Postmortem Eyefluid Analysis in Dogs, Cats and Cattle as an Estimate of Antemortem Serum Chemistry Profiles

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ABSTRACT

This study was carried out to determine the diagnostic usefulness of postmortem eyefluid analysis in estimating antemortem concentrations of serochemical constituents. A total of 31 cattle, 18 dogs and 22 cats were selected from routine elective euthanasia submissions to a diagnostic laboratory. For all cases, a biochemical profile, including determinations for electrolytes, glucose, urea, creatinine, enzymes, cholesterol, bilirubin, protein and osmolality was performed on antemortem serum, and postmortem aqueous and vitreous humors at 0 and 24 h incubation periods. The association between serum and postmortem eyefluid chemistry values was examined using simple linear regression. A strong correlation between serum and postmortem eyefluid urea and creatinine concentrations was demonstrated in the three species examined over a 24 h postmortem interval. We concluded that an accurate estimate of antemortem serum urea or creatinine can be made from the analysis of aqueous or vitreous fluid at necropsy. An estimation of antemortem serum electrolytes (including calcium in cattle) cannot be made with a high degree of accuracy due to the amount of variability in the relationship between serum and eyefluid electrolyte values. For large molecules such as proteins, enzymes, cholesterol and bilirubin there was very poor correlation between serum and eyefluid values.

RÉSUMÉ

Cette étude consistait à déterminer la valeur diagnostique de l'analyse des fluides oculaires postmortem afin d'estimer les concentrations antemortem des constituants biochimiques. Au total, 31 bovins, 18 chiens et 22 chats ont été sélectionnés des cas soumis pour euthanasie au laboratoire de diagnostic. Pour tous ces cas, un profil biochimique était effectué sur le serum antemortem et les humeurs aqueuse et vitrée obtenues à 0 et 24 heures après la mort. Le profil biochimique incluait la détermination des électrolytes, du glucose, de l'urée, de la créatinine, des enzymes, du cholestérol, de la bilirubine, des protéines et de l'osmolalité. L'association entre les valeurs chimiques du sérum et des fluides oculaires postmortem a été examinée par régression linéaire simple. Une forte corrélation a été démontrée entre les concentrations de l'urée et de la créatinine du sérum et celles des fluides oculaires, et ce chez les trois espèces étudiées sur un intervalle de 24 heures postmortem. En conclusion une estimation précise des valeurs sériques antemortem de l'urée et de la créatinine peut être obtenue à partir des humeurs aqueuse et vitrée lors de nécropsie. Les concentrations des électrolytes sériques antemortem, incluant le calcium chez les bovins, ne peuvent être estimées avec précision dû à la variabilité des rapports entre les valeurs électrolytiques du sérum et des fluides oculaires. Pour les protéines, les enzymes, le cholestérol et la bilirubine, la corrélation était très faible entre les valeurs du sérum et celles des fluides oculaires. ***(Traduit par Dr Sylvie D'Allaire).***

INTRODUCTION

Many diseases of animals are associated with biochemical alterations. Antemortem serum chemistry values indicating these biochemical changes are frequently required to support or confirm a postmortem diagnosis. In many instances however, the antemortem chemistry values are unavailable to the pathologist. In these cases a postmortem test that could accurately estimate the antemortem chemistry values would be useful.

There is evidence suggesting that postmortem eyefluid analysis could provide a reliable estimate of antemortem chemistry values (1-9). Eyefluid, which is a filtrate of the blood, is in a protected anatomical location and is infrequently contaminated by blood during collection. In human medicine, postmortem eyefluid has been used to estimate the time of death (postmortem interval), antemortem serum chemistry values, antemortem drug and toxin levels, and to investigate "sudden infant death syndrome" (8-11).

In veterinary medicine, there have been several recent studies attempting to correlate postmortem eyefluid chemistry to corresponding antemortem chemistry values (2-7). With few exceptions, these studies have correlated values from slaughterhouse

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specimens which were presumed to have normal serum chemistry ranges. The exceptions to this involve correlation of antemortem serum values to postmortem eyefluid values of urea and/or creatinine in both uremic and nonuremic animals (1,6,7). One study examined the lag time for equilibration of increased serum urea across the blood-retinal barrier (5).

The purpose of this study was to determine the diagnostic usefulness of postmortem eyefluid analysis as an estimate of antemortem serum chemistry concentrations in dogs, cats and cattle. Animals having a variety of antemortem serum chemistry abnormalities were used.

MATERIALS AND METHODS

A total of 31 cattle, 18 dogs and 22 cats were sampled from routine elective euthanasia submissions to the diagnostic laboratory at the Atlantic Veterinary College. Some cases, e.g. parturient dairy cows and uremic dogs and cats were specifically selected because of prior knowledge of their particular serum biochemical abnormality.

In all cases, a venous blood sample was collected from the cephalic or jugular veins within 5 min before euthanasia. Animals were then euthanized by intravenous injection of sodium pentobarbitone (Euthanyl-MTC Pharmaceuticals, Cambridge, Ontario) into the cephalic or jugular veins. No later than 5 min after death, samples of aqueous and vitreous humor were collected from one of the eyes *in situ*. Sampling, from left or right eyes, was alternated on consecutive cases of the same species.

Aqueous humor was collected from the anterior chamber by gentle aspiration into a 3 mL syringe following corneal penetration with a 23-gauge needle in small animals or a 21-gauge needle in cattle. Vitreous humor was collected from the central portion of the vitreous body following insertion of a 16-gauge needle through the sclera and aspiration into a 10 mL syringe. The blood and vitreous samples were centrifuged at approximately 833 *g* for 7 to 10 min within 30 min of collection. A biochemical profile (defined below) was performed on the serum and aqueous and vitreous humors within 4 h of the time of collection. If a delay in sample analysis occurred, the samples were either refrigerated at 4°C (overnight) or frozen at -20°C (over weekend).

Following eyefluid collection, a thorough necropsy was performed on each carcass. The eye from which fluid had been aspirated was removed and fixed in 10% neutral buffered formalin for histological examination. The half portion of the head that contained the unsampled eye was placed on a stainless steel tray and stored at 4°C. Care was taken during handling of the skull to maintain the integrity of the periorbital tissues (eyelids, skin, etc.) and to simulate the normal postmortem conditions to which the globe may be exposed. After a period of 24 h (or 6 h with seven of the feline cases), the second eye was sampled and eyefluid analyzed in a manner identical to that of the opposite eye.

All biochemical constituents were determined using an automated chemical analyzer (DACOS, Coulter Electronics, Hialeah, Florida). The constituents examined, followed in parentheses with the specific methodology, included: sodium and potassium (direct potentiometry), chloride (modified Schoenfeld and Lewellen), calcium (modified Connerty and Briggs), phosphorus (modified Daly, Ertingshausen), magnesium (magnesium assay kit, Diagnostic Chemicals Limited, Charlottetown, Prince Edward Island), urea (modified Talke and Shubert), creatinine (kinetic Jaffe), glucose (hexokinase-modified CDC reference method), cholesterol (enzymatic), total and direct bilirubin (modified Walters and Gerard), amylase (enzymatic amylase, Beckman Instruments Inc, Carlsbad, California), alkaline phosphatase (modified Bowers and McComb), creatinine phosphokinase (modified Oliver and Rosalki), aspartate aminotransferase and alanine aminotransferase (modified IFCC), gamma-glutamyltransferase (modified Szasz method), total protein (modified Biuret) and albumin (modified Doumas). The chemical analyzer was calibrated with company standards (DACOS, Coulter Electronics, Hialeah, Florida) and daily controls were performed.

A paired, two-tailed *t* test was used to detect differences between mean concentrations of specific eyefluid chemicals taken from separate eyes of a single individual at different postmortem intervals. An unpaired, two-tailed *t* test was used to detect differences in mean eyefluid and/or serum chemistry concentrations from different individuals. The association between serum and eyefluid biochemical constituents was examined using simple linear regression. F-tests were used to test the significance of both the slopes of the regression lines and the correlation coefficients (12). The slopes of different regression lines were compared using *t*-tests. Tests were considered significant if $p < 0.05$.

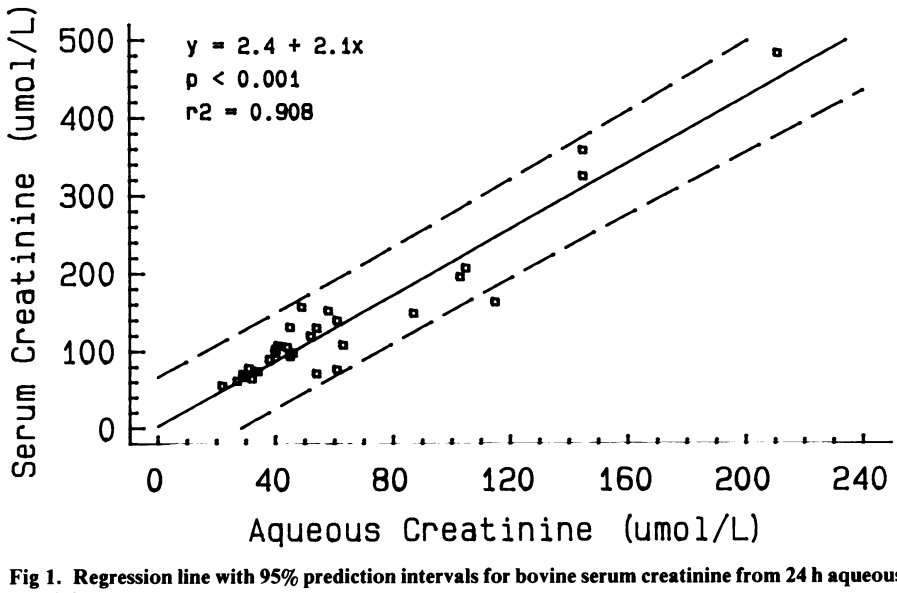

Fig 1. Regression line with 95% prediction intervals for bovine serum creatinine from 24 h aqueous creatinine.

RESULTS

The biochemical profiles indicated a broad range of values for most of the parameters measured, with many values outside the "normal range". The significant relationships between serum and eyefluid values are described below.

The histopathological examination of the eyes from all cases revealed no lesions. However, as many of the animals in the study had been submitted for euthanasia, a wide variety of lesions were present in many of the other organs.

UREA AND CREATININE

The regression of serum urea and creatinine on their respective postmortem eyefluid values in dogs, cats, and cattle indicated a high correlation and a highly significant slope ($p < 0.001$). Figure 1 is a plot of bovine serum creatinine on 24 h aqueous creatinine values, with a least squares regression line and 95% prediction intervals. Extrapolation from this graph or direct calculation from the regression equation gives a critical eyefluid value above which we can be at least 97.5% confident (since the lower tail of the confidence interval is 2.5%) that an individual animal's antemortem serum creatinine concentration was above the upper limit of the normal range. For example, in Fig. 1 the critical value of 24 h aqueous creatinine is 111 μmol/L. This was chosen as the critical value because its corresponding point on the lower boundary of the 95% prediction interval has as its ordinate the upper limit of the normal range for serum creatinine (175 μmol/L). Thus, for any aqueous fluid creatinine value above 111 μmol/L, there is a 97.5% probability that the antemortem serum creatinine value in that animal was above the upper limit of the normal range.

Figure 2 is a plot of feline serum urea concentration on 24 h aqueous urea values, where similar extrapolations as those described for creatinine values can be made. With the feline data, seven of 22 subjects were sampled at a 6 h postmortem interval rather than the usual 24 h interval. For both urea and creatinine, no statistically significant differences were detected between the respective 6 or 24 h sets of eyefluid values and their

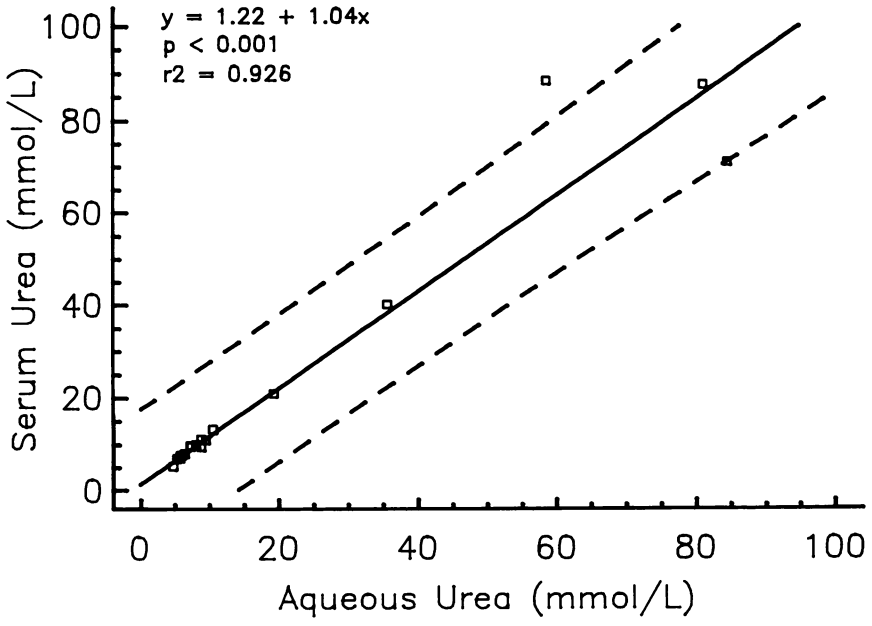

Fig 2. Regression line with 95% prediction intervals for feline serum urea from 24 h aqueous urea.

TABLE I. Regression analysis for urea and creatinine

Independent variable[a]	Regression equation[b]	Coeff deter (r^2)	Critical value[c] (95% C.I.)
Bovine			
0Aq-Urea	y = 1.49 + 0.95x	91.4	14 mmol/L
24Aq-Urea	y = 1.12 + 0.98x	82.7	17 mmol/L
0Vit-Urea	y = 1.24 + 1.04x	88.8	14 mmol/L
24Vit-Urea	y = 1.54 + 1.06x	72.2	18 mmol/L
0Aq-Cre	y = 40.0 + 1.46x	91.5	135 μmol/L
24Aq-Cre	y = 2.44 + 2.12x	90.8	111 μmol/L
0Vit-Cre	y = 15.1 + 2.48x	86.1	109 μmol/L
24Vit-Cre	y = 56.1 + 1.49x	61.1	154 μmol/L
Canine			
0Aq-Urea	y = 0.127 + 1.10x	99.6	11 mmol/L
24Aq-Urea	y = -0.57 + 1.09x	99.9	11 mmol/L
0Vit-Urea	y = 0.04 + 0.97x	99.4	13 mmol/L
24Vit-Urea	y = -0.47 + 1.10x	99.8	12 mmol/L
0Aq-Cre	y = 7.11 + 2.91x	97.8	76 μmol/L
24Aq-Cre	y = 14.0 + 2.10x	98.6	89 μmol/L
0Vit-Cre	y = 14.1 + 2.74x	96.0	92 μmol/L
24Vit-Cre	y = 8.00 + 2.68x	92.3	108 μmol/L
Feline[d]			
0Aq-Urea	y = 2.03 + 1.02x	97.1	18 mmol/L
24Aq-Urea	y = 1.22 + 1.04x	92.6	25 mmol/L
0Vit-Urea	y = 1.43 + 1.07x	95.6	21 mmol/L
24Vit-Urea	y = -1.90 + 1.70x	67.5	28 mmol/L
0Aq-Cre	y = -1.6 + 2.27x	98.5	149 μmol/L
24Aq-Cre	y = 31.8 + 1.93x	96.3	188 μmol/L
0Vit-Cre	y = 22.6 + 2.94x	77.4	230 μmol/L
24Vit-Cre	y = 65.3 + 2.80x	75.1	218 μmol/L

[a]Where 0 and 24 are the postmortem inteval in h, Aq is aqueous, Vit is vitreous and Urea and Cre are the urea nitrogen and creatinine concentrations respectively

[b]Where y is the serum value, x is the independent variable and there is a significant trend ($p < 0.001$) in all cases

[c]The critical eyefluid value above which we can be at least 97.5% confident that the individual animal's antemortem serum urea or creatinine concentration was above the upper limit of the normal range

[d]The feline 24 h values include seven 6 h values

TABLE II. Mean concentrations between 24 and 0 h postmortem interval electrolyte and osmolality concentrations in aqueous and vitreous humor of cattle

Eyefluid/variable[a]	Mean eyefluid concentration[b] 24 h	0 h	n	p value[c]	% change from 0 h
Aq/Sodium	138.0	135.7	29	0.21	1.75
Vit/Sodium	128.8	131.6	31	0.039	2.08
Aq/Potassium	9.25	4.03	29	< 0.0001	129.7
Vit/Potassium	7.35	4.58	31	< 0.0001	60.0
Aq/Chloride	115.6	113.1	30	0.12	2.21
Vit/Chloride	109.6	112.2	31	0.07	2.30
Aq/Calcium	1.52	1.33	24	< 0.0001	14.8
Vit/Calcium	1.46	1.54	29	0.0088	5.54
Aq/Phosphorus	1.66	1.43	30	0.0067	16.2
Vit/Phosphorus	0.84	0.40	30	< 0.0001	108.6
Aq/Magnesium	1.09	0.80	30	< 0.0001	36.3
Vit/Magnesium	1.18	1.15	31	0.05	2.04
Aq/Osmolality	284.4	275.9	27	0.022	3.08
Vit/Osmolality	264.5	268.0	31	0.22	1.31

[a]Where Aq is aqueous humor and Vit is vitreous humor
[b]Where electrolyte values are in mmol/L and osmolality values are in mmol/K
[c]p value of the two-tailed paired *t* test between the 24 and 0 h postmortem eyefluid values

corresponding 0 h values. Consequently the 6 h data for both urea and creatinine were pooled with the 24 h data.

Table I gives the regression equations, p values of the trend, coefficients of determination and the critical eyefluid values (at 97.5% confidence), which estimate the upper limit of the normal serum range for the 0 and 24 h aqueous and vitreous urea and creatinine concentrations.

No statistically significant differences were observed between individual 0 and 24 h creatinine values in either the aqueous or vitreous humor, as predictors of serum values in the three species examined. A similar result was found in the urea data, with a single exception occurring with canine aqueous urea. In that case the difference was so small (mean difference of 0.759) as to be diagnostically trivial. In some cases for both urea and creatinine, there was a small but statistically significant ($p < 0.05$) difference between the slopes of the regression lines for the serum vs 0 h and serum vs 24 h data. These slope differences resulted in slightly different 95% prediction intervals and consequently, different critical eyefluid values which estimate the upper limit of the normal range for antemortem serum values. In these cases the highest critical eyefluid value of the two was chosen to give the most conservative estimate for the 0 to 24 h postmortem interval. Choosing the larger of the two critical values guarantees at least 97.5% probability that a 0 or 24 h eyefluid value above the critical level is associated with a serum value above the normal range.

TABLE III. Regression analysis for electrolytes and osmolality. Regression equations, p value of the trend, and coefficients of determination between serum and 24 h aqueous/vitreous electrolyte concentrations in cattle

Independent variable[a]	Regression equation[b]	p value trend	Coeff deter (r^2)	Serum values range[c]
0Aq-Na+	y = 18.9 + 0.878x	< 0.001	60.8	122-158
24Aq-Na+	y = 93.9 + 0.321x	0.005	26.2	
0Vit-Na+	y = 90.9 + 0.357x	0.010	20.9	
24Vit-Na+	y = 124 = 0.106x	0.419	2.3	
0Aq-K+	y = 1.52 + 0.705x	< 0.001	59.4	2.4-8.0
24Aq-K+	y = 1.91 = 0.268x	0.001	35.6	
0Vit-K+	y = 1.91 + 0.535x	0.011	20.3	
24Vit-K+	y = 0.46 + 0.531x	< 0.001	37.9	
0Aq-Cl-	y = 19.5 + 0.687x	< 0.001	59.7	73-110
24Aq-Cl-	y = 63.5 + 0.293x	0.004	26.1	
0Vit-Cl-	y = 58.3 + 0.349x	0.005	24.4	
24Vit-Cl-	y = 74.7 + 0.208x	0.047	12.9	
0Aq-Ca++	y = 0.59 + 1.26x	0.001	40.0	< 1.00-2.98
24Aq-Ca++	y = 1.43 + 0.538x	0.006	26.8	
0Vit-Ca++	y = 0.70 + 0.965x	0.001	30.8	
24Vit-Ca++	y = 1.03 + 0.802x	0.002	29.6	
0Aq-Phos	y = 0.86 + 0.977x	< 0.001	35.8	1.34-4.69
24Aq-Phos	y = 0.406 + 1.01x	0.001	34.8	
0Vit-Phos	y = 0.94 + 3.27x	0.014	19.7	
24Vit-Phoss	y = 1.16 + 1.25x	0.030	16.3	
0Aq-Mg++	y = -0.08 + 1.38x	< 0.001	59.3	0.22-1.96
24Aq-Mg++	y = -0.20 = 1.12x	< 0.001	39.6	
0Vit-Mg++	y = 0.05 + 0.863x	0.002	27.7	
24Vit-Mg++	y = -0.26 + 1.10x	0.003	26.9	
0Aq-0sm	y = 39.7 + 0.878x	< 0.001	63.7	241-328
24Aq-0sm	y = 158 + 0.483x	0.004	29.3	
0Vit-0sm	y = 189 + 0.351x	0.054	12.7	
24Vit-0sm	y = 253 + 0.114x	0.465	1.9	

[a]Where 0 and 24 are the postmortem interval in h, Aq is aqueous, Vit is vitreous and 0sm is osmolality
[b]Where y is the serum value, x is the independent variable
[c]The electrolyte values are in mmol/L and osmolality (osm) is in mmol/K

ELECTROLYTES AND OSMOLALITY

For many of the electrolyte and osmolality concentrations in the three species examined, there was a significant difference between the means of the individual 0 and 24 h postmortem eyefluid values for both the vitreous and aqueous humors. For some constituents such as potassium and phosphorus, the differences were relatively large (frequently greater than 70% change in mean value). For others, such as calcium and osmolality the means were either not significantly different or significantly different but diagnostically inconsequential. Table II lists the mean differences between these levels at the 0 and 24 h postmortem intervals for the bovine and indicates significant difference. The canine and feline data showed a similar trend.

The regression equations, p values of the trend, and coefficients of

determination for the relationships between serum and eyefluid electrolytes and osmolality for the bovine are given in Table III. A similar trend was present in the canine and feline data. In general, there was a statistically significant trend between serum and eyefluid electrolyte values, however the relatively low coefficient of determination indicated substantial variability of the serum value for any given eyefluid value. This was reflected in the relatively wide prediction intervals for estimation of serum electrolyte values from postmortem eyefluid values, as was the case for the prediction of canine sodium (see Fig. 3).

Many of the mature bovine subjects were selected on the basis of having serum calcium concentrations below the normal range, i.e. less than 2.11 mmol/L. Further, the mean ratio of 0 h aqueous to serum calcium concentration in mature ($\geq$ 1 year) cattle was 0.616, while the same ratio in immature ($<$ 1 year) cattle was 0.555. The mean ratios of 24 h aqueous to serum calcium concentration in mature cattle vs immature cattle were 0.734 and 0.587 respectively. For both the 0 and 24 h postmortem intervals, the difference between the ratios for mature and immature cattle was significant ($p < 0.01$). The relationship between serum and 24 h postmortem aqueous calcium concentrations for mature cattle is shown in Fig. 4.

In the mature cattle the mean 0 and 24 h aqueous calcium concentrations for the nine cattle with serum calcium values less than 2.11 mmol/L (1.27 and 1.34 mmol/L respectively) were significantly different ($p < 0.05$) than the respective mean 0 and 24 h aqueous calcium concentrations in the eight cattle with serum calcium values in the normal range (1.36 and 1.65 mmol/L respectively). There was however considerable overlap in the range of eyefluid calcium concentrations between the hypocalcemic and normocalcemic groups of cattle. For example, at the 24 h postmortem interval the range of aqueous calcium concentrations in the hypocalcemic group was 1.02 to 1.68 mmol/L, while in the normocalcemic group the range was 1.32 to 2.12 mmol/L.

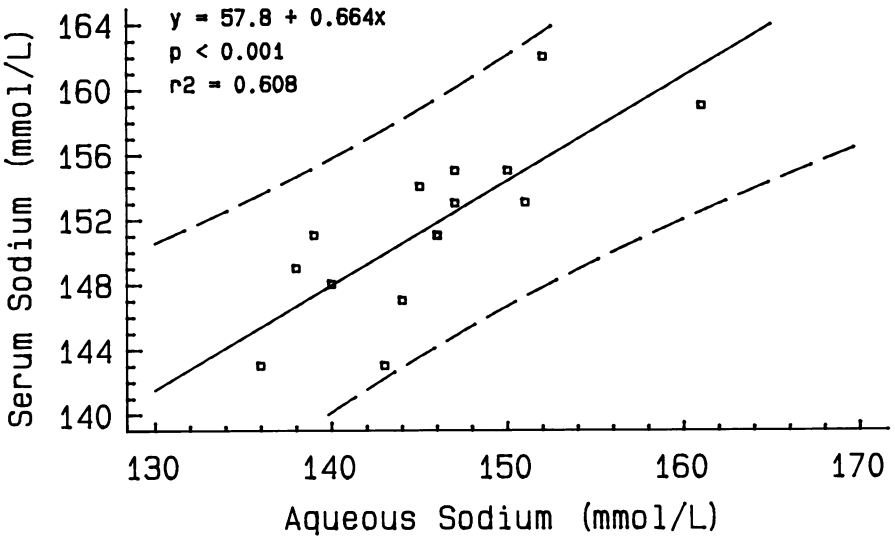

Fig 3. Regression line with 95% prediction intervals for canine serum sodium from 24 h aqueous sodium.

In the feline data, in spite of the relatively wide prediction intervals, two aqueous humor osmolality values were elevated above the point at which we could be 97.5% confident that the individuals serum value was above the normal range (see Fig. 5). As with the feline urea and creatinine data there were no significant differences between the 6 and 0 h or the 24 and 0 h aqueous humor osmolality values, so the 6 h data were pooled with the 24 h data.

GLUCOSE

In the three species examined there was a high correlation and a significant trend ($p < 0.05$) for the regression of serum on eyefluid glucose values at both 0 and 24 h. However, there were statistically ($p < 0.001$) and diagnostically significant differences between 0 and 24 h eyefluid glucose concentrations in all cases (see Table IV).

CHOLESTEROL, BILIRUBIN, ENZYMES AND PROTEIN

For most of these large molecule constituents, the eyefluid concentrations were very small compared with the serum levels. For the enzymes amylase, creatine kinase, and aspar-

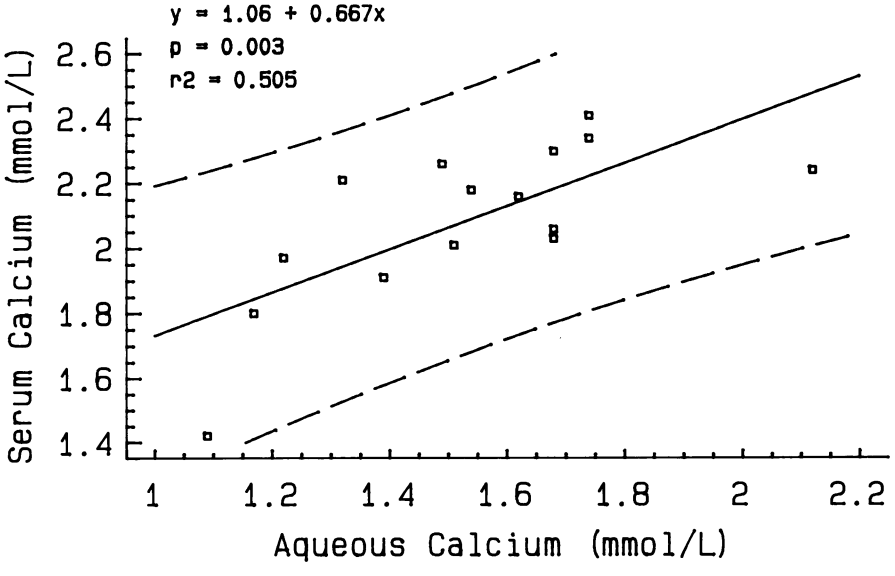

Fig 4. Regression line with 95% prediction intervals for serum calcium from 24 h aqueous calcium in mature cattle.

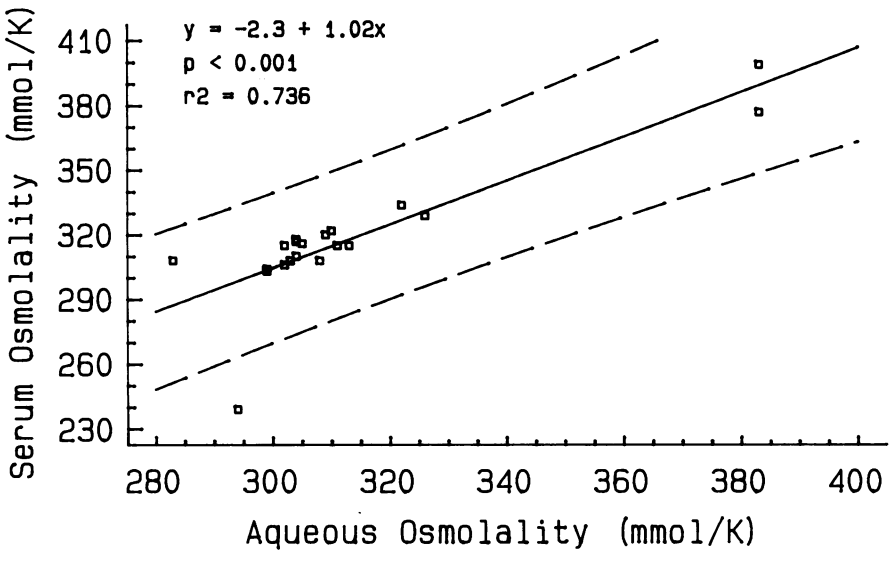

Fig 5. Regression line with 95% prediction intervals for feline serum osmolality from 24 h aqueous osmolality.

tate aminotransferase at least some of the eyefluid values were a significant portion of, or greater than, the corresponding serum concentration. However, the correlation between serum and eyefluid values for all these constituents was poor and there was no significant linear trend.

DISCUSSION

Evaluations of organ function are difficult at the time of postmortem examination. Changes in morphology often do not reflect functional abnormalities that were evident before death. Analysis of eyefluid during the postmortem examination provides the pathologist with the means to estimate some of these antemortem functional changes. Furthermore, newer analytic technology, which allows a wide range chemical profile on a relatively small volume of fluid, enables the examination of aqueous humor in addition to the more traditionally examined vitreous humor. Aqueous humor is also much easier to collect and analyze than vitreous humor in diagnostic situations (5,7).

This study has shown that for most of the chemical constituents examined, the correlation between serum and aqueous humor was the same or stronger than the correlation between serum and vitreous humor. With a few cats in this study, an insufficient quantity of aqueous humor was available from a single eye for a complete chemical profile. In a necropsy situation however, aqueous humor could be harvested from both eyes and the analysis could be limited to oniy those biochemical constituents that are diagnostically useful. Under these conditions it would be unlikely that the volume of aqueous humor would be a limiting factor.

The sampling protocol in this study has some advantages over those previously employed to compare serum and postmortem eyefluid chemistry (3-7). The analysis of serum followed by 0, 24 and occasionally 6 h postmortem eyefluid on each subject controls for the variation among animals, as well as monitoring to some extent the variation in eyefluid concentrations over the entire postmortem interval. Other studies have shown that the concentrations of urea, creatinine and several of the electrolytes in the aqueous and vitreous humor of several species are relatively stable for 24 h or longer after death (2-6). Thus the relationships described in this study, which showed no significant difference between the 0 and 24 h eyefluid levels, should be valid throughout the entire 24 h interval.

During the postmortem interval of this study the eyes were stored at 4°C. Other studies have shown that the concentrations of urea, creatinine and several of the electrolyes in the eyefluid do not vary significantly in the first 24 h after death at temperatures between 4°C and 20°C (2-4). At 37°C however, changes in eyefluid concentrations of most electrolytes become more apparent. As it is recommended procedure that carcases be refrigerated during the postmortem interval to prevent autolysis, the results described here can be applied to most diagnostic situations.

The eye that was to be sampled after the postmortem interval was left in the skull following necropsy to more accurately simulate natural conditions. Furthermore, histological examination of both eyes following sampling showed no lesions and thus gave some assurance of the structural integrity of blood-ocular barriers. Several studies have shown that damage to these barriers from a variety of causes can alter the composition and dynamics of the intraocular fluids (13-16).

As a result of the selection process, there was adequate representation of abnormal values for the serum constituents that were considered to have the most potential diagnostic value; that is, urea, creatinine and calcium. In the three species examined, there were a total of 23 subjects with elevated serum urea values and subjects with elevated serum creatinine values. Of the 17 mature cattle sampled, 9 had serum calcium values below the lower boundary of the normal range (i.e. < 2.11 mmol/L). In general, for the other serum constituents examined, there were fewer or no values outside the normal range.

TABLE IV. Mean concentrations between 24 and 0 h glucose concentration in aqueous and vitreous humor

Eyefluid	Mean eyefluid glucose conc[a] 24 h	0 h	n
Bovine			
Aqueous	0.972	4.890	29
Vitreous	1.348	3.155	31
Canine			
Aqueous	0.277	4.747	17
Vitreous	0.844	3.912	16
Feline			
Aqueous	1.520	5.787	15
Vitreous	0.892	3.854	13

[a]Glucose concentration in mmol/L

UREA AND CREATININE

Previous studies have shown a strong correlation between serum and postmortem eyefluid concentrations for both urea (1,5-7) and creatinine (6) in dogs and cattle in both normal and abnormal ranges. This study has shown that elevated antemortem serum urea and creatinine concentrations in cattle, dogs and cats can be estimated at a specific high level of confidence from the analysis of postmortem eyefluid.

An increase in the variability between the serum and eyefluid urea concentrations was observed in cattle and cats when levels were markedly elevated (see Fig. 2). This can be explained by the known lag phase in the equilibration of changing concentrations of urea across the blood-eye barriers. In fact, such a lag phase has been demonstrated for the equilibration of urea between the blood and vitreous humor in the dog (5). This does not reduce the diagnostic value of this test, but it does lessen the accuracy of the estimate at extremely elevated values. Additionally, it suggests that the critical values for cats and cattle listed in Table I are overly conservative (i.e. are likely lower), since the regression equations from which they were derived assume equal variability of serum values over the entire range of eyefluid values.

ELECTROLYTES AND OSMOLALITY

Two main factors provide major impediments to the use of eyefluid electrolyte levels for predicting antemortem serum values. The first is the fluctuation in the concentration of many of the electrolytes in the eyefluid and the second is the relatively poor correlation between serum and eyefluid values.

As noted in the results, there were consistent increases or decreases in the mean eyefluid concentrations of many of the electrolytes over the 24 h postmortem interval. This observation is consistent with the findings in many previous studies on domestic animals (2-5). For both potassium and phosphorus, marked increases in their respective eyefluid concentrations over the postmortem interval severely limit their usefulness in estimating antemortem serum levels. For example, an elevated eyefluid potassium or phosphorus value taken early in the postmortem interval would not likely be considered elevated later in the interval, when the mean concentrations have increased twofold. This situation was exhibited in the feline phosphorus data. Thus, the use of eyefluid potassium or phosphorus concentrations in predicting serum levels would have to be restricted to eyefluid samples taken very shortly after death. Furthermore, if the time of death was known it might be possible to extrapolate eyefluid electrolyte concentrations back to a corresponding 0 h level and then analyze this value for elevations. This latter method adds a new variable and further studies would be needed to determine if it is a worthwhile approach. For osmolality and electrolytes other than potassium and phosphorus, the changes in eyefluid concentrations over the 24 h postmortem interval were small or not apparent.

The weak correlation between serum and eyefluid electrolyte and osmolality values renders them less valuable diagnostically. Thus, an extremely elevated or depressed eyefluid electrolyte concentration would be required to maintain a high degree of confidence in estimating abnormal serum values prior to the animal's death. This was demonstrated in the feline data with the observation of some extremely elevated osmolality values in the serum and eyefluid. Thus, in addition to the limitations provided for by the change in electrolyte values in eyefluid over time, the relatively low correlation between serum and eyefluid electrolyte concentrations further restricts the use of eyefluid as a predictor of serum electrolyte levels.

Based on previous studies, postmortem eyefluid analysis showed promise as a method to diagnose hypocalcemia in postpartum cows at necropsy (3,5). The present study demonstrates some of the potential limitations of the procedure. While a significant trend exists in the correlation of serum and eyefluid calcium values in the mature cattle sampled in this study, there is still substantial variation of serum values for any given eyefluid value. This is indicated in the data by the significantly ($p < 0.05$) lower mean eyefluid calcium values in cattle with serum calcium values below the normal range compared to cattle with normal serum calcium concentrations. Yet because of an overlap in the range of eyefluid calcium values in these two groups it is only possible to identify hypocalcemic individuals with extremely low serum and eyefluid calcium concentrations. This is further reflected in the regression analysis by the wide distribution of values above and below the regression line and consequently by the wide 95% prediction intervals (see Fig. 4). For example, with an aqueous calcium value of 1 mmol/L within a 24 h postmortem interval, the regression equations indicate that we can be 97.5% confident that an individual cow's antemortem serum calcium value was less than 2.2 mmol/L; however this is still within the range of normal values. Since very few of the hypocalcemic cattle in this study had eyefluid values less than 1 mmol/L, the usefulness of estimating antemortem serum calcium values from postmortem eyefluid appears to be severely limited. A similar result has been reported from a study on human eyefluid (17).

The regression analysis for calcium was limited somewhat by the inability of the chemistry analyzer employed to detect calcium values of less than 1 mmol/L. Thus, the few cases where the serum or eyefluid calcium concentration was less than 1 mmol/L could not be included in the regression analysis. These low values however did follow the trend established by the other calcium data, i.e. an eyefluid calcium value less than 1 mmol/L corresponded to serum calcium values below the normal range. Because these missing points followed the trend, their absence from the analysis would not substantially alter the prediction intervals over the range of eyefluid values given. In summary, our data suggest that a value somewhat less than 1 mmol/L would be required to make a postmortem diagnosis of hypocalcemia in the bovine with a high degree of confidence.

GLUCOSE

A significant trend and a strong correlation was shown to exist between serum and eyefluid glucose

concentrations at both 0 and 24 h postmortem intervals. However, the continuous decrease of the glucose concentration over the postmortem interval (due to continued utilization) severely limits its use for estimating antemortem serum glucose levels within a specific postmortem time interval. This finding is similar to those observed previously in studies on humans (18). Its use could be limited to cases of sudden death in which confirmation of suspected diabetic coma or hyperinsulinemia was required. In these cases, the samples would have to be taken and analyzed within a short period after death. Because glucose values are steadily falling after death, it would be unlikely to have a falsely elevated glucose level, however a low glucose level would have to be interpreted with caution.

CHOLESTEROL, BILIRUBIN, ENZYMES AND PROTEIN

Knowledge of the blood ocular barriers indicates that large molecules present in the serum are greatly restricted in their entry into the ocular fluid (19-22). This study has shown that only a small percentage of the relatively large serum molecules such as cholesterol, bilirubin, most enzymes and protein pass into the eyefluid through the blood-ocular barrier. A few of the serum enzymes, namely amylase, creatine kinase and aspartate aminotransferase, were found in substantial concentrations in postmortem eyefluid. Their presence appears to be associated with their release into the eyefluid from the cytoplasm of the surrounding cells due to damage of the plasma membranes during eyefluid collection or associated with autolysis. Some studies on human eyes have reported similar findings (10,23). For all these large molecules, regardless of their postmortem eyefluid levels, there was a poor correlation between serum and eyefluid values.

The present study demonstrated that postmortem eyefluid can be used to accurately estimate antemortem serum levels of urea and creatinine in cats, dogs and cattle in the first 24 h after death. Although either aqueous or vitreous fluid can be used in this regard, the aqueous fluid was at least as accurate as the vitreous and was much easier to collect and analyze. At extremely elevated concentrations of urea and creatinine there was increased variability in the relationship between serum and eyefluid values. This may relate to the lag time for the equilibration of changing concentrations of these small molecules across the blood-ocular barriers which has been previously demonstrated experimentally (5).

Additionally, this study has shown that while antemortem serum calcium levels can be estimated from eyefluid values in mature cattle, this cannot be done with a high degree of accuracy.

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