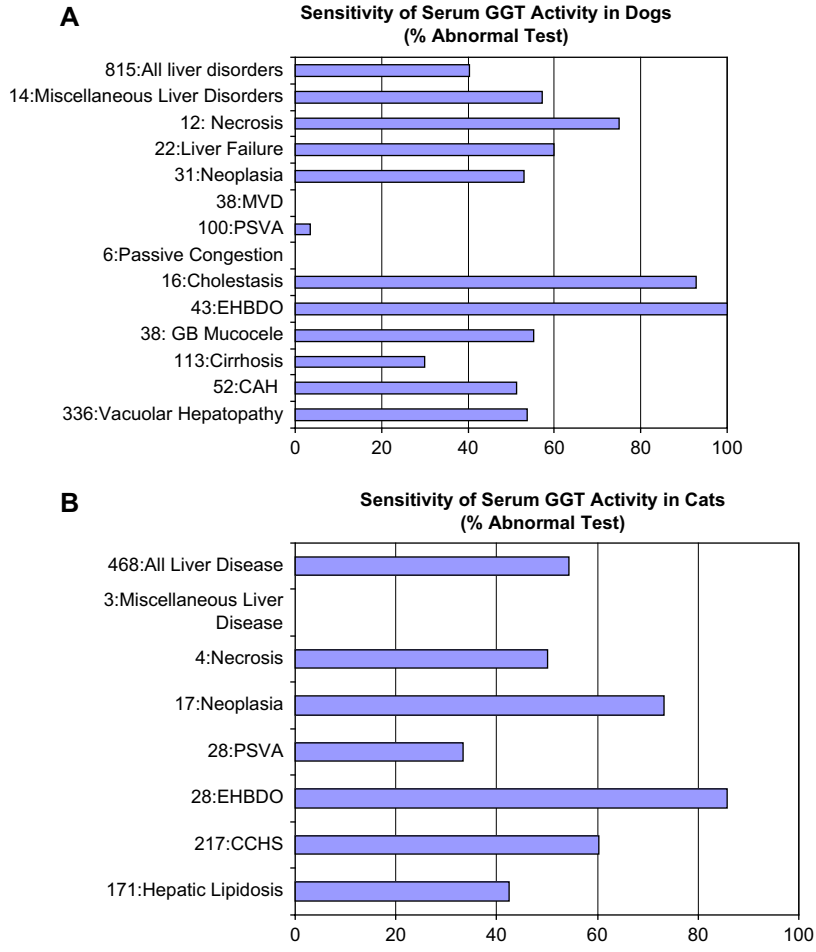

Fig. 17. Sensitivity of serum γ-GT (GGT) activity for the detection of hepatobiliary syndromes in the dog (*A*) and cat (*B*). The number preceding the disease description indicates the number of cases included. Miscellaneous liver disorders included disorders that could not be classified in other categories and for which there were fewer than five cases. CAH, chronic ''active'' hepatitis; CCHS, cholangitis or cholangiohepatitis syndrome of cats; EHBDO, extrahepatic bile duct occlusion; GB, gallbladder; MVD, microvascular dysplasia; PSVA, portosystemic vascular anomaly. All diagnoses were confirmed by liver biopsy or definitive imaging studies in dogs with a PSVA. (*Data from* the New York State College of Veterinary Medicine, Cornell University, Ithaca, NY, 2006.)

and pancreatic ducts), because these likely have greater potential for γ-GT production.

Neonatal animals of several species, including the dog but not the cat, develop high serum γ-GT activity secondary to colostrum ingestion, as discussed in detail elsewhere in this article [96–98].

LACTATE DEHYDROGENASE

LDH has a wide tissue distribution in all species. The highest tissue concentrations, in descending order, occur in the skeletal muscle, heart, and kidney, with lesser amounts in the intestine, liver, lung, and pancreas [99]. Each tissue has been shown to contain at least five isozymes [99]. LDH_5 predominates in the liver and is believed to be a major contributor to serum LDH activity. Serum biochemistry profiles report total LDH, however, negating the utility of this enzyme for detection of hepatobiliary abnormalities. High LDH activity is often observed in animals with diffuse severe hepatic necrosis or inflammation, myositis or muscle trauma, and lymphosarcoma external to the liver.

ARGINASE

Arginase is considered to be a liver-specific enzyme because it exists in higher concentrations in hepatocytes than in any other tissue (Fig. 18). It functions as a major catalyst of the urea cycle, with large quantities located in mitochondria. With severe hepatic insults, damaged mitochondrial membranes acutely release preformed arginase into the systemic circulation [15,100]. Tissue concentrations of arginase in several species have demonstrated specificity for the liver [15]. Although a simplified method for arginine analysis has adapted the test for clinical practice, its utility is limited to detection of severe acute hepatic insults because of its transient appearance in serum. Consequently, it has never gained the popularity needed to support its routine measurement economically.

With acute severe necrosis, ALT and arginase are immediately released from hepatocytes, causing a marked sharp rise in their serum activities [100,101]. If plasma arginase and transaminase activities become persistently increased, a progressive necrotizing lesion is surmised [15]. In dogs and cats, experimentally induced acute hepatic necrosis with CCl_4^- causes a 500- to

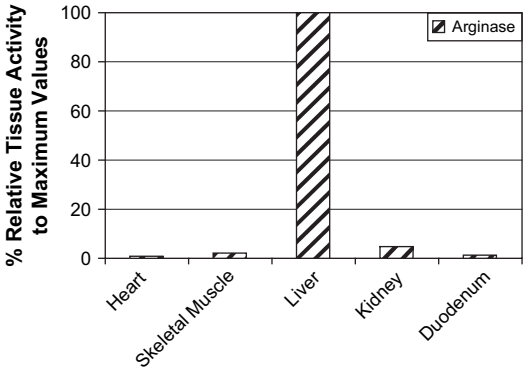

Fig. 18. Comparative tissue distribution of arginase in a dog. (*Data from* Mia AS, Koger HD. Comparative studies on serum arginase and transaminases in hepatic necrosis in various species of domestic animals. Vet Clin Pathol 1979;8:9–15.)

1000-fold increase in arginase that persists for only 2 to 3 days. During recovery, sustained increases in serum transaminase activity reflect continued enzyme leakage and longer plasma $t_{1/2}$. During recovery, leakage of transaminases but not arginase continues, with ALT and AST activity persisting for 1 week or longer [15,101,102].

Dogs treated with dexamethasone (3 mg/kg once daily for 11 days) developed a transient 5- to 8-fold increase in arginase activity by day 4 [74]. With treatment chronicity, a steady increase in serum arginase was maintained. At study termination (day 12), serum arginase activity was 10-fold normal [74]. Glucocorticoid induction of catabolic adaptations may contribute to this high arginase activity, as reported in other species [15,74,100–103].

SORBITOL DEHYDROGENASE

Sorbitol dehydrogenase (SDH) is a cytosolic enzyme released during hepatic degeneration or necrosis or secondary to altered membrane permeability. The concentration of SDH is greater in the liver than in all other tissues [3]. Although it may be a useful test for recognition of hepatocellular injury, it offers no advantage over determination of serum ALT activity. There also is concern over its lability in vitro as compared with ALT. Consequently, there has been little use of this enzyme in small animal diagnostic enzymology.

SERUM ENZYME PATTERNS IN EXTRAHEPATIC BILE DUCT OCCLUSION AND ACUTE HEPATIC NECROSIS

Typical enzyme patterns in dogs and cats sequentially sampled after experimentally induced acute hepatic necrosis (CCl_4^- initiated) and surgically created extrahepatic bile duct obstruction are displayed in Figs. 19 and 20, showing routinely used enzymes as well as arginase and SDH.

ENZYMATIC MARKERS OF HEPATIC NECROSIS: THE HEALING PHASE

Hepatic necrosis, a common reaction to liver injury, is followed by a reactive regenerative response driven by a multitude of interacting cells, cytokines, regulatory factors, and extracellular matrix molecules [104,105]. The extent or degree of hepatic regeneration depends on the type of injurious agent or event, the nature of the underlying liver disease, and the number of affected cells. Hepatocytes and bile duct epithelium maintain the potential to replicate when challenged with appropriate stimuli. A population of pluripotential cells (oval cells) also contributes to parenchymal and ductal repair [104,105]. Cell replication during the healing process explains the delayed onset or late phase and sustained increases in liver enzymes (especially of the membrane-affiliated ALP and γ-GT) after severe diffuse hepatocellular injury.

INFLUENCE OF AGE ON LIVER ENZYME ACTIVITY

Age-appropriate reference intervals for serum liver enzyme activity are essential in puppies and kittens. Plasma enzyme activities of ALP and γ-GT in the

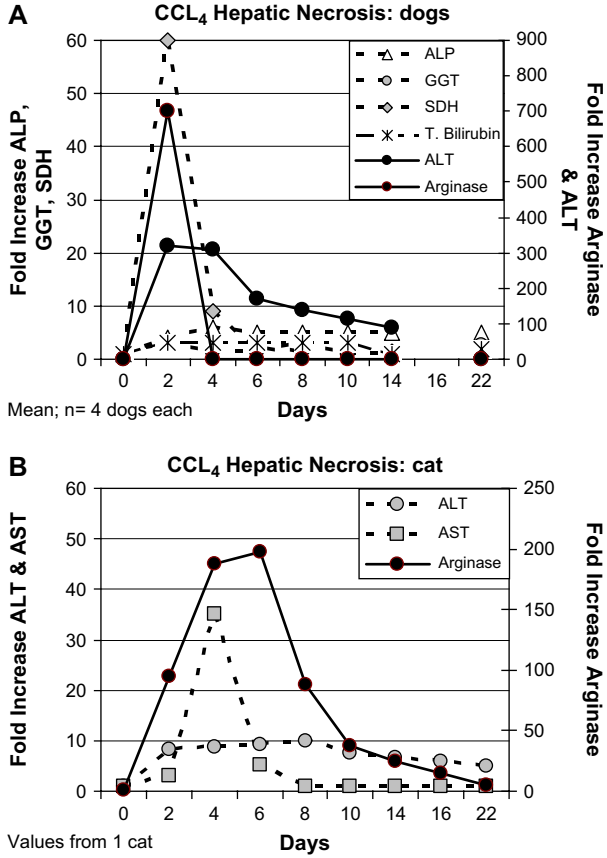

Fig. 19. Enzymology associated with severe acute hepatic necrosis induced by administration of CCl_4^- in dogs and a cat and chronic bile duct obstruction in dogs showing the clinical utility of routinely measured enzymes as well as arginase and SDH. (*Data from* Noonan NE, Meyer DJ. Use of plasma arginase and gamma-glutamyl transpeptidase as specific indicators of hepatocellular or hepatobiliary disease in the dog. Am J Vet Res 1979;40:942–47; and Mia AS, Koger HD. Comparative studies on serum arginase and transaminases in hepatic necrosis in various species of domestic animals. Vet Clin Pathol 1979;8:9–15.)

dog and cat are significantly influenced by age. Neonates have markedly higher serum enzyme activities than adults (Fig. 21) [4,96–98]. These differences reflect physiologic adaptations during the transition from fetal and neonatal life stages, colostrum ingestion, maturation of metabolic pathways, growth effects, differences in volume of distribution and body composition, and nutrition [97]. An important factor influencing serum liver enzyme activity in neonatal dogs and cats is the enteric absorption of colostral macromolecules during the first day of life (Fig. 22) [96–98]. In neonates, serum activity of ALP, AST, CK, and LDH usually increases greatly during the first 24 hours after

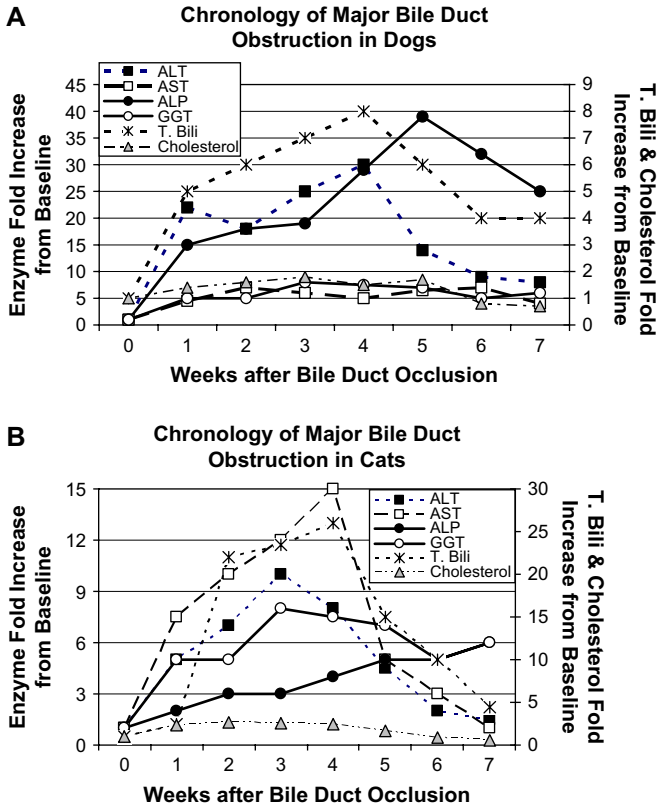

Fig. 20. Enzymology associated with chronic experimentally induced extrahepatic bile duct occlusion in the dog and cat. GGT, γ-glutamyltransferase.

birth. In kittens, serum activity of ALP, CK, and LDH exceeds adult values through 8 weeks of age. Early increases in AST, CK, and LDH are proposed to reflect muscle trauma associated with birth, whereas ALP activity reflects the bone isoenzyme (early bone growth). Serum ALP markedly increases in 1-day-old puppies and kittens subsequent to colostrum ingestion, however [96,97]. One-day-old pups (n = 5) had serum γ-GT activities 29-fold greater and ALP activities 5-fold greater than 2- and 7-month-old dogs [4]. Further study demonstrated increased ALP (30-fold) and γ-GT (100-fold) in 1- to 3-day-old pups relative to normal adult dogs [96]. Significant differences between γ-GT and ALP activities developed between colostrum-deprived and suckling pups within 24 hours. At 10 and 30 days after birth, serum γ-GT and ALP activities were less than values before suckling in all pups. Colostrum had substantially higher γ-GT and ALP activities compared with bitches' serum (γ-GT was 100-fold greater and ALP was 10-fold greater in colostrum and milk than in serum

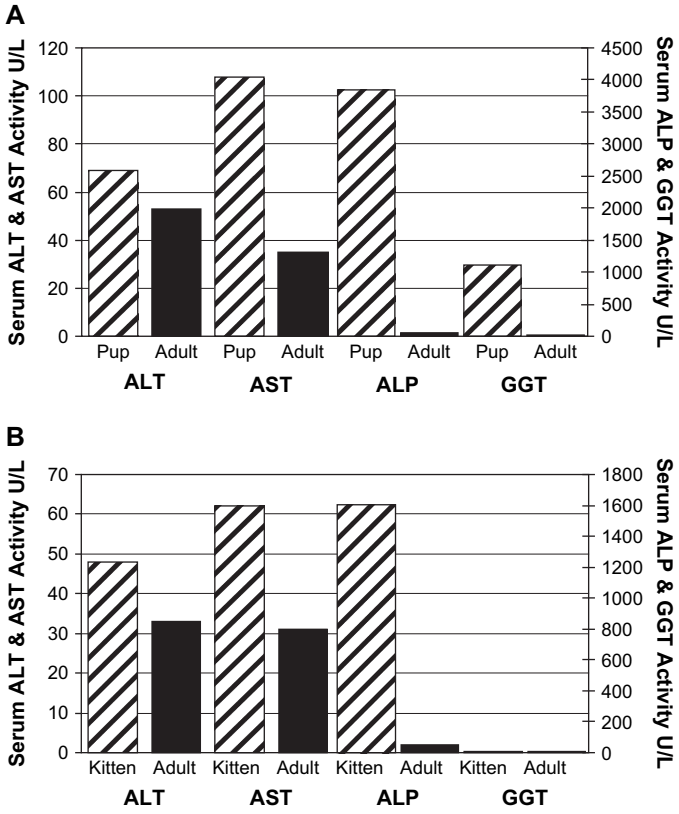

Fig. 21. The serum transaminase, ALP, and γ-GT (GTT) activity in 1- to 3-day-old puppies and kittens compared with healthy adults. (*Data from* references 96–98.)

through day 10). By day 30, γ-GT and ALP activity in milk was less than before suckling had started. Although a marked influence of colostrum on serum ALP activity in neonatal kittens also occurs, the effect on γ-GT is modest (see Fig. 22) [97,98]. The analysis of milk and colostrum (Fig. 23) from bitches and queens demonstrates the remarkable concentrations of enzymes imbibed and resorbed by the 1-day-old neonate.

HEPATOCELLULAR CARCINOMA AND SERUM ENZYME ACTIVITY

Dogs with HCCA or hepatoma commonly display increased serum liver enzyme activity. In those with HCCA, serum ALP or ALT is most often increased. More than one liver enzyme is elevated in 90% of such dogs (Fig. 24); the median magnitude of serum enzyme abnormalities in dogs with massive HCCA is shown in Fig. 25 [106]. In dogs undergoing successful surgical resection, liver enzymes normalize within 2 to 3 weeks. Markedly

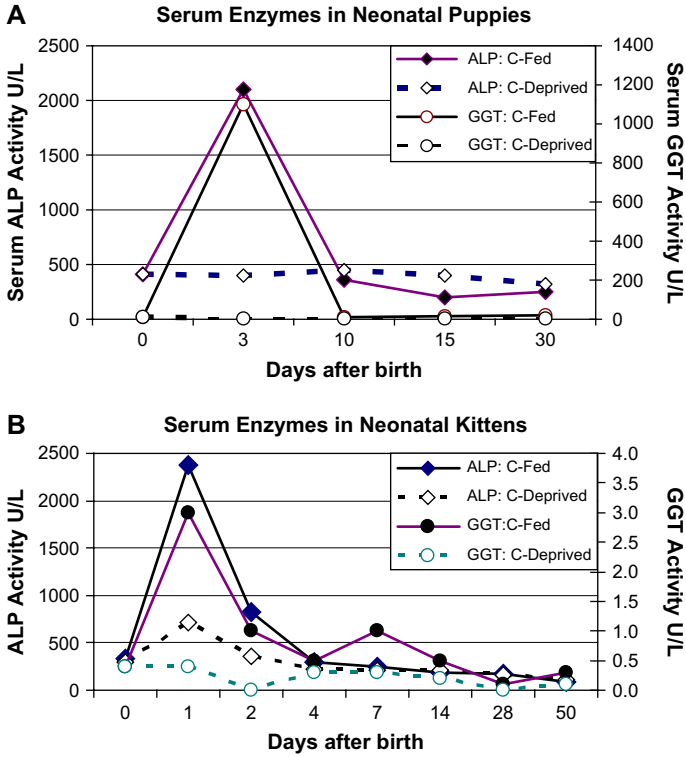

Fig. 22. The serum transaminase, ALP, and γ-GT (GTT) activity in 1- to 3-day-old puppies and kittens compared with healthy adults. The sharp increase in ALP and GTT in dogs and ALP in cats is derived from colostrum. (*Data from* Refs. [96–98].)

increased ALT and AST activity indicates a poor prognosis, because high transaminase activity reflects aggressive tumor behavior, fast growth rate, and large tumor size. High serum bile acid concentrations indicate loss of functional hepatic mass, release of cytokines from neoplastic cells causing paraneoplastic cholestasis, or invasion or compression of the porta hepatis compromising circulation or bile flow. The full spectrum of biochemical abnormalities commonly associated with hepatic dysfunction or liver disease (eg, hypoalbuminemia, hypercholesterolemia, coagulopathy) is infrequently encountered in dogs with massive HCCA. These tumors may be nodular (approximately 29%) or diffuse (approximately 10%) and more commonly involve the left liver lobes. Although large-volume surgical debulking is difficult, a median survival time of 4 years has been reported [106]. Dogs not undergoing surgical tumor excision had a median survival time of 270 days [106]. Prognostic indicators for poor surgical outcome include high ALT and AST activity and right-sided tumor invasion (right-sided involvement is technically more difficult to extirpate).

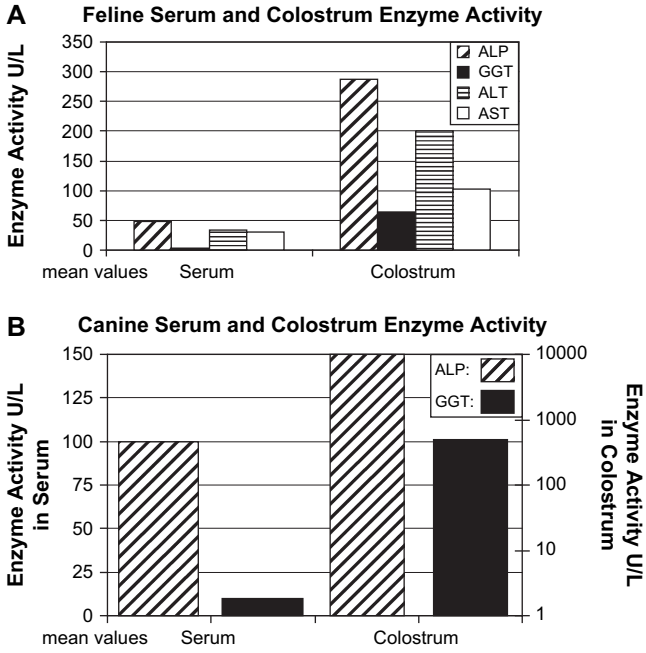

Fig. 23. The relative concentration of transaminases, ALP, and γ-GT (GTT) activity in the serum and colostrum from lactating queens and bitches. (*Data from* Center SA, Randolph JR, Man-Warren T, et al. Effect of colostrums ingestion on gamma-glutamyltransferase and alkaline phosphatase activities in neonatal pups. Am J Vet Res 1991;52:499–504; and Crawford PC, Levy JK, Werner LL. Evaluation of surrogate markers for passive transfer of immunity in kittens. J Am Vet Med Assoc. 2006;228:1038–41.)

SUMMARY

Abnormalities in liver enzymes are commonly encountered in clinical practice. Knowledgeable assessment requires a full understanding of their pathophysiology and provides an important means of detecting the earliest stage of many serious hepatobiliary disorders. The best interpretations are achieved using an integrated approach, combining historical and physical findings with routine and specialized diagnostic procedures and imaging studies. In some cases, liver enzyme abnormalities initiate early assessment of liver function using serum or urine bile acid determinations that help to prioritize the need for liver biopsy and definitive disease characterization. Several unique syndromes have been described in which liver enzymes direct diagnostic and therapeutic decisions. These include the ALP/γ-GT ratio in the feline hepatic lipidosis syndrome, the induction of the glucocorticoid-ALP isoenzyme with steroidogenic hormones, the development of the canine vacuolar hepatopathy syndrome, the apparent paraneoplastic association between ALP and some mammary neoplasia, the prognostic value of transaminases in dogs with massive HCCA, the origin

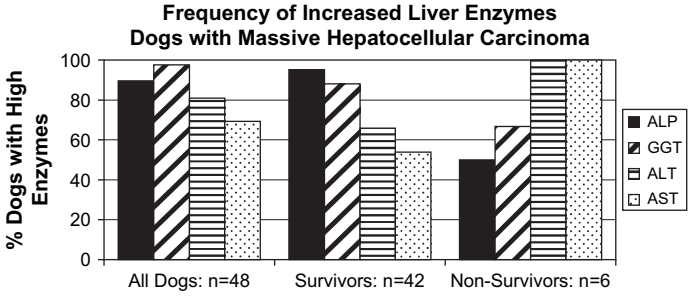

Fig. 24. Frequency of increased serum enzyme activities in dogs with large-volume or ''massive'' HCCA. GGT, γ-glutamyltransferase. (*Data from* Liptak JM, Dernell WS, Monnet E, et al. Massive hepatocellular carcinoma in dogs: 48 cases (1992-2002). J Am Vet Med Assoc 2004;225:1225–30.)

of increased serum ALP in hyperthyroid cats, the inhibition of transaminase synthesis by certain toxins (eg, aflatoxin, microcystin) blunting evidence of lethal hepatocellular injury, and the influence of colostrum ingestion on serum enzyme activity in neonatal puppies and kittens. Information in this article provides the foundation, by example, for understanding the reliability of single time point enzyme measurements, the value of sequential measurements, the importance of interpreting the activity of enzymes in light of their $t_{1/2}$ and tissue of origin, and the influence of the induction phenomenon. Understanding the contribution of the proliferative reparative process that follows severe liver injury explains why a protracted increase in cholestatic enzyme activity is observed during the recovery process.

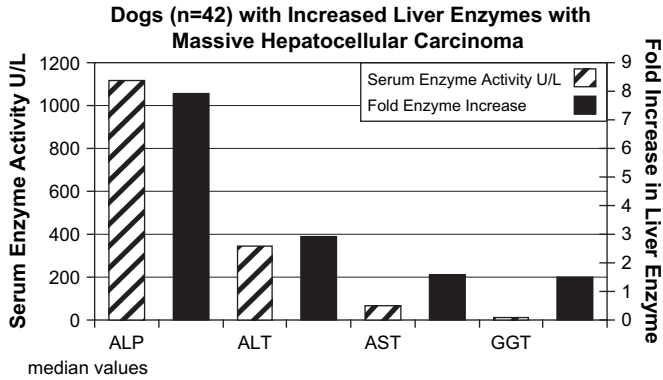

Fig. 25. Magnitude of increased serum enzyme activities in dogs with large-volume or ''massive'' HCCA. (*Data from* Liptak JM, Dernell WS, Monnet E, et al. Massive hepatocellular carcinoma in dogs: 48 cases (1992-2002). J Am Vet Med Assoc 2004;225:1225-30.)

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