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From the Department of Biochemistry, Norwegian College of Veterinary Medicine, Oslo, Norway.

SERUM BILE ACIDS AS AN INDICATOR OF LIVER DISEASE IN DOGS

By

Jens Gabriel Hauge and *Signe Videm Abdelkader*

HAUGE, JENS GABRIEL and SIGNE VIDEM ABDELKADER: *Serum bile acids as an indicator of liver disease in dogs.* Acta vet. scand. 1984, *25*, 495—503. — Total serum bile acids were determined in 62 dogs with different primary or secondary liver diseases, using 3α-hydroxysteroid dehydrogenase coupled to nitrobluetetrazolium in a centrifugal analyzer. A reaction time of 4 min was sufficient, yielding a within run coefficient of variation of 7 % at 6 μmol/l and 3 % at 27 μmol/l. A reference range of 0—4.4 μmol/l 2 h post prandially was observed. The sensitivity of bile acids as a liver function test was superior to that of alanine and aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase and combinations of two of these. The bile acids test detected 36 of 39 patients with a morphological or clinical liver diagnosis. For dogs with heart failure the bile acids test was a markedly more sensitive indicator of secondary liver involvement than alanine aminotransferase or alkaline phosphatase. For secondary liver affections associated with pyometra or epilepsy medication the opposite was the case. Bile acid values in the pooled patient material was not correlated to any of the 4 enzymes measured. For cirrhosis there was positive correlation, however, with the amino transferase values.

diagnostic test; sensitivity; serum enzymes; liver function; canine.

Serum bile acids determination is, in human medicine, perhaps the most sensitive single liver function test (*Annoni et al.* 1981, *Skrede et al.* 1978). The physiological basis for the test is the normally very rapid removal of bile acids from the blood as they pass the liver. Reduced ability of the hepatocyte to transport bile acids out of the blood and into the bile, as well as impaired blood circulation in the liver will increase the serum bile acid concentration. *Answer et al.* (1976) showed that serum bile acid elevation follows CCl_4 induced liver damage in dogs, sheep, calves and ponies. A sensitive enzymatic test is now available which measures bile acids directly in the serum without an extraction step (*Mashige et al.* 1981). We have adapted this test for auto-

matic analysis and we have, in order to determine the usefulness of this test in canine medicine, measured bile acids in dogs with a range of clinical conditions.

MATERIALS AND METHODS

Clinical material

The main clinical material consisted of 39 patients with a liver affection, 37 of which were admitted to the Department of Small Animal Medicine and 2 patients admitted to the Department of Obstetrics, all during a period of 14 months in 1982—83. Twenty-eight of the cases were classified histologically upon necropsy into 4 groups (Tables 1 and 2). The remainder had liver disease as the sole or partial clinical diagnosis. In addition, 23 patients from the Department of Small Animal Medicine and the Department of Obstetrics, presumed by us to have a secondary liver affection on the basis of their primary diagnosis and their liver test values, were studied.

Methods

The tests listed in Tables 1 and 2 were carried out using a centrifugal analyzer (Gemsaec Fast Analyzer from Electronucleonics, Inc.). A control serum for monitoring the day to day repeatability was included in each run. Alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP) and γ-glutamyl transferase (γGT) were determined at 37°C according to methods recommended by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology.

Determination of bile acids (BA)

The method utilizes a bacterial 3α-hydroxysteroid dehydrogenase (3 α-HSD; EC 1.1.1.50), where reduction of NAD^+ is coupled to reduction of nitrobluetetrazolium with diaphorase* (EC. 1.6.4.3). In adapting the method for the centrifugal analyzer, the stop reagent was omitted. The Enzabile 25 μmol/l bile acid standard was used throughout. It consists of a 1:1:1 mixture of glycocholate, glycodeoxycholate and taurochenodeoxycholate in a bovine serum albumin solution. The values for a blank run

* Reagents (the kit "Enzabile") were obtained from Nyegaard & Co. A/S, Oslo.

without 3α-HSD were subtracted by the analyzer. With the accurate temperature and time control of the centrifugal analyzer, the reaction time could be reduced from 15 to 4 min without affecting the precision or the linearity of the assay. Fig. 1 shows the kinetics of the reaction. The within-run coefficient of variation was 7 % at 6 μmol/l and 3 % at 27 μmol/l. The day-to-day coefficient of variation during 1 month periods with a control serum at 24 μmol/l was 5 %. Samples were diluted if values above 150 μmol/l were obtained.

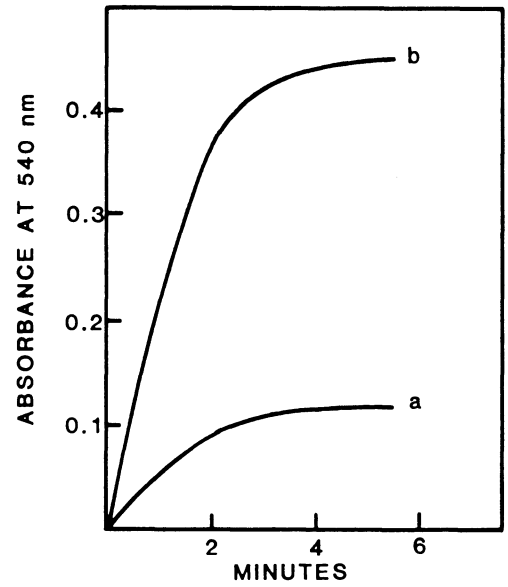

Figure 1. Kinetics of formazan production. The reaction was run in a recording spectrophotometer under the same conditions as in the centrifugal analyzer. The analyzer reaction mixture contained 100 μl standard, 50μl water (sample flush) and 300 μl reagent or blank reagent. Curve a, 25 μmol/l, curve b, 100 μmol/l in the standard.

Reference ranges

Twenty-five healthy dogs brought to the outpatient service of the Department of Obstetrics, usually for vaccination, were used as reference group. For bile acids the reference range observed was 0—2.1 μmol/l. Bile acid determination in 50 dogs admitted to the Department of Small Animal Medicine with problems other than liver problems yielded a reference range 0—4.4. These blood samples were taken approximately 2 h after the

morning feeding. We have observed that the BA concentration in serum rises 3—4 fold during these 2 h. It is possible that many of the dogs brought in for vaccination had not received a morning meal, in contrast to the dogs in the patient groups. We have therefore chosen to use 0—4.4 as the relevant reference range for bile acids.

RESULTS

Table 1 shows that BA values were above normal in all patient groups. The values are, however, scattered over a wide range. This results in a considerable overlap in values between the groups. The highest value recorded, 747 μmol/l, was observed in a case of lymphosarcomatosis. Values above 200 μmol/l have also been found for cirrhosis, and for liver changes secondary to heart failure. For comparison, Table 1 also shows the values for 4 enzymes used widely to measure hepatocyte injury or cholestasis. For fatty liver, enzyme values were often in the normal range, while serum bile acids still revealed the liver affection. The mean value was 17 times the upper reference limit.

Changes in bile acids over time for 2 liver patients are shown in Fig. 2. Fig. 2a shows BA observations, as well as ALAT and

Table 1. Bile acids and some other blood parameters in patients with primary or secondary liver affections[a].

Group	No. of patients	BA (μmol/l)	ALAT	ASAT	ALP	γGT
			U/l			
Liver neoplasms	10	110 (2—747)	244 (31—900)	247 (36—1534)	2390 (31—8220)	28 (4—79)
Chronic hepatitis	3	79 (46—109)	194 (69—386)	85 (56—149)	3839 (436—9998)	29 (5—50)
Cirrhosis	6	75 (2—210)	284 (17—1246)	120 (38—415)	1363 (124—4915)	11 (2—28)
Fatty liver	6	76 (14—169)	98 (21—235)	43 (10—80)	334 (162—525)	12 (3—31)
Other patients with liver disease	14	81 (0—236)	515 (17—1765)	140 (32—293)	1242 (49—4980)	38 (0—290)
Normal animals (reference range)[b]		0—4.4	0—69	17—42	0—207	0—11

[a] The values are arithmetic means (range in parenthesis).
[b] Mean value ± 2 s.

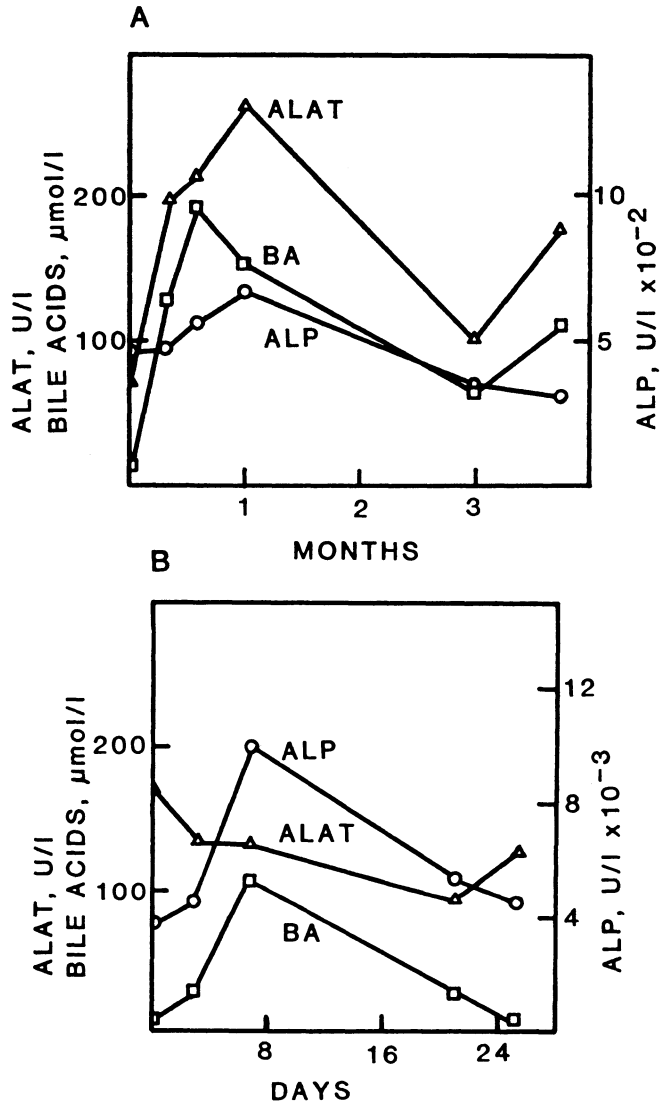

Figure 2. Changes in BA, ALAT and ALP in 2 patients A) Liver cirrhosis in a dog (4 months). B) Chronic hepatitis in a dog (11 years).

ALP values, during 4 months for a young dog with liver cirrhosis. BA is seen to vary in parallel with ALAT. Fig. 2b represents a case of chronic hepatitis in an 11 year old dog. The ALP values are here much higher and BA changes in parallel with ALP. The first blood sample in this patient is a case where the value was

in the normal range while the other liver parameters were pathological.

Statistical values for the pooled patient material did not show significant positive correlation between bile acids and any of the 4 enzymes. For cirrhosis there was, however, positive correlation between bile acids and ALAT as well as ASAT ($r = 0.89$, and 0.87, respectively, $P < 0.05$).

Table 2 shows a comparison of the sensitivity of the bile acid test to that of other liver function tests, given as the per cent of results above the upper reference limit. Bile acids scores as the most sensitive single parameter in the total material, 36 of 39 (92 %) patients having pathological values. When combined with ALP, all cases were detected. Combination with ALAT or ASAT detected 97 %, while the best combination of enzyme tests alone, ALAT + ASAT + ALP detected 95 %.

Table 2. Sensitivity of the serum bile acid test as compared with some other tests of liver function[a].

	No. of patients	Bile acids	ALAT	ASAT	ALP	γGT
Liver neoplasms	10	90	70	70	90	67
Chronic hepatitis	3	100	67	100	100	67
Cirrhosis	6	83	83	83	83	33
Fatty liver	6	100	50	33	33	33
Other patients with liver disease	14	93	86	93	86	54
Total material	39	92	74	77	79	51

[a] The numbers are percentages of results above the upper limit for normal animals, given in Table 1.

In addition to the material discussed above, 3 other types of secondary liver affections have been studied, namely dogs with heart failure, epilepsy or pyometra (Table 3). BA is seen to be the most sensitive parameter of the 3 listed for detecting liver changes secondary to heart failure. This may reflect a lowered liver circulation directly and/or lowered oxygen tension and metabolism in the hepatocytes.

Dogs with epilepsy were treated with phenytoin or primidone. This usually resulted in pathological ALP values, however. For

Table 3. Mean values and sensitivity of serum bile acids, ALAT and ALP in some secondary liver affections.

		Bile acids		ALAT		ALP	
	No. of patients	sensitivity[a]	mean[b] (μmol/l)	sensitivity	mean (U/l)	sensitivity	mean (U/l)
Heart failure	4	100	73 (15—225)	25	192 (31—630)	50	181 (121—644)
Epilepsy	13	38	8 (0—36)	54	120 (37—390)	85	866 (167—3133)
Pyometra	6	16	10 (1—49)	17	35 (15—88)	100	514 (327—762)

[a] Sensitivities are given as in Table 2.
[b] The range is given in paranthesis.

the even milder liver disturbance seen in pyometra dogs, BA was increased in only 16 %, ALP again being the most sensitive test.

DISCUSSION

The level of serum bile acids is an interesting parameter for liver disease because it, in contrast to ALAT, ASAT and ALP, is specific for the liver. In addition, our study demonstrates that the serum bile acid concentration is a very sensitive parameter for the detection of liver disease in dogs, more sensitive than other more used tests or combinations of these.

Introduction of the 3α-HSG-diaphorase-tetrazolium assay (*Mashige* 1981) has increased the precision and sensitivity of the assay. The feasability of adapting this method for automatic analysis, as demonstrated in the present work, makes it practicable to include BA determination in the routine screening for liver disease. Because of the high sensitivity of the test, secondary liver involvement as in diabetes (fatty liver) or heart failure often was revealed only by the BA test. In the majority of liver patients, however, increases in BA were accompanied by increases in the transaminases, ALP, and often γGT. Occasionally, BA was found normal in dogs with diagnosis of liver disease. The 3 examples of this in our material was a two month old dog with liver cirrhosis, an 11 year old dog with liver metastases from stomach cancer and an 8 year old dog with a liver hematoma and some liver cell necrosis. In these patients the structural

changes in the liver apparently were not sufficient to affect the bile acid absorption/secretion processes.

A situation with normal BA values together with pathological enzyme values may also arise during a recovery phase, because clearance of enzymes from the blood is slower than that of the bile acids. We have seen this for a dog recovering from cortisone-induced hepatosis when cortisone treatment was stopped.

While values for bile acids and liver enzymes in serum usually increase together, the relative increases were often different, resulting in lack of significant positive correlation between bile acids and the other parameters in the pooled material. Low correlation with other liver parameters is observed also in the human (*Skrede et al.* 1978). An exception in the present material was the group of 6 dogs with cirrhosis, which showed high correlation between BA and ALAT. Such correlation was also observed over time in a cirrhosis dog (Fig. 2a).

Two kinds of secondary liver involvement showed lower sensitivity for BA than for ALP, namely dogs treated for epilepsy and dogs with pyometra. Use of anticonvulsant drugs leads to induction and leakage of some enzymes from the liver (*Meyer & Noonan* 1981). It is possible, however, that the source of some or all of the increased ALP seen is the skeletal system. In man anticonvulsant drugs are known to cause oesteomalacia, possibly through induction of vitamin D inactivating enzymes (*Hoikka et al.* 1981). ALP isoenzyme studies in dogs would be required to settle this question.

Pyometra in dogs is accompanied by a mild intrahepatic cholestasis (*Börresen & Skrede* 1980). These authors found that the mean ALP value was about doubled, while the small increase in BA was not significant. The present results are in agreement with these findings. Whereas mild intrahepatic cholestasis in pyometra dogs fails to disturb the ability of the liver to handle bile acids, we have observed marked BA increases for intrahepatic cholestasis in other patients.

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SAMMENDRAG

Gallesyrer i serum som indikator for leversykdom hos hund.

Totale gallesyrer i serum ble bestemt for 62 hunder med forskjellige primære eller sekundære leversykdommer ved bruk av 3α-hydroxysteroiddehydrogenase koblet til nitrobluetetrazolium i en centrifugalanalysator. En reaksjonstid på 4 min var tilstrekkelig og ga en variasjonskoeffisient innen en kjøring på 7 % ved 6 μmol/l, 3 % ved 27 μmol/l. Referanseområdet 2 timer postprandielt var 0—4.4 μmol/l. Gallesyremålingenes sensitivitet som leverfunksjonsparameter var høyere enn sensitiviteten for alanin- og aspartataminotransferase, alkalisk fosfatase, γ-glutamyltransferase eller kombinasjoner av 2 av disse. Gallesyretesten detekterte 36 av 39 pasienter med en morfologisk eller klinisk leverdiagnose. For hunder med hjertesvikt var gallesyretesten en betydelig mer sensitiv indikator for sekundær leverpåkjenning enn alaninaminotransferase eller alkalisk fosfatase. For sekundære levereffekter assosiert med pyometra eller medikamentell epilepsibehandling var det motsatte tilfelle. Gallesyreverdier i det samlede materiale var ikke signifikant korrelert til noen av de 4 målte enzymene. For cirrhose var det imidlertid positiv korrelasjon til aminotransferaseverdiene.

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Reprints may be requested from: J. G. Hauge, the Department of Biochemistry, Norwegian College of Veterinary Medicine, P. O. Box 8146, Dep., 0033 Oslo 1, Norway.