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Clinical, radiographical and haematobiochemical changes in growing dogs with retained cartilage core

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ABSTRACT

The study was conducted in 11 growing dogs of up to 1 year affected with retained cartilage core. All the 11 cases were observed for the clinical signs of the disease and subjected to different haemato-biochemical and hormone analysis to find out the status of the disease. The incidence recorded was 2.87% (among the 387 growing dogs). Great Dane (7) was more commonly affected breed than others. Majority of the affected dogs (7) were less than 3 months. Males and females were almost equally affected. Lameness, focal hard swellings proximal to the carpus, bowing of the radius, carpal valgus and stunted growth were the clinical signs observed in most of the dogs. A radiolucent inverted cone shaped area in the distal metaphysis of ulna was the characteristic radiographic sign. Hyperphosphataemia, low Ca: P ratio, hypovitaminosis-D_3 and increased alkaline phosphatase activity were the common biochemical findings. The diagnosis was made on the basis of radiographical signs. The dogs were treated with preparations of vitamin D_3, calcium and multivitamins and an appreciable improvement in 7 dogs could be noticed within 45 days.

Key words: Dogs, Haematobiochemical changes, Radiographical changes, Retained cartilage core

Bone growth abnormalities particularly of forelimb(s) are common in the large breed dogs, which result in bowing of limb(s), lateral deviation of the paw, varying degree of lameness and stunted growth (Burk and Ackerman 1986). In a long bone, longitudinal bone growth occurs by a process of endochondral ossification of the growth plate and an abnormality in this process is thought to produce the lesion of retained cartilage core, which is responsible for the development of forelimb deformity. Retained cartilage core (RCC) is more frequently reported in the fast growing large breed dogs like Great Danes (Konde 1994, Montgomery 2002). Though the radiographic picture of retained cartilage core like the presence of an inverted radiolucent cone at the distal ulna has been very well described in the literature (Montgomery 2002, Farrow 2003), plasma biochemical profile and therapy of retained cartilage core have not been described earlier. Further, there is no study on the incidence of RCC in Indian subcontinent. The present study was, therefore, aimed to record the incidence, clinical signs, radiographic features and haemato-biochemical profile in growing dogs affected with RCC and to record their response to calcium and vitamin D_3 therapy.

MATERIALS AND METHODS

The growing dogs aged up to one year, presented to the referral polyclinic of Indian Veterinary Research Institute, Izatnagar (UP) during April 2002 to March 2003 were examined for the presence of any skeletal growth abnormality. Retained cartilage core (RCC) was found in 11 dogs that made the subject for the present study.

Detailed history regarding the age, sex, breed, duration of illness, diet, litter size, previous treatments etc. were recorded. All the affected dogs were subjected to clinical, radiographical and haemato-biochemical examinations. Clinical signs like pain, lameness, bowing of the radius-ulna, enlargement of the distal metaphysis of the radius-ulna, hind quarter weakness, angular deformity and carpal valgus were recorded on the day of admission and at 15 days interval thereafter. All the clinical signs were graded on a 0–3 scale as normal (0), mild (1), moderate (2), and severe (3). The angle of carpal valgus was measured as per the method of Ramadan and Vaughan (1978). Physiological parameters like respiratory rate (RR), heart rate (HR) and rectal temperature (RT) were also recorded on day 1 and at an interval of 15 days, when the animals were presented to the clinic for follow up examination.

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Radiographs of the fore limbs were taken in antero-posterior (A/P) and medio-lateral (M/L) planes on the day of admission and subsequently at an interval of 15 days up to 45 days. Diagnosis of the case was made on the basis of changes seen on radiographic examination.

The haematological parameters viz. haemoglobin (Hb), differential leukocyte count (DLC) and packed cell volume (PCV) were estimated by standard procedures (Jain 1986). The plasma calcium (Webster 1962), phosphorus (Gomorri 1942), magnesium (Gindler and Dwayne 1971), total protein and albumin (Dumas *et al.* 1971), and alkaline phosphatase (Kind and King 1954) were estimated by standard methods. Vitamin D_3 was estimated by RIA method (Bouillon *et al.* 1987), whereas iPTH (Bouillon *et al.* 1990) and calcitonin were estimated by IRMA method (Rougier *et al.* 1983). All the affected dogs were subjected to haemato-biochemical evaluation on the day of presentation and at an interval of 15 days up to 45 days. The plasma value of different parameters on the days of admission was compared with plasma value of the normal dogs (Kaneko *et al.* 1997, Kushwaha 2003), whereas plasma values obtained on subsequent days were compared with the previous plasma values of the same dogs obtained on the day of admission.

Treatments given to all the 11 dogs included preparation of calcium, vitamin D_3, multivitamins and minerals. Vitamin D_3 (vit.D_3 in oily base 300,000 IU (7.5 mg) and 600,000 IU (15 mg) per mL)] was administered intramuscularly @ 3 lakh IU in the dogs weighing up to 10 kg and 6 lakh IU in the dogs weighing more than 10 kg at weekly intervals for a minimum of 3–4 weeks. Syrup or tablet containing elemental calcium 250 mg, vitamin D_3 125 IU @ ½ tsf bid or 1 tab bid orally was used for calcium supplementation for a minimum of 30 days. The anti-inflammatory-analgesic drug nimesulide was administered orally @ ½ tab (50 mg) bid for 1 week.

The incidence (age, sex, breed etc.) was expressed in percentage. The data of different haemato-biochemical parameters recorded in all the dogs on day 1 were expressed in mean±SE. Some of the dogs were not presented for follow up examinations at definite intervals. The data obtained from 4 dogs, which were presented at regular intervals (up to 45 days) were analyzed for haemato-biochemical parameters by paired 't' test (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

In the present study, RCC was recorded in 11(2.87%) out of 387 growing dogs. Great Dane was the most commonly affected breed (63.63%) followed by non-descript (18.18%)), German Shepherd and Doberman (9.09% each) breeds. Johnson (1981) and Konde (1994) has also reported that retention of cartilage occurs in young large and giant breed of dogs like Great Dane, where physeal hypertrophic chondrocytes fail to mature and mineralize adjacent matrix and accumulate in large columns in the primary spongiosa. Johnson *et al.* (1995) have observed that signs of RCC appear at the age of 5 months and lesions are usually bilateral. However, in the present study dogs aged up to 3 months were affected more (63.63%) followed by those aged between 3-6 months (36.36%), which suggest that signs of RCC could be seen in very young dogs also. Among the dogs affected with RCC, 6 were males and 5 were females.

The dog owners reported that the clinical signs started to appear about 15–30 days prior to presentation to the clinics. Eight dogs suffering from RCC were vaccinated and dewormed prior to presentation. Six dogs were fed on milk, bread and chapati. In remaining 5 dogs, meat and eggs were also given. Two Great Dane dogs also had femoral fracture, where osteopenic bone probably predisposed for fracture. The status of littermates and dam was not known to the owners in any case. In most of the dogs, general clinical signs seen were pain, lameness, broadening of distal metaphyses of radius-ulna, bowing of the radius, hind quarter weakness and carpal valgus (Fig. 1). Similar signs were also reported by Johnson *et al.* (1995) and Farrow (2003). Mild pain on palpation of the affected bone was recorded in 5 cases and moderate pain in 1 case. Mild and moderate lameness was seen in 2 cases each and severe lameness in 1 case during progression. Retarded or discontinued growth at the distal ulnar physis leads to cranial bowing of radius, due to shortened ulna acting as a bowstring. In the present study mild bowing was recorded in 7 cases and moderate bowing was seen in 4 cases, which may result in hyperextended carpus. The radius bows medially, forcing the limb to deviate laterally into valgus. This results in external rotation of paw (Ramadan and Vaughan 1978, Newton 1985), which was noticed in 5 cases. The angle of carpal valgus was mild (5–

Figs 1–2. **1.** A 3½ month-old female Great Dane pup affected with retained cartilage core (RCC) shows bilateral carpal valgus and focal hard swelling proximal to carpus (arrow). **2.** Radiograph of a retained cartilage core (RCC) in medio-lateral view shows an inverted 'V' shaped cone extending from physis deep into metaphysis (arrow).

10°) in 3 cases, moderate in remaining 2 cases, 1 had 25° and another had 30° angle of carpal valgus. Mild broadening of distal metaphysis was seen in 4 cases and moderate broadening was seen in 7 cases. Mild and moderate hindquarter weakness was seen in 3 cases each. The mean rectal temperature, respiration rate and pulse rate recorded were within the normal physiological range.

The characteristic radiographic feature observed in all the cases of RCC was the presence of a radiolucent inverted cone shaped area in the distal metaphysis and broadening of metaphysis (Fig. 2). The cone shaped radiolucent area was seen mostly at the distal metaphysis of ulna which is due to failure in endochondral ossification. This results in slow growth of ulna which causes variable degree of bowing of radius (Montgomery 2002, Farrow 2003).

The mean ±SE of different haemato-biochemical parameters recorded in plasma of all the 11 growing dogs are shown in Table 1. Haemoglobin (106±5.89g/L) was found below normal physiological range (120–180 g/L) for the species; 4 dogs showed normal value and remaining 7 dogs had below normal range. The packed cell volume (0.34±0.02 L/L) was also below normal physiological range (0.37–0.55 L/L); 5 dogs had normal levels and 6 dogs had low levels. The differential leukocyte count was found normal, although 3 dogs showed neutropenia and lymphocytosis. This indicates that the RCC does not influence the haematological parameters. Plasma biochemical status of the RCC affected dogs revealed normal calcium level (mean 2.404±0.321 mmol/L), however, hypercalcaemia and hypocalcaemia were recorded in 3 cases each. The plasma phosphorus level was found high in all the 11 cases with the range of 2.23–7.93 mmol/L. The Ca: P ratio was found below normal (0.756 ±0.151); however, 2 dogs, despite disturbed plasma Ca and P level, showed normal Ca: P ratio. The significant reduction in Ca: P ratio was mainly due to hyperphosphataemia. Earlier studies showed that both high calcium and high phosphorus level might result in the failure of endochondral ossification (Hazewinkel *et al.* 1987, Nap *et al.* 1993). In this study the plasma magnesium was found normal (0.795 ±0.135 mmol/L), however, 4 dogs showed hypomagnesemia and 2 dogs showed hypermagnesemia. The total protein, albumin, globulin and A:G ratio were below normal (46.15 ±2.87 gm/L), normal (29.53 ±3.44 gm/L), below normal (16.63 ±1.91 gm/L) and above normal (2.26 ± 0.532) respectively. The low level of plasma protein indicated that dogs were not fed on proteinous diets. The mean plasma alkaline phosphatase (370.36±132.4 U/L) showed marked increase in the cases of retained cartilage core. Increased alkaline phosphatase activity is probably due to increased osteoblastic activity, which is a sign of incomplete mineralization of organic matrix (Johnson *et al.* 1988, Grondalen *et al.* 2004). The iPTH level increased (10.67±5.16 pg/mL), indicating hyperparathyroidism. The available literature does not show any correlation between RCC and secondary hyperparathyroidism. This may be attributed to hyperphosphataemia, which reduces blood calcium levels, which in turn stimulate parathyroid glands. Reduced vitamin D_3 level was recorded in the present study. Vitamin D_3 and its metabolites normally increase the absorption of calcium and phosphorus from the intestine, thereby maintain adequate level of these electrolytes in the extra cellular fluid that permits proper mineralization of bone matrix. In young animals, vitamin D_3 is required for orderly growth and mineralization of cartilage in growth plate. In the deficiency of vitamin D, mineralization of cartilagenous matrix fails to occur and hence the formation of bone is blocked (Hazewinkel and Tryfonidou 2002). The

Table 1. Haematobiochemical parameters in plasma of the dogs affected with retained cartilage core (RCC)

Breed	Hb (g/L)	PCV (L/L)	N (%)	L (%)	M (%)	E (%)	B (%)	Ca (mmol/L)	P (mmol/L)	Ca:P	Mg (mmol/L)	TP (g/L)	A (g/L)	G (g/L)	A:G	ALP (U/L)	PTH (pg/mL)	CT (pg/mL)	Vit-D_3 (mmol/L)
GD	105	0.33	65	30	2	2	1	0.838	3.21	0.26	0.80	60.37	45.76	14.67	3.13	84.09	3.94	4.07	44.85
GD	120	0.38	51	40	8	1	0	4.38	2.91	1.50	0.54	48.50	30.00	18.50	1.62	168.06	6.89	52.9	83.87
GD	59	0.20	48	47	3	2	0	2.80	7.93	0.35	1.69	44.10	31.52	12.67	2.42	144.77	–	99.6	–
GD	98	0.30	66	22	12	0	0	2.22	3.07	0.72	0.36	48.40	32.50	15.90	2.04	137.25	1.27	4.45	247.7
ND	105	0.32	70	25	3	2	0	1.50	3.25	0.44	0.41	60.37	48.40	11.97	3.64	190.22	–	–	–
DB	115	0.36	72	20	6	1	1	1.44	2.59	0.55	0.41	46.00	27.27	18.80	1.44	141.26	5.17	5.39	220.12
GSD	115	0.43	65	20	8	6	1	–	–	–	–	–	–	–	–	–	–	–	–
GD	120	0.38	67	25	7	1	0	2.16	2.23	0.96	0.95	41.16	17.53	23.36	0.74	737.13	35.6	35.1	140.06
GD	125	0.38	70	18	9	3	0	3.09	3.14	0.97	1.01	39.61	15.89	23.71	0.64	603.50	11.2	41.9	215.00
GD	85	0.29	65	30	5	0	0	3.09	2.34	1.31	1.21	30.50	26.48	0.431	6.12	119.85	–	45.8	220.00
ND	120	0.47	47	43	8	2	0	2.53	5.06	0.50	0.57	42.50	20.00	22.50	0.88	1377.5	–	–	86.00
Mean (±SE)	106.09± 5.89	0.34 ±0.02	62.36 ±2.75	29.09 ±3.02	6.45 ±0.91	1.81 ±0.50	0.27 ±0.14	2.404 ±0.32	3.57 ±0.54	0.756 ±0.131	0.795 ±0.135	46.15 ±2.87	29.53 ±3.44	16.63 ±1.9	12.26 ±.53	370.36 ±132.4	10.67 ±5.16	36.15 ±11.50	157.825 ±27.41
Ref. range	120-180#	0.37–0.55#	60–70#	12–30#	3–10#	2–8#	0–1#	2.20–3.00#	1–2#	1.2–2.0#	0.74–0.99#	0.74–0.99#	26–40#	21–37#	0.702–1.904#	20–156#	2.04 ±0.50#	19.12 ±8.46#	235.65 ±31.96#

GD- Great Dane, ND-non-descript, DB-Doberman, GSD- German Shepherd; # Kaneko *et al.* (1997); * plasma value obtained in normal growing dogs recorded in the present study.

Table 2. Mean (±SE) values of plasma levels of different biochemical parameters at different intervals of treatment in dogs affected with RCC (n=4)

	Interval (days)			
Parameters	0	15	30	45
Ca (mmol/L)	2.07±0.45	2.17±0.38	2.63±0.23	2.74±0.08P
P (mmol/L)	2.74±0.19	2.80±0.65	2.03±0.40	1.66±0.10*
Ca:P	0.79±0.18	1.25±0.55	1.41±0.31	1.70±0.14
Mg (mmol/L)	0.87±0.17	0.55±0.14	0.51±0.08	0.67±0.10
TP (g/L)	44.20±6.28	50.99±8.84	48.18±4.46	53.31±5.24
A (g/L)	28.85±6.21	33.99±7.21	33.82±6.91	32.03±4.67
G (g/L)	15.35±4.12	17.01±4.97	14.36±4.28	21.28±1.72
A:G	2.85±1.21	2.67±0.89	2.71±0.04	1.52±0.23
ALP (U/L)	237.05±122.33	264.12±96.84	173.84±110.22*	121.61±60.82
PTH (pg/mL)	12.73±6.15	30.87±10.12	–	–
CT (pg/mL)	13.96±9.32	29.61±3.69	–	–
Vit-D_3 (nmol/L)	122.22±38.03	208.39±78.17	–	–

*Differ significantly (P<0.05) from base value.

exact cause of RCC is not clearly understood (Konde 1994, Farrow 2003) and it is thought to be due to failure in endochondral ossification (maturation and mineralization of matrix), which might occur due to increased calcium and increased phosphorus intake, hypercalcitonism and disorders in vitamin-D_3 metabolism (Hedhammar *et al.* 1974, Nunez *et al.* 1974, Schoenmakers *et al.* 2000). The epiphyseal plate becomes irregularly thickened as progressively more cartilagenous matrix accumulates and fails to mineralize. In the present cases, the reduced level of vitamin D_3 and disturbed Ca: P ratio might have caused defects in mineralization resulting in retention of cartilage core.

The animals with RCC showed varied response to treatment. Among the 8 dogs presented for follow-up treatment, 5 dogs showed moderate improvement clinically in about 30–45 days, 2 dogs showed slight improvement, whereas another dog did not show any improvement till 30th day. Response to treatment in the remaining 3 dogs was not known as the owners did not turn-up for follow-up treatment. Rectal temperature, heart and respiratory rates recorded at different post-treatment intervals did not show any significant change (P>0.05).

Follow-up radiographs could be taken in only 6 cases. Among these, slight improvement in terms of ossification of retained cartilage was seen in 2 cases and moderate improvement in another case. However, no improvement was seen in 3 cases up to 45 days of observation.

Mean haemoglobin, PCV and DLC values recorded at different intervals did not show any significant change. The plasma biochemical parameters estimated at different intervals of treatment are shown in Table 2. Plasma calcium increased at different intervals up to day 45. Mean phosphorus value recorded on day 0 was above the normal range. It decreased gradually to reach the normal range on day 45. Ca: P ratio, which was below normal on day 0, increased gradually to attain normal range on day 30 and it remained within normal range on day 45. The supplementation of vitamin D_3 and calcium might have corrected the calcium: phosphorus ratio and hence improved the condition. Plasma Mg values recorded at different treatment intervals were below the base value recorded on day 0. Mean plasma total protein increased at different treatment intervals. Albumin and globulin also increased at different intervals except on day 30, where globulin slightly decreased. A: G ratio showed a decreasing trend to attain the normal range on day 45. Alkaline phosphatase activity increased on day 15, but significant decrease was noticed on day 30 and subsequently reduced well below the base level on day 45. The decreased alkaline phosphatase activity indicates mineralization of the cartilage osteoid matrix. The mean plasma levels of iPTH, calcitonin and vitamin D_3 increased on day 15 as compared to 0 day values. These results indicate that most of the haemato-biochemical parameters showed signs of improvement following treatment.From the present study, it can be concluded that RCC is a bone disease of young growing dogs occurs probably due to failure in endochondral ossification. Hyperphosphataemia, disturbed Ca: P ratio, low level of vitamin D_3, increased iPTH and alkaline phosphatase are significant changes in the blood. Supplementation of vitamin D_3 and calcium preparations may be useful in the treatment of RCC, though the response is not uniform.

REFERENCES

Bouillon R A, Auwerx J D, Lissens W D and Pelemans W K. 1987. Vitamin D status in the elderly, seasonal substrate deficiency causes 1,25- dihydroxycholecalciferol deficiency. *American Journal of Clinical Nutrition* **45**: 755–63.

Bouillon R, Caopmans W, D E Groole D E H, Radous D and Eliard P H. 1990. Immunoradiometric assay of parathyrin with polyclonal and monoclonal region -specific antibodies. *Clinical*

Chemistry **36**: 271–76.

Burk R L and Ackerman N. 1986. *Small Animal Radiology: A Diagnostic Atlas and Text* p. 277. Churchill Livingstone, New York, Edinburgh, London, Melbourne.

Dumas B T, Watson W A and Biggs H G. 1971. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinical Chemistry Acta* **31**: 87–96.

Farrow C S. 2003. Congenital and Developmental bone diseases. *Veterinary Diagnostic Imaging The Dog and cat-Vol 1*. pp. 169. Mosby, United State.

Gindler E M and Dwayne A H. 1971. Colorimetric Determination with bound "Calmagite" of magnesium in human blood serum. *Clinical Chemistry* **17**: 662.

Gomorri G. 1942. A modification of calorimetric phosphorus determination. *Journal of Laboratory Clinical Medicine* **21**: 955–58.

Grondalen J. Trangerud C, Indrebo A and Moe L. 2004. The Skeleton project continues. *Norsk-Veterinaertidssk-rift* **226** (7): 544–45.

Hazewinkel H A W and Tryfonidou M A. 2002. Vit-D_3 metabolism in dogs. *Molecular-and-cellular-endocrinology* **197** (1/2): 23–33.

Hazewinkel H A W, Hackeng W H L, Goedegebuure S A, Voorhout G and Van den Brom W E. 1987. Influence of different calcium intakes on calciotropic hormones and skeletal development in young growing dogs. Front. *Hormone Research* pp. 221–32.

Hedhammar A, Wu F M, Krook L, Schryver H F, De Lahunta A, Whalen J P, Kallfelz f A, Nunez E A, Hintz H F, Sheffy B E and Ryan G D. 1974. Overnutrition and skeletal disease. An experimental study in growing Great Dane dogs. *Cornell Veterinary* **64**: Suppl- 160.

Jain N C. 1986. *Schalm's Veterinary Haematology*. 4th edn. Lea and Febiger, Philadelphia.

Johnson K A, Church D B, Barton R J and Wood A K W. 1988. Vitamin D dependent rickets in a Saint Bernard dog. *Journal of Small Animal Practice* **29**: 657–66.

Johnson K A, Watson A D J and Page R L. 1995. Skeletal diseases. *Textbook of Veterinary Internal Medicine* pp. 2077–03. (Eds) Ettinger S J and Feldman E C. W B Saunders, Philadelphia.

Johnson K A. 1981. Retardation of endochondral ossification at the distal ulnar growth plate in dogs. *Australian Veterinary Journal* **57**: 474–78.

Kaneko J J, Harvey J W and Bruss M L. 1997. *Clinical Biochemistry of Domestic Animals*. 5th edn, pp. 885–05. San Diego, London, Boston, New York, Sydney, Tokyo, Toronto.

Kind P R N and King E J. 1954. Estimation of plasma phosphate by determination of hydrolyzes phenol with aminoantipyrine. *Journal of Clinical pathology* **7**: 322.

Konde J. 1994. Deseases of the Immature skeleton. *Text Book of Veterinary Diagnostic Radiology*. 2nd edn. (Eds) Thrall D E. W B Saunders, Philadelphia. pp. 94–104.

Kushwaha R B. 2003. 'Pathophysiology and Management of Skeletal Abnormalities in Growing Dogs'. M.V.Sc. Thesis, Deemed University, IVRI, Izatnagar–243 122.

Montgomery R. 2002. Miscellaneous orthopaedic Diseasee. *Text Book of Small Animal Surgery*. Vol 2, 3rd edn, pp. 2251–60. (Eds) Douglas Slatter. Saunders, Philadelphia.

Nap R C, Hazewinkel HAW and Van den Borm W E. 1993. Growth and skeletal development in Miniature Poodles fed different levels of calcium: radiographic, histologic, and endocrine aspects. *Utrecht University* pp. 75–93.

Newton C D. 1985. Radial and Ulnar Osteotomy. *Text Book of Small Animal Orthopaedics*, pp. 533–44. (Eds) Newton C D and Nunamaker D M. J B Lippincott, Philadelphia.

Nunez E A, Hedhammar A, Wu F M, Whalen J P and Krook L. 1974. Ultrastructure of the parafolicular (C) cells and the parathyroid cell in growing dogs on a high calcium diet. *Laboratory Invest* **31**: 96–108.

Ramadan R O and Vaughan L C. 1978. Premature closure of the distal ulnar growth plate in dogs: A review of 58 cases. *Journal of Small Animal Practice* **19**: 647–67.

Rougier P H, Calmetes A, Laplanche A, Travagli J P, Lefevre M, Parmentier C, Milhaud G and Tubiana M. 1983. The values of calcitonin and carcinoembryonic antigen in the treatment and management of non familial medullary thyroid carcinoma. *Cancer* **51**: 856–62.

Schoenmakers I, Hazewinkel H A W, Voorhout G, Carlson C S and Richardson D. 2000. Effects of diets with different calcium and phosphorus contents on the skeletal development and blood chemistry of growing Great Danes. *Veterinary Record* **147**: 652–60

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th edn. Oxford and IBH Publishing Co., New Delhi.

Webster J W W. 1962. A simple microspectrophotometric method for the determination of serum/plasma calcium. *American Journal of Clinical Pathology* **31**: 330–33.