Acta vet. scand. 1986, 27, 59—70.

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SERUM ENZYME DETERMINATION IN THE STUDY OF LIVER DISEASE IN DOGS

By

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ABDELKADER, SIGNE VIDEM and JENS GABRIEL HAUGE: *Serum enzyme determination in the study of liver disease in dogs.* Acta vet. scand. 1986, 27, 59—70. — Glutamate dehydrogenase (GLDH), sorbitol dehydrogenase (SDH), 5'-nucleotidase (5'-ND) and cholin esterase (CHE) were determined in the sera of 37 dogs with various liver diseases. The values for these parameters were compared with the values for aspartate and alanin aminotransferase (ASAT, ALAT), alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT) and albumin (ALB). GLDH was found to be the most sensitive enzyme parameter (sensitivity 92 %), its values also reflecting the degree of liver cell necrosis well. Next in sensitivity followed ASAT and ALP. SDH values were correlated to ALAT values but had a low sensitivity (43 %). 5'-ND discriminated markedly better between biliary tract disturbance and other lesions than ALP and somewhat better than γ-GT. CHE had a wide reference range, low sensitivity, and was not correlated to ALB. Single determinations of CHE may thus not be of diagnostic value in dogs.

liver function; canine; diagnostic test; histopathology.

Screening for liver disease in the dog is often done by measuring the serum activity of alanine aminotransferase and alkaline phosphatase. Some also include aspartate aminotransferase in the serum enzyme profile (*Hoe & O'Shea* 1965, *Palm et al.* 1982, *McConnel & Lumsden* 1983). In a recent contribution we have shown that total serum bile acids is an even more sensitive liver function parameter than these, in addition to being liver specific (*Hauge & Abdelkader* 1984). The bile acids test does not distinguish between different types of liver disorders, however. Increased aminotransferase is known to reflect general hepatocyte damage (*Cornelius et al.* 1959), while increased alkaline phosphatase is more indicative of biliary stasis (*Schall* 1976, *Noonan*

& Meyer 1979, *Cornelius* 1979). Since aspartate aminotransferase and alkaline phosphatase are not liver specific and alanine aminotransferase lacks the desirable sensitivity, it was of interest to study some alternative enzymes for their possible clinical usefulness. We chose to examine glutamate dehydrogenase and sorbitol dehydrogenase as possible general hepatocyte injury tests (*Zinkl et al.* 1971, *Keller* 1981), γ-glutamyl transferase and 5′-nucleotidase as cholestasis indicators (*de Broe et al.* 1975, *Shull & Hornbuckle* 1979) and cholinesterase as a possible indicator of impaired liver protein synthesis (*Vorhaus et al.* 1953, *Keller* 1981).

MATERIALS AND METHODS

Clinical material

The clinical material comprised 35 dogs admitted to the Department of Small Animal Medicine and 2 dogs admitted to the Department of Obstetrics over a period of 15 months. Their are range was 0.5—11 years. All the patients had liver disease as the sole or partial diagnosis. Of the total of 37 patients, 29 underwent necropsy.

In accordance with the final diagnosis based on the clinical and pathological case reports, the dogs were arranged into 6 groups relevant to liver disease. The patient groups and the clinical parameters included in this study are listed in Table 1.

Methods

The clinical chemical tests were carried out in a centrifugal analyzer (Gemsaec Fast Anolyzer, Electronucleonics, Inc.), using control sera or pooled sera to monitor the day-to-day repeatability. Alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), and γ-glutamyltransferase (γ-GT) were determined using commercial kits, which followed the recommendations of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. 5′-nucleotidase (5′-ND), glutamate dehydrogenase (GLDH), sorbitol dehydragenase (SDH) and cholinesterase (CHE) were determined using commercial kits prepared for the Gemsaec analyzer by the manufacturer or modified for this analyzer by us.

The 5′-ND assay measures the ammonia produced with added

adenosine deaminase in a reaction with glutamate dehydrogenase (*Arkesteijn* 1976). 5 min were allowed before the first absorbance reading, in order to establish steady state in the coupled enzyme assay and complete the NADH consumption due to other enzymes and endogenous substrates. For GLDH determination, 2 min, and for SDH, 6 min of reaction took place before the first reading. The CHE assay was based on the absorbance change at 420 nm of the indicator meta-nitrophenol when acetylcholine was hydrolyzed (*Rappaport* 1959). All enzymes were determined at 37°. Serum albumin (ALB) was determined by the bromcresol green method (*Doumas et al.* 1971).

ALAT, ASAT, ALP and ALB were determined on the day of blood sampling, the other parameters on sera which were stored up to 5 months at —80°C (mean storage time 1.5 months). The stability at this temperature of the most labile of the enzymes, SDH, was investigated by redetermination of a group of sera after 2—4 months. The decay rate varied in this group between 1 and 13 % per month, with a mean of 5 %. All SDH values were recalculated assuming 5 % decay per month.

Reference ranges and diagnostic sensitivities

Twenty-five healthy dogs brought to the outpatient service of the Department of Obstetrics, usually for vaccination, were used as reference group. The group consisted of 15 males and 10 females, aged 3 months—10 years. Their mean age was 4.1 years, as compared to 6.9 years in the clinical material. The reference ranges listed in Table 1 are the arithmetic mean value ± 2 s, except for ALP and CHE, where the logarithm of the values were used in the calculations.

Diagnostic sensitivities for the enzymes are given as the percentage of cases with values above the upper reference limit. For albumin and cholinesterase diagnostic sensitivity is given by the percentage of cases below the lower reference limit.

Histology

For the 29 dogs where necropsy was performed relatively shortly after blood sampling, histological liver sections from each patient were examined by light microscopy. The presence of necrosis, fatty change, fibrosis, cholestasis and congestion was recorded. An evaluation of the grade of necrosis was also made.

RESULTS

Enzyme observations on groups and total material

Table 1 shows the observed range, mean value, reference limits and diagnostic sensitivity for the various tests. In the total material GLDH is seen to be the most sensitive parameter, followed by ASAT, ALP and ALAT. Determination of GLDH detect-

Table 1. Serum enzymes and albumin for patients with liver affections.[1]

	Liver neoplasms (n = 13)	Chronic hepatitis (n = 3)	Cirrhosis (n = 5)	Fatty liver (n = 5)	Heart failure (n = 4)	Other patients with liver disease (n = 7)	Reference range Overall sensitivity
ASAT	27—1534	49—149	38—415	26—80	30—545	56—249	17—42
(U/l)	238	84	134	49	177	123	
	85	100	80	40	75	100	81
ALAT	31—900	69—386	72—1246	21—235	31—630	29—1590	0—69
(U/l)	273	194	353	108	192	574	
	69	67	100	60	25	86	70
GLDH	11—250	12—80	12—180	5—27	2—36	9—89	0—9
(U/l)	61	35	49	16	20	37	
	100	100	100	80	75	86	92
SDH	4—28	6—10	4—9	9—20	5—16	1—41	1—9
(U/l)	11	7	8	12	10	15	
	54	33	0	40	50	57	43
ALP	31—8220	436—9998	124—4915	162—709	112—644	49—4980	39—222
(U/l)	2093	3839	1275	360	282	1500	
	92	100	80	40	50	71	76
5'-ND	4—63	5—22	4—46	5—16	4—15	4—18	1—11
(U/l)	17	13	14	9	8	10	
	46	67	20	20	25	29	35
γ-GT	0—79	5—50	2—28	3—31	3—12	2—57	0—11
(U/l)	29	29	11	13	8	12	
	69	67	40	40	25	14	46
CHE	1.9—12.8	3.5—8.4	2.4—6.0	2.0—4.4	2.6—4.7	2.5—9.3	2.0—5.0
(kU/l)	4.6	5.3	4.3	3.0	3.6	4.2	
	31(8)	33(0)	20(0)	0(0)	0(0)	14(0)	19(3)
ALB	19—33	17—22	23—32	27—35	28—44	18—35	25—36
(g/l)	26	19	27	31	33	27	
	0(23)	0(100)	0(40)	0(0)	25(0)	0(29)	3(27)

[1] For each parameter and group observed range, mean value and diagnostic sensitivity (percentage of cases above upper reference limit) are listed. For CHE and ALB the percentage of cases below lower reference limit is given in parenthesis. The last column lists the reference range and the overall diagnostic sensitivity.

ed all cases of liver neoplasm, chronic hepatitis and cirrhosis. SDH, 5′-ND, γ-GT and CHE had sensitivities in the range 19—46 %.

Table 2 shows some selected correlations. The two transaminases were well correlated in cirrhosis and heart failure, but not in the total material. Good correlation was found, however, for ALAT with GLDH and SDH in the total material as well as in some groups. For the cholestasis parameters the correlation was especially high between ALP and 5′-ND. Good correlation was also observed in the collected material between 5′-ND and GLDH.

Table 2. Correlation between some serum enzyme values.[1]

	Liver neoplasms (n = 13)	Cirrhosis (n = 5)	Fatty liver (n = 5)	Heart failure (n = 4)	Other patients with liver disease (n = 7)	All patients
ALAT-ASAT	0.02	0.99**	0.68	0.99*	0.49	0.20
ALAT-GLDH	0.92**	1.00***	0.64	0.78*	0.70	0.67***
ALAT-SDH	0.54*	0.40	0.63	0.77	0.64	0.55***
GLDH-ASAT	0.10	0.99***	—0.10	0.82	—0.19	0.25
ALP-5′-ND	0.92***	1.00***	0.78	0.96*	0.84*	0.77***
ALP-γGT	0.67*	0.37	0.98**	0.66	0.08	0.58***
γGT-5′ND	0.66*	0.33	0.71	0.65	0.03	0.56***
5′-ND-GLDH	0.46	1.00***	0.84	0.64	0.06	0.60***

[1] Pearson linear correlation coefficients are given. ***$P<0.001$; **$P<0.01$; *$P<0.05$; no symbol $P>0.05$

Histological observations and enzyme values

Hepatic cell necrosis was a very common feature in our material, being present in every case of liver neoplasm, cirrhosis and chronic hepatitis. Otherwise, the histopathological findings revealed considerable variation not only between groups, but also within the groups. In most liver sections necrosis, fatty change, fibrosis, biliary stasis and congestion did not appear singly, but in combination with one or more of the other lesions.

In Table 3 the relationship between enzymes and histopathological changes are listed. GLDH again reveals itself as the most sensitive parameter. ALAT and ASAT showed similar sensitivities, but somewhat lower than that of GLDH. In necrosis, fibrosis, cholestasis and congestion of the liver, SDH showed

Table 3. Relationship between serum enzymes and histopathological findings.[1]

	Necrosis (n = 26)	Fatty change (n = 8)	Fibrosis (n = 6)	Biliary (n = 6)	Congestion (n = 12)
ASAT	26—1534	26—80	38—415	26—415	26—293
	175	48	126	172	88
	88	50	88	83	75
ALAT	31—1590	21—235	69—1246	73—1246	52—900
	317	101	313	481	207
	77	50	88	100	75
GLDH	11—250	5—27	12—180	12—250	2—250
	49	16	54	95	37
	100	83	100	100	92
SDH	4—28	9—20	4—13	6—28	4—20
	11	12	7	12	10
	42	50	13	33	25
ALP	31—9998	162—950	124—8220	525—9998	118—9998
	1711	459	2015	3230	1501
	81	50	88	100	75
5′-ND	4—63	5—16	4—63	8—46	4—32
	15	9.3	19	23	12
	38	17	38	83	33
γGT	0—79	3—31	2—79	17—50	3—64
	23	12	22	28	19
	58	33	50	100	58
CHE	1.9—12.8	1.9—3.4	2.4—8.4	4—8.4	2—12.8
	4.3	2.8	5	5.3	4.4
	27	17	38	33	8

[1] For each parameter and type of change, observed range, mean and percentage of observations outside the reference limits (see Table 1) are given.

considerably lower sensitivity than ASAT, ALAT and GLDH. Only 42 % of the instances with hepatic cell necrosis gave abnormal SDH. In fatty change, however, SDH was as sensitive as ALAT and ASAT (50 %).

Both ALP and γ-GT detected every occurrence of intrahepatic bile stasis and showed the highest mean values with this lesion present. The ALP values, however, also rose above normal in more than 80 % of the instances with hepatic cell necrosis and fibrosis. 5′-ND showed a somewhat lower sensitivity than ALP and γ-GT for biliary stasis, having abnormal values in 83 % of the observations.

CHE had values above the normal range in about 1/3 of the dogs showing fibrosis or biliary stasis, the percentage being lower for the other types of pathological change. Only one patient, a dog with fatty change and necrosis of the liver associated with neoplastic infiltration, had a CHE level below the normal range. Its ALB level, however, was normal.

Hepatic necrosis was present in 26 of the 29 cases investigated. The extent of necrosis as found by subjective assessment differed, however, from case to case. In Table 4 the values of 5 enzymes are correlated with the grade of necrosis. GLDH and ALAT values are seen to reflect the grade of necrosis well. Their means for the 3 levels of necrosis were significantly different ($P<0.05$) and increased about 10 fold from mild to severe necrosis. Some of the ALAT values were within the normal range, while all GLDH values were abnormal. The means for ASAT and ALP increased about 5 fold from mild to severe necrosis, for SDH only 2 fold. The differences for ASAT and SDH were, however, not statistically significant.

Table 4. Serum enzyme values in relation to the grade of liver cell necrosis.[1]

	Mild (n = 12)	Moderate (n = 9)	Severe (n = 5)
ASAT	38—88	26—1534	149—415
	60[x]	217[x]	281[x]
	89	83	100
ALAT	58—178	31—540	386—1590
	97[x]	203[y]	986[z]
	67	75	100
GLDH	11—22	12—95	47—250
	15[x]	38[y]	140[z]
	100	100	100
SDH	5—15	4—20	6—28
	8[x]	10[x]	15[x]
	22	50	60
ALP	121—950	31—8220	1083—4915
	467[x]	2542[y]	2269[y]
	67	83	100

[1] For each parameter and grade of necrosis, range, mean and percentage of observations above the reference limits (see Table 1) are given. Means in the same row followed by different letters are significantly different ($P<0.05$).

DISCUSSION

Through experimental approaches it is possible to cause liver changes like bile stasis, hepatic lipidosis or necrosis to occur singly, allowing a study of the effect of each type of lesion on biochemical parameters. In spontaneous liver disease, however, several histological changes often exist simultaneously, making the relation between the individual lesions and the blood chemistry more difficult to interpret. This is especially so when a majority of the patients suffer from advanced liver disease, as was the case for our material. The mean age was somewhat higher in the clinical material than in the reference group. With these limitations in mind, and the limitation implicit in the relatively small number of animals studied, certain tentative conclusions can nevertheless be drawn regarding the diagnostic and prognostic usefulness of the various enzyme parameters.

A L A T. A relatively high overall diagnostic sensitivity was found for this parameter (70 %). This is not unexpected, since 26 of the 29 cases studied histologically had some degree of necrosis, ALAT having pathological values in 77 % of these. The highest proportion of abnormal ALAT values (100 %), was found when biliary stasis was present. This may merely reflect that cholestasis often develops simultaneously with degenerative and necrotisizing process of the hepatocytes. The magnitude of the ALAT values gave a good measure of the degree of necrotic change, making it possible to predict whether necrosis was mild or more advanced. A similar approach to diagnosis of mild or severe liver damage for various liver diseases, using ALAT, ALP or a combination of these, was employed by *Hoe & O'Shea* (1965).

A S A T. In the present study ASAT was found to be somewhat more sensitive as a detector of liver disease than ALAT. It showed abnormal values in 88 % of the dogs with necrosis. It should be remembered, however, that differences in sensitivity for any two parameters are influenced by the uncertainties implicit in the determination of reference limits. The lack of correlation between ASAT and ALAT in the total material, and particularly for the group with neoplasms, suggests that other tissues than hepatocytes often contribute to the ASAT measured. It could be the neoplastic cells themselves, as indicated by the study of *McConnel & Lumsden* (1983). Our largest ASAT value was recorded in a case where there, in addition to liver metastasis, was a massive invasion of neoplastic tissue in the dia-

phragm. In order to reveal muscle as the source of increased ASAT, one could include the muscle specific parameter creatinine kinase in the profile, as was done by *McConnel & Lumsden* (1983).

S D H. This parameter was included as a potential liver specific indicator of mild hepatocyte injury, the enzyme being confined to the cytosol. SDH was indeed correlated to ALAT in the total material. In contrast to observations after experimental liver injury (*Zinkl et al.* 1971, *Noonan & Meyer* 1979), the increases in SDH were relatively small, resulting in a low overall diagnostic sensitivity (43 %). The lability of the enzyme, even when stored frozen, is also a drawback.

G L D H. This parameter was also included because of its liver specificity (*Keller* 1981). It revealed itself to be the diagnostically most sensitive of all parameters tested, detecting 34 of the 37 cases (92 %). All instances necrosis, whether mild moderate or advanced, were accompanied by abnormal GLDH values, the magnitude of the values reflecting the degree of necrosis well. GLDH values were correlated with ALAT values but not ASAT values, confirming the usefulness of GLDH as a hepatocyte damage parameter. It was unexpected that GLDH, being a mitochondrial enzyme (*Keller* 1981), should be so sensitive an indicator of hepatic injury. The fact that necrosis was observed in 26 of the 29 cases studied histologically largely explains this. Its narrow normal range contributes to its sensitivity. GLDH appears to be the parameter of choice in detecting liver cell necrosis in the dog, as found earlier for ruminants (*Boyd* 1962).

A L P. ALP is used primarily as an indicator of biliary stasis (*Schall* 1976). In line with this we found the highest values of ALP for dogs having this disturbance. All cases had abnormal ALP values. A large fraction (75 %) of the dogs having liver necrosis without recognizable biliary stasis, however, also had pathological ALP values. Many of the latter dogs had liver neoplasms, whereof two suffering from bile duct carcinoma showed extremely high plasma ALP values. It is indeed an experience that space occupying lesions of the liver, such as metastasis, result in increased ALP (*Cornelius* 1979). Liver tissue in itself contains very little ALP (*Keller* 1981). Elevated plasma ALP-levels reflect an increase in synthesis and release of the enzyme (*Strombeck* 1978, *Cornelius* 1979). When liver cell necrosis is accompanied by an elevation of plasma ALP, it could be the

result of simultaneous regenerative process. Hypercortisonism has also been associated with abnormal ALP in earlier studies (*Rogers & Ruebner* 1977) as well as in the present one. ALP values in the present study did not distinguish between moderate and severe necrosis.

γ-GT, 5′-ND. These parameters were included as more liver specific, and possibly more biliary stasis specific substitutes or supplements for ALP (*de Broe et al.* 1975, *Shull & Hornbuckle* 1979). Table 2 shows that they in our material indeed were well correlated with ALP and with each other. They resemble ALP in having their highest means and highest sensitivities for biliary stasis, while also sowing increased values for neoplasms in the absence of biliary stasis. The highest value for both of these parameters and the second highest for ALP was observed in a dog having bile duct carcinoma. When cases of biliary stasis and biliary carcinoma were seen together (8 dogs), the diagnostic sensitivity for γ-GT, 5′-ND and ALP was 100 %, 88 % and 100 %, respectively, while the sensitivities of these parameters for the remaining 21 dogs were 38 %, 19 % and 67 %. 5′-ND thus appears to be the enzyme parameter of choice in discriminating between biliary disturbances and other liver lesions.

The relatively high correlation between 5′-ND and GLDH in the pooled material is at first glance surprising. The main contributions to this are, however, the cases of cirrhosis, liver neoplasms and fatty change, pathological changes which may effect both hepatocytes and biliary epithelium.

CHE. In human medicine this parameter is reported to be a valuable supplement to albumin as an indicator of liver protein synthesis ability, its advantage being a more rapid response to changes in liver function (*Vorhaus & Kark* 1953). The normal variation is high, however, limiting its use to longitudinal studies, each patient being his own control. A large normal range was also found for our canine material, contributing to the very low diagnostic sensitivity observed for CHE. There was, furthermore, no correlation with ALB values. Abnormally high CHE values are known to occur in the human when protein loosing conditions (nephrosis, intestinal disorders) induce liver protein synthesis. Increased CHE levels were found in 19 % of our material, without any apparent connection to such protein loosing conditions. Thus the present data do not indicate that CHE in single tests has any diagnostic value in dogs.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the scientific and technical staff of the Department of Pathology for providing the desired histological sections and assisting in the interpretation of these.

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SAMMENDRAG

Serumenzymbestemmelser ved studium av leversykdom hos hunder.

Glutamatdehydrogenase (GLDH), sorbitoldehydrogenase (SDH), 5'-nukleotidase (5'-ND) og cholinesterase (CHE) ble bestemt i sera fra 37 hunder med forskjellige leversykdommer. Verdiene for disse parametrene ble sammenliknet med verdiene for aspartat- og alanin-aminotransferase (ASAT, ALAT) alkalisk fosfatase (ALP), γ-glutamyltransferase (γ-GT) og albumin (ALB). GLDH ble funnet å være den mest sensitive enzymparameter (sensitivitet 92 %) og GLDH-verdiene var samtidig et godt mål for graden av levercellenekrose. I sensitivitet fulgte dernest ASAT og ALP. SDH-verdiene var korrelert til ALAT-verdiene, men hadde lav sensitivitet (43 %). 5'-ND skilte vesentlig bedre mellom hepatobiliære og hepatocellulære skader enn ALP, og noe bedre enn γ-GT. CHE hadde et bredt referanseområde (normalområde), lav sensitivitet og var ikke korrelert til ALB. Enkeltbestemmelser av CHE er derfor trolig uten diagnostisk betydning for hund.

(Received November 11, 1985).

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