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The effects of different anticoagulants on routine canine plasma biochemistry

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Abstract

The effects of heparin, ethylenediaminetetraacetic acid (EDTA), sodium citrate and sodium fluoride/potassium oxalate on plasma biochemistry results in dogs were studied and compared with serum. Blood specimens from 10 apparently clinical healthy dogs were collected and placed in different tubes containing each anticoagulant tested. Differences in albumin, acetylcholinesterase, ionized calcium and potassium were found between serum and heparinized plasma. Most metabolites and enzymes did not show any variation, but significant decreases in electrolytes, alkaline phosphatase, acetylcholinesterase, bile acids, fructosamine and albumin were found when EDTA was used. Sodium citrate produced a 10–15% decrease in most metabolites and enzymes, possibly due to a sample dilution effect. Sodium fluoride/potassium oxalate produced haemolysis which may have influenced changes in some biochemical parameters.

Keywords: Heparin; EDTA; Sodium citrate; Sodium fluoride; Dog

1. Introduction

In recent years there has been an increasing effort by international committees and working groups to develop quality standards for the pre-analytical phase including the proper use of anticoagulants in sample collection (Guder et al., 1998; WHO, 1999). Anticoagulants are additives that inhibit the clotting of blood and/or plasma, thereby ensuring that the concentration of the substance to be measured is changed as little as possible before the analytical process (Guder, 2001). A number of different anticoagulants are used in routine medical and veterinary practice depending on the parameters to be quantified. These include: ethylenediaminetetraacetic acid (EDTA), which is recommended for routine haematology; heparin for clinical biochemistry; sodium citrate for coagulation studies and sodium fluoride for blood glucose analysis (Burtis and Ashwood, 2000). Anticoagulation is achieved either by the binding of calcium ions (EDTA, citrate and fluoride) or by inhibition of thrombin (heparin).

Usually, only serum or plasma with heparin is used for routine biochemistry since heparin causes very little interference with tests (Burtis and Ashwood, 2000; Thorensen et al., 1992). However, specimens mixed with other anticoagulants such as EDTA, sodium fluoride or sodium citrate are sometimes submitted to veterinary clinical pathology laboratories for biochemical analysis. Although ideally analysis of these samples should be refused, there may be times when the collection of a further specimen is not feasible (Pay and Cyr-Manthey, 1991).

In contrast with the increasing interest in the study and proper use of anticoagulants in human clinical biochemistry, in dogs there are few data on the effects of anticoagulants other than heparin on routine biochemical tests, and such as there have been focused on selected parameters. For example, Myers and Pierce (1972) studied the influence of EDTA on alkaline phosphatase (AP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and Aktas et al. (1994), in a

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detailed study on the causes of creatine kinase (CK) variation, analyzed the influence of EDTA, sodium citrate and fluoride on this enzyme.

The purpose of the present study was to determine and compare how the main anticoagulants, which are commercially available for use in veterinary clinical pathology laboratories, may affect the results of routine biochemistry in canine plasma specimens.

2. Material and methods

2.1. Animals and sampling procedure

Ten healthy dogs of different breeds (five German Braco and five Beagles), four males and six females, aged 1–3 years, located at the Animal Resources Center of Murcia University were used in the study. All animals were fasted for 12 h prior to blood sampling. The study was approved by the University of Murcia Animal Care Committee.

Blood samples were obtained by jugular venepuncture using a disposable syringe and a 21 G needle. Twenty millilitres of blood were obtained from each animal and aliquots were distributed and placed in different types of tubes in the following sequence:

(1) Lyophilized lithium heparin at a concentration of 17 UI/mL, in a 4 mL tube, (BD Vacutainer, LH PST).
(2) Tripotassium methylenediaminetetraacetic acid (EDTA K3), 0.072 mL at 7.5%, in a 3 mL tube, (BD Vacutainer).
(3) Trisodium citrate, 0.3 mL at 3.8%, in a 3 mL tube, (Venoject Na_3 citrate buffered).
(4) Sodium fluoride and dipotassium oxalate, 5 mL tube, (Venoject $NaF+K_2O_x$). The only data provided by the company was that the total anticoagulant final concentration was 4.5 mg/mL.
(5) Clot activator, 5 mL tube (Tapval) for serum.

All samples were centrifuged at 2000*g* for 10 min, and serum and plasma were immediately separated prior to analysis on the same day. The time between collection and centrifugation was approximately 30 min. No haemolysis or lipaemia were detected in any of samples analyzed, with the exception of samples placed in sodium fluoride and potassium oxalate tubes which were haemolyzed.

2.2. Reagents and apparatus

Details of the analytical methods used for metabolites and minerals are shown in Table 1, and the enzymatic methods are outlined in Table 2. An automated biochemical analyzer (Cobas Mira Plus) was used for the analysis of all metabolites, minerals and enzymes. Sodium, potassium and ionized calcium were determined in a blood gases analyzer (Radiometer ABL System) using selective membrane electrodes. The following control material samples were used to test accuracy of parameters analyzed: ABX Diagnostics Human Control N, Enzyme Control and Fructosamine Control (ABX Inc.). All parameters showed an inaccuracy of <10%.

A previous blood sampling was performed in one dog one week before the study and serum and plasma samples with the four anticoagulants were obtained as previously described and used to test within-run precision. The biochemical parameters were analyzed five times in each of the five samples in the same analytical series. All parameters showed a within-run imprecision of <10%, with the exception of bile acids, triglycerids, GGT, phosphorus and Ca in fluoride (35%, 15%, 33%, 21% and 46%, respectively), AP in EDTA (12.2%) and lipase in all plasmas (fluoride 12%, citrate 16%, EDTA 19% and heparin 15.5%). Spinreact (Spinreact SA) reagents were used for total bilirubin as these provided a higher precision.

Table 1
Details of analytical methods used for metabolites and minerals

Metabolite	Method	Reference
Albumin	Green bromcresol (BCG)	Doumas et al. (1971)
Bile acids	Diaphorase method	Mashige et al. (1976)
Cholesterol	CHOD (cholesterol oxidase)/PAP(4-aminoantipyrine)	Allain et al. (1974)
Creatinine	Kinetic modification of the Jaff method	Vassault et al. (1992)
Fructosamine	Nitrotetrazolium blue method	Johnson et al. (1983)
Glucose	GOD (glucose oxidase)/PAP(4-aminoantipyrine)	Trinder (1969)
Phosphorus	Phosphomolyddate method	Daly and Ertingshausen (1972)
Total bilirubin	DMSO (dimethylsulphoxide) method	Walters and Gerarde (1970)
Total calcium	Arsenazo III	Bauer (1981)
Total proteins	Biuret reaction (copper salts in an alkaline medium)	Weichselbaum (1946)
Triglycerides	GPO (glycerol 3 phosphate oxidase)/PAP(4-aminoantipyrine)	Fosatti and Prencipe (1982)
Urea	Urease/GLDH (glutamate dehydrogenase)	Hallet and Cook (1971)

All analysis were made using ABX kits with the exception of bile acids which were measured using Randox kits and total bilirubin which was measured using Spinreact kits.

Table 2
Details of analytical methods used for enzymes

Enzyme	EC number	Substrate	Reference
Ache	3.1.1.7	Acetylthiocoline	Tecles et al. (2000)
ALT	2.6.1.2	α-Ketoglutarate/L-alanine	Thefeld et al. (1974)
Amylase	3.2.1.1	2-Chloro-4-nitrophenyl maltotrioside	Winn-Deen et al. (1988)
AP	3.1.3.1	4-Nitrophenylphosphate	Mathieu et al. (1982)
AST	2.6.1.1	α-Ketoglutarate/L-aspartate	Thefeld et al. (1974)
Bche	3.1.1.8	Butyrilthiocoline	Tecles et al. (2000)
CK	2.7.3.2	Creatine phosphate and reactivation with *N*-acetylcysteine	Szasz et al. (1976)
G-GT	2.3.2.2	L-γ-glutamyl-3-carboxy-4-nitroanilide	Szasz (1969)
Lipase	3.1.1.3	1,2-Diglyceride	Imamura et al. (1989)

All determinations were made using kinetic spectrophotometric methods at 37 °C. ABX kits were used except for AchE and BchE which were determined with home made reagents.

Ache, acetylcholinesterase; ALT, alanine aminotransferase, AP, alkaline phosphatase; AST, aspartate aminotransferase; Bche, butyrilcholinesterase; CK, creatine kinase, G-GT: γ-glutamyl transferase.

2.3. Statistical study

Data were tested for normal distribution by Kolmogorov–Smirnová's test. In each parameter which followed normal distribution, confidence intervals were calculated as mean $\pm$ 1.96SD/$\sqrt{n}$ (SD = standard deviation and n = number of animals) and a paired t test (significance level $p < 0.05$) was used to assess differences between results obtained in serum and each other different anticoagulants (heparin, EDTA, citrate and fluoride + oxalate). For values which did not meet the normal distribution criteria (GGT, ALT, total and ionized Ca, K and Cl), limits of confidence intervals were calculated as 25 and 75 percentiles and the Wilcoxon matched pair test was performed to assess differences between serum and different anticoagulants. SPSS software, version 10.0 (SPSS Inc.) was used as our statistical programme.

3. Results

The metabolite, mineral and electrolyte, and enzyme values are shown in Tables 3–5, respectively. Similar values with no significant differences were found between heparin and serum in all parameters tested with

Table 3
Effect of anticoagulant on canine metabolites

	Serum	Heparin	EDTA	Citrate	Fluoride	Serum–heparin	Serum–EDTA	Serum–citrate	Serum–fluoride
Albumin (g/L)	35.1 (32.9–37.2)	36.1 (34.8–37.3)	34.5 (33.3–35.8)	31.1 (30.1–32.2)	32.3 (31.1–3.5)	–**	–***	–***	–***
Bile acids (μmol/L)	5.84 (1.82–9.86)	5.74 (1.43–10.5)	4.73 (0.82–8.64)	4.59 (1.57–7.61)	6.00 (3.32–8.68)	N.S.	–*	–*	N.S.
Bilirubin (total) (μmol/L)	4.37 (2.87–5.87)	3.76 (1.77–5.74)	4.61 (2.86–6.36)	3.48 (2.05–4.92)	7.72 (6.07–9.38)	N.S.	N.S.	–*	–***
Cholesterol (mmol/L)	4.73 (3.58–5.88)	4.70 (3.88–5.51)	4.60 (3.84–5.37)	4.06 (3.37–4.75)	4.24 (3.51–4.97)	N.S.	N.S.	–***	–***
Creatinine (μmol/L)	67.8 (51–84.6)	67.7 (61.2–74.1)	65.6 (60.3–71.0)	71.2 (68.4–73.9)	56.4 (48.6–64.3)	N.S.	N.S.	N.S.	–***
Fructosamine (μmol/L)	233 (234–253)	224 (211–236)	211 (198–223)	200 (188–213)	207 (195–220)	N.S.	–**	–***	–***
Glucose (mmol/L)	5.70 (5.45–5.95)	5.82 (5.62–6.03)	5.73 (5.41–6.05)	5.16 (4.86– 5.46)	5.69 (5.41–5.97)	N.S.	N.S.	–***	–*
Total protein (g/L)	62.6 (56.5–68.7)	64.5 (60.7–68.3)	62.2 (58.2–66.3)	56.4 (52.8–59.9)	59.6 (55.8–63.4)	N.S.	N.S.	–***	–***
Triglycerids (mmol/L)	0.57 (0.34–0.80)	0.52 (0.38–0.66)	0.53 (0.39–0.67)	0.50 (0.37–0.63)	0.52 (0.38–0.65)	N.S.	N.S.	N.S.	N.S.
Urea (mmol/L)	12.1 (9.1–15.1)	12.2 (10.0–14.4)	12.1 (9.93–14.3)	11.3 (9.36–13.2)	12.4 (10.2–14.6)	N.S.	N.S.	–***	N.S.

Results appear as mean values and confidence intervals. N.S., not significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 4
Effect of anticoagulants on canine minerals and electrolytes

	Serum	Heparin	EDTA	Citrate	Fluoride	Serum–heparin	Serum–EDTA	Serum–citrate	Serum–fluoride
Calcium (Ca)	2.44 (2.39–2.61)	2.47 (2.32–2.63)	0.04 (0.02–0.04)	1.98 (1.91–2.13)	0.04 (0.04–0.07)	N.S.	–***	–***	–***
Phosphorus (P)	1.59 (1.38–1.81)	1.53 (1.34–1.71)	1.44 (1.27–1.62)	1.26 (1.10–1.42)	1.41 (1.26–1.57)	N.S.	–**	–**	–**
Ionized calcium	1.28 (1.26–1.31)	1.21 (1.17–1.24)	0.00 (0–0)	0.02 (0.01–0.03)	0.00 (0–0)	–**	–**	–**	–**
Sodium (Na)	143.9 (142.4–145.4)	146.1 (145.3–146.8)	139.1 (138.1–140.1)	161.7 (160.4–162.9)	152 (150.1–153.9)	N.S.	–***	–***	–***
Potassium (K)	4.4 (4.1–4.9)	4.05 (3.67–4.22)	22.3 (21.2–22.9)	3.45 (3.15–3.62)	43.8 (40.4–48.1)	–**	–**	–**	–***
Chloride (Cl)	108.5 (106.7–109)	110.5 (108.0–111.2)	108.5 (106.0–110.2)	92.5 (91.5–94.2)	107.0 (105.0–108.2)	N.S.	N.S.	–***	–*

Results appear as mean values and confidence intervals. All results are in mmol/L. N.S., not significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 5
Effect of anticoagulants on canine enzymes

	Serum	Heparin	EDTA	Citrate	Fluoride	Serum–heparin	Serum–EDTA	Serum–citrate	Serum–fluoride
Ache	1592 (1092–2092)	1421 (909–1933)	1420 (889–1951)	1270 (947–1593)	1212 (820–1604)	–**	–**	–***	–**
ALT	37.5 (31.2–41.2)	33.5 (29.1–37.3)	38.0 (33.0–41.7)	33.5 (28.2–36.5)	33.5 (30.2–39.2)	N.S	N.S.	N.S.	N.S.
Amylase	755 (525–985)	766 (597–935)	710 (542–877)	673 (520–825)	691 (535–846)	N.S.	N.S.	–***	–***
AP	82.4 (42.1–122)	79.6 (51.7–107)	12.4 (0.43–24.3)	68.9 (44.1–93.6)	70.7 (44.7–96.6)	N.S.	–***	–***	–**
AST	23.4 (19.4–27.4)	21.5 (17.8–25.1)	21.6 (18.0–25.1)	20.6 (17.5–23.6)	23.5 (20.2–26.7)	N.S.	N.S.	N.S.	N.S.
Bche	2926 (1977–3875)	2829 (2180–3478)	2822 (2082–3562)	2542 (2093–2991)	2063 (1508–2617)	N.S.	N.S.	–***	–***
CK	89.7 (64.1–115)	84.2 (74.0–94.3)	79.7 (70.9–88.4)	78.6 (70.1–87.1)	86.1 (73.6–98.5)	N.S.	N.S.	N.S.	N.S.
GGT	4.0 (3.00–4.25)	3.50 (2.75–4.00)	4.00 (3.00–4.00)	3.00 (2.75–4.00)	2.00 (2.00–3.00)	N.S.	N.S.	N.S.	–**
Lipase	222 (101–343)	220 (131–308)	214 (127–300)	190 (118–262)	199 (123–276)	N.S.	N.S.	–**	–**

Results appear as mean values and confidence intervals. All results in U/L. N.S., not significant.
** $p < 0.01$.
*** $p < 0.001$.

the exception of an increase in albumin and a decrease in potassium, ionized calcium and acetylcholinesterase in heparin.

Serum and EDTA plasma samples yielded broadly similar values for all metabolites tested and no statistically significant differences were found with the exception of a decrease in albumin, bile acids and fructosamine in EDTA-plasma. A statistically significant decrease in total and ionized calcium and sodium and an increase in potassium were found in plasma collected with EDTA. Of the enzymes, only AP and Ache showed a significant decrease when EDTA was used.

Metabolites generally showed a statistically significant decrease when citrate was used compared to serum with the exception of creatinine (which showed a non-significant increase) and triglycerides (which showed a non-significant decrease). A significant decrease in total and ionized calcium, phosphorus and chloride and an increase in sodium were also observed. A statistically significant decrease was found in most enzymes with the exception of ALT, AST, CK and GGT.

Fluoride–oxalate produced an evident haemolysis in all samples and significant decreases in most metabolites with exception of bile acids, triglycerides, urea and total bilirubin (which showed a significant increase). There

was a statistically significant increase in sodium and potassium, and a decrease in total and ionized calcium, phosphorus and chloride. A significant decrease occurred in most enzymes with the exception of ALT, AST and CK.

4. Discussion

The use of serum or plasma in clinical pathology remains controversial. Serum is preferred by many laboratories for biochemical tests since it avoids the addition of anticoagulants that can interfere with some analytical methods or change the concentration of the parameters being measured. There is no interference by fibrinogen in heterogeneous inmunoassays and serum is also recommended for electrophoresis and selected protein analysis (Guder, 2001). Furthermore, in stored samples, the formation of fibrin strands is lower than when plasma is used and there is less risk of occlusion of automated biochemical analyzers. However the use of plasma is preferred in some centres as it saves time (plasma samples can be centrifuged directly after sample collection and, unlike serum, it is not necessary to wait until coagulation is completed); secondly, 15–20% more plasma than serum can be obtained from the same volume of blood and, thirdly, with plasma there are no coagulation-induced changes or interferences (Guder, 2001).

Heparin has been generally recommended as the most suitable anticoagulant for plasma collection (Burtis and Ashwood, 2001) although in previous reports significant differences in selected parameters have been found between heparinized plasma and serum (Ladenson et al., 1974; Thorensen et al., 1992). In our study, serum and heparinized plasma yielded similar results for most of parameters tested with the exception of the decreases in ionized calcium, potassium and Ache and the increase in albumin found with heparin. Ladenson et al. (1974) indicated that potassium could be artifactually elevated in serum due to platelet activation. An artifactual increase in albumin in heparinized plasma, compared with serum and other anticoagulants, using a bromcresol green assay (BCG) has recently been described in canine samples due to the combination of heparin and fibrinogen (Stokol et al., 2001). This could explain the significant increases obtained in albumin when heparin was used in our study. A sample blank and a reaction time of less than 1 min for albumin measurements in heparinized plasma of dogs using a BCG method, should be recommended to prevent such artifactual increases (Stokol et al., 2001).

Although most veterinary clinical pathology laboratories only recommend the use of serum or heparinized plasma for biochemical analysis, in some cases, only samples collected with EDTA, sodium fluoride or sodium citrate are submitted for routine clinical biochemistry. The reasons are varied and include the failure to obtain enough blood to fill all required anticoagulant tubes, or if additional biochemical tests (not previously expected) to the initial required haemogram, glucose or coagulation factors analysis are subsequently requested, or by simple mistake. The ideal approach would be to take a further sample but unfortunately this is not always possible and the laboratory then has no option other than to analyze the specimen collected. Knowledge of how anticoagulants may influence final results can therefore be of considerable practical significance.

EDTA acts as a chelating agent forming complexes with calcium (which is essential for coagulation) and is recommended for routine haematology since it provides a very good staining quality of blood cells. It can be used as the disodium, dipotassium or tripotassium salts, the latter two being preferred as they are more soluble (Burtis and Ashwood, 2001). In our study, no differences in metabolite values were detected with EDTA and serum with the exception of albumin, bile acids and fructosamine. However, EDTA produced significant changes in total and ionized calcium and most electrolytes such that clinical interpretation could be affected. Decreases in total and ionized calcium may reflect EDTA chelating properties and increases in potassium could be due to the use of the more soluble tripotassium salts, since the sodium salt of EDTA yields similar K levels to heparin (Schmidt et al., 1953). Comparable changes in minerals and electrolytes induced by EDTA have been described in humans (Guder, 2001).

Of the enzymes measured, EDTA induced significant decreases only in AP and acetylcholinesterase. Decreases in AP associated with EDTA have been previously described in humans, dogs, and ruminants (Burtis and Ashwood, 2001; Myers and Pierce, 1972; Jones, 1985a,b) and could be due to the acidifying effect of EDTA (Myers and Pierce, 1972). Alternatively, the chelating properties of EDTA may have influenced results, since AP is a zinc-dependent, magnesium-activated enzyme (Jones, 1985b; Guder, 2001). An increase in transaminases (ALT and AST) has been described in EDTA-plasma samples from sheep, but the EDTA-canine samples in our work showed no variation in transaminase concentrations as has been found with humans and cattle (Jones, 1985a,b). Our CK results agreed with previous reports which found no differences in CK between EDTA and serum in cattle samples (Jones, 1985a), unlike human EDTA-treated samples which showed a decrease (Burtis and Ashwood, 2001). Distribution of specific isoenzymes could be responsible for these discrepancies between species.

Overall, with some selected and important exceptions (electrolytes, AP, Ache, albumin, bile acids and fructosamine) all other enzymes and metabolites in the

EDTA collected samples showed no differences compared with serum samples. Although the use of serum or heparin is mostly recommended for biochemistry analysis, in some instances (such as with glycosylated haemoglobin or polymerase chain reaction determinations) the use of EDTA as anticoagulant is preferable (Togni et al., 2002).

Sodium citrate solution at a concentration of 3.2–3.8 g/dL in a ratio of 1 part to 9 parts of blood, is generally used for the analysis of factors related to haemostasis because its effect is easily reversible by the addition of ionized calcium. In our study, citrate produced an increase in sodium (since a sodium salt was used) and a decrease in almost all other parameters compared to serum. However, most of these decreases were in the range of 10–15%, and could be simply due to a dilution (1:9) effect when the blood was mixed with the anticoagulant in liquid form. A similar negative effect due to dilutional error has been described in humans associated with the use of liquid instead lyophilized heparin (O'Leary and Langton, 1989). The negative effect of citrate described in humans on parameters susceptible to its chelating properties such calcium or AP (Burtis and Ashwood, 2001), could have contributed to the decreases observed in these analytes in our work. However, it seems that citrate has a more negative effect on ionized than on total calcium. The use of a reduced concentration of citrate (3.2%; as recommended nowadays in human laboratories NCCLS, 1998), could lead to less variation in biochemical parameters due to this anticoagulant.

Sodium fluoride is a weak anticoagulant, which is added as an antiglycolytic agent (it inhibits several glycolytic enzymes by complexing with the magnesium ion cofactor) to preserve blood glucose (Christopher and O'Neill, 2000) and is usually combined with others stronger anticoagulants such as potassium oxalate. Concentrations of 2 mg/mL blood are said to be effective when it is used with potassium oxalate, but when used alone for anticoagulation, 3–5 times higher concentrations are required (Burtis and Ashwood, 2001).

The haemolysis observed in our study with sodium fluoride and potassium oxalate could be explained by the shrinkage and partial haemolysis of human RBCs when the final concentration of each compound exceeded 2–3 mg/mL blood; at even lower concentrations the same effects have been observed in other vertebrate species (Castellini et al., 1992). The manufacturer did not specify the concentration of each individual anticoagulant, but the concentrations used in our work (although <10 mg/mL blood as recommended by Kaneko et al., 1997) were high enough to produce shrinkage and/or lysis. PCVs obtained in our study with fluoride were in a range of 10–20% lower than EDTA-blood samples. How much this decrease could be attributed to lysis or shrinkage was not determined but, unlike in horses (Ferrante and Kronfeld, 1994), visual haemolysis was evident.

Cell shrinkage and/or haemolysis will produce a shift in RBC components and dilution of the plasma, and three main effects may be associated with this fact: firstly, a false decrease in the level of analytes present at higher concentrations in plasma than in RBCs; secondly, a false increase in the concentration in plasma of analytes that are normally present in higher concentrations within canine red cells such as AST, CK, ALT, Na and K—only in Akita dogs—(Alleman, 1990); and, thirdly, colour interference and chemical interactions between red blood cell components and analytes that can alter the final results.

It would be difficult to identify which of the above mechanisms were responsible for the variations in the results that appeared in our study. However, AST could be affected by the second mechanism which might explain the slight increase found with fluoride–oxalate. Additionally, the increase in total bilirubin could be influenced by colour interference produced by haemoglobin (third mechanism) (Alleman, 1990). Overall, it seems that the effects produced by the anticoagulant mixture in canine plasma were of less magnitude than those found in other species such as in cattle (with a significant inhibition of several enzymes such as CK, transaminases and AP (Jones, 1985a)); or in humans where it has been described as a potent inhibitor of many blood enzymes such as alkaline and acid phosphatase, amylase and lactic dehydrogenase, and metabolites such as uric acid, and it can also interfere with some methods used to measure BUN (Caraway, 1962; Benjamín, 1978; Burtis and Ashwood, 2001). Studies with fluoride–oxalate in dogs where no negative effect on CK (Aktas et al., 1994) or amylase (Mc Geachin et al., 1957) was observed would seem to support this hypothesis. However, the haemolysis detected in all samples treated with this anticoagulant in our study, plus the high imprecision and significant changes observed in many parameters suggests that fluoride and oxalate are not suitable as anticoagulants for general clinical biochemistry analysis.

This work has focused only on healthy animals, but further studies looking at diseased animals with significant altered biochemistry values would be desirable to ascertain the influence of different anticoagulants in these cases. Our results to date should be interpreted with caution since we used specific commercial kits and biochemical autoanalyzers, and significant variation in values and results may be found using other reagents, methods, analyzers or even blood extraction systems (Almagor and Lavid-Levy, 2001). For example a very significant decrease (86% of values obtained with serum) in lipase with EDTA was found when samples were subsequently analyzed with a turbidimetric method (Roche kit) using triolein as substrate

(unpublished data), and no significant differences in CK were found between serum and different plasma samples in our work, unlike previous reports made in dogs (Aktas et al., 1994; Thorensen et al., 1992). Moreover in studies comparing different anticoagulants, significant differences in results have been found depending on the autoanalyzer (Bolten et al., 1992) and the relative amounts of anticoagulant used (Jones, 1985a).

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Book review

Physiology of the Graafian Follicle and Ovulation. Hunter, R.H.F. Cambridge, Cambridge University Press, 2003. 397pp. £65 (hard) ISBN 0521781981.

There is a fashion presently in advertising to assert that a product performs in the manner claimed for it. 'It does exactly what it says on the tin'. This book claims to '…provide comprehensive coverage of the field, integrating research findings from animal and human studies and condensing the vast published literature into a meaningful and digestible physiological account that highlights the key role played by the oocyte in influencing all stages of follicular development'.

The first chapter gives a fascinating account of the history of ovarian physiology, which the author rather self-effacingly suggests may seem an indulgence. In fact it adds greatly to the understanding of the present position of the field; how should we know where we are, if we know not how we got here? The rest of the book then follows a logical developmental arrangement, dealing with the formation and structure of the ovaries and Graffian follicles, the endocrine function of the follicles and the stages they go through during maturation. There are chapters on ovulation, post-ovulatory events, the induction of ovulation and syndromes causing failure to ovulate. As its title suggests, this is a book on physiology, and although it contains some pathophysiology it will not satisfy a clinician looking for a comprehensive account of ovarian pathology. But it is a very comprehensive review of ovarian physiology.

The book has many attributes. First, the author writes clear and polished English. The care he has taken with the language is obvious. Second, it is well illustrated by black-and-white line drawings and photographs (I counted 88), and numerous tables in addition to colour pictures. Third, it is up-to-date – it includes for example a discussion of the role of oocyte growth factors, follicle temperature (like testes, follicles are cooler than their surrounding tissues) and apoptosis. Lastly, it draws on the author's long interest and study of the ovary to pick out the important facts, as highlighted by the comparative physiology. Because the author has been a long time travelling this road, the book contains many interesting observations and viewpoints which he has acquired along the way, and which appropriately illustrate aspects of the subject.

The science of follicle function is currently moving fast, and many groups are involved. There are several reasons for this: fertility in some domestic species remains a problem, and studies at the interfaces between follicle function and nutrition, genetics and gamete manipulation are burgeoning. The clinical syndromes arising from ovarian malfunction continue to cause concern both in human clinical and veterinary practice. Technical developments in cell and molecular biology have opened new approaches. As a result ovarian physiology is attracting many new recruits. This book will be as useful to molecular biologists wanting to review the physiology, as to physiologists needing an up-to-date review of the molecular biology, and it gives a careful, balanced and enjoyable introduction to the field. This book 'does exactly what it says on the tin'.

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