Calcium plays two important physiological roles in the avian subject. First, it provides the structural strength of the avian skeleton by the formation of calcium salts. Second, it plays vital roles in many of the biochemical reactions within the body via its concentration in the extracellular fluid. The control of calcium metabolism in birds has developed into a highly efficient homeostatic system, able to quickly respond to increased demands for calcium required for both their ability to produce megalecithal eggs and their rapid growth rate when young. Parathyroid hormone (PTH), metabolites of vitamin D, and calcitonin, regulate calcium as in mammals, acting on the main target organs of liver, kidney, gastrointestinal tract and bone. Estrogen and prostaglandins also appear to have an important role in calcium regulation in the bird.

There are distinct differences between the mammalian and avian systemic regulations of calcium. The most dramatic difference between the two phyletic groups is in the rate of skeletal metabolism at times of demand. The domestic chicken will respond to hypocalcemic challenges within minutes compared with response to similar challenges in mammals that can take over 24 hours. This is best demonstrated by an egg-laying bird where 10% of the total body calcium reserves can be required for egg production in a 24-hour period. This calcium required for eggshell production is mainly obtained from increased intestinal absorption and a highly labile reservoir found in the medullary bone, normally visible radiographically in female birds. The homeostatic control of the medullary bone involves estrogen activity. Due to the rapid metabolic responses in the avian skeleton, it has become a common model for skeletal studies concerning the regulation of calcium.
Abnormalities of calcium metabolism are common in the poultry industry, leading to poor production and growth defects, in particular, tibial dyschondroplasia in broiler chickens kept indoors. Due to the economic status of the poultry industry, calcium metabolism has been well researched in production birds, including the assessment of the importance of dietary calcium, vitamin D₃ and their interaction with ultraviolet light, especially in the UV-B spectrum (315-280 nm).⁸⁻¹³,²⁻¹⁴

In captive pet birds, disorders of calcium metabolism also are common, ranging from osteodystrophy in young birds (due in part to the greater calcium requirement in young growing birds) to hypocalcemic seizures and egg binding in adults.⁵⁻⁶,⁷,¹⁶ Although African grey parrots (Psittacus erithacus) are considered to be especially susceptible to disorders of calcium metabolism, problems have been reported in a variety of captive species. The husbandry requirements with respect to calcium have been poorly researched in pet birds in the past, and much of our present knowledge is extrapolated from work with poultry.

**Calcium Regulation in Birds**

**CALCIUM**

Calcium exists as three fractions in the avian serum: 1) the ionized salt, 2) calcium bound to proteins, and 3) as complexed calcium bound to a variety of anions (citrate, bicarbonate and phosphate). Ionized calcium is the physiologically active fraction of serum calcium, with a role to play in bone homeostasis, muscle and nerve conduction, blood coagulation and control of hormone secretions such as vitamin D₃ and parathyroid hormone. The majority of the protein-bound calcium is bound to albumin and considered to be physiologically inactive. Any change in serum albumin levels will directly affect the total calcium level. The binding reaction is strongly pH-dependent, so an increase or decrease in pH will respectively increase or decrease the protein-bound calcium fraction. The significance of the small, complexed calcium fraction is unknown at the present time, but any disease state that affects the levels of the binding anions would be expected to significantly alter calcium levels.⁸⁻¹¹,²⁻¹²

The ionized calcium level appears to be maintained within a tight range in the normal individual compared with the total calcium level, which fluctuates with serum protein concentrations, and any major change in the serum ionized calcium level is likely to be of pathologic significance. Ionized calcium concentrations are regulated by the interaction of PTH, vitamin D₃ metabolites and calcitonin in response to changing demands.

**VITAMIN D₃**

The vitamin D₃ metabolism of birds has been extensively reviewed.¹³,¹⁴,²⁻¹⁵ Following the identification of vitamin D₃ metabolic pathways, vitamin D₃ now is considered to be a steroid hormone exhibiting feedback relationships in response to calcium requirements.⁶ The main role of vitamin D₃ is in the control of bone metabolism by strictly regulating mineral absorption, but more recently it has been found to have profound effects on the immune system, skin and cancer cells.¹ Due to the importance of the vitamin’s role in bone development and the requirement for UV light in the metabolic conversion of provitamin D₂ to vitamin D₃, the commercial availability of dietary vitamin D₃ has been essential to allow the indoor production of poultry.⁷ Vitamin D occurs naturally in plants as ergocalciferol (vitamin D₂) and this will function in mammals as well as vitamin D₃, but birds do not respond well to dietary vitamin D₂. This is due to increased renal clearance of vitamin D₂ rather than lack of intestinal absorption.¹⁰

It has been established that the domestic chicken secretes 7-dehydrocholesterol (provitamin D₃) onto the featherless skin. It has recently been shown that there are ten times more provitamin D₃ on the featherless leg skin than on the back, indicating the importance of this area for vitamin D₃ metabolism.²⁻¹⁰ Conversion of the provitamin D to cholecalciferol (vitamin D₃) occurs by a UV-B light-dependent isomerization reaction. Cholecalciferol is a sterol prohormone that is subsequently activated by a two-stage hydroxylation process.

Cholecalciferol is initially metabolized to 25-hydroxycholecalciferol in the liver; 25-hydroxycholecalciferol is transported to the kidney via carrier proteins and converted to either 1,25-dihydroxycholecalciferol or 24,25-dihydroxycholecalciferol, the active metabolites of cholecalciferol in the domestic fowl. The most significant active metabolite of vitamin D₃ in domestic chickens is 1,25-dihydroxycholecalciferol, which displays a hypercalcemic action.⁸⁻¹⁰ While not significant in mammals, 24,25-dihydroxycholecalciferol is thought to have a special active role in the laying hen.⁸ The synthesis of 1,25-dihydroxycholecalciferol is tightly regulated by PTH, depending on the calcium status of the bird. The metabolite regulates calcium absorption across the intestinal wall by inducing the formation of the carrier protein calbindin-D28k. The presence of this protein reflects the ability of the intestine to absorb calcium. Calbindin-D28k also is found in the wall of the oviduct, and levels rise during egg laying, although this process is not directly related to the actions of 1,25-dihydroxycholecalciferol. Bone formation is stimulated by 1,25-dihydroxycholecalciferol, which induces osteoclastin production from osteoblasts. In egg-laying birds, 30 to 40% of the calcium required...
for eggshell formation is acquired from medullary bone. The control of this highly labile pool of calcium involves both 1,25- dihydroxycholecalciferol and estrogen activity. The function of vitamin D₃ is reliant on the presence of normal vitamin D₃ receptors. The receptors have been found in bone, skin, skeletal muscle, gonads, pancreas, thymus, lymphocytes and pituitary gland.

If vitamin D₃ is supplied in the diet, it can be absorbed with 60 to 70% efficiency. In most situations, there is a compromise between the amount of dietary vitamin D₃ administered (on the basis of economy and toxicity) and UV light provided for natural vitamin D₃ formation.

PARATHYROID HORMONE (PTH)

The parathyroid glands produce PTH from the chief cells in response to a low ionized calcium level. As in mammals, PTH has an essentially hypercalcemic action in birds, and if a parathyroidectomy is performed in quail, the birds respond with severe hypocalcemia unless calcium is given in the diet. Birds appear more sensitive to PTH than mammals, reacting to intravenous injections of the hormone within minutes with a rise in blood calcium levels. The blood ionized calcium level feeds back on the chief cells in the parathyroid gland to tightly control secretion of the hormone. The hormone uses the kidney and the bone as its main target organs in birds. In the bone, the hormone has rapid effects measurable within 8 minutes of administration compared with hours in mammals, so PTH is probably at least partially responsible for the speed of calcium metabolism in the bird.

PTH directly stimulates osteoclasts to resorb bone. The hormone also will actively stimulate osteoblast activity, and it is thought that PTH-stimulated osteoblasts regulate osteoclast activity, providing the precise control system necessary in avian skeletal turnover.

PTH binds to osteoclasts, increasing bone resorption by stimulating their metabolic activity and division. PTH also has direct influence on both calcium and phosphorus excretion in the bird. Calcium excretion is increased and phosphorus decreased following parathyroidectomy. These changes can be reversed by injections of PTH.

PTH has a different structure in birds than in mammals. It consists of an 88-amino acid chain, compared with 84 in mammals, and there is a lack of homology between the two molecules. The greatest similarity is found in the 1-34 segment of the chain, which also is responsible for much of the activity of the hormone. Unfortunately, this segment has a short half-life, and mammalian assays tend to assess the middle and carboxyl-terminal regions of the amino acid chain where homology with avian PTH is poor. This may explain why PTH has appeared difficult to measure in the avian subject and is poorly researched at the present time, although circulating levels are believed to be low compared with mammals. An inverse relationship has been found between PTH concentrations and ionized calcium levels in the laying chicken, indicating that PTH has at least an important role in calcium regulation during this period. This response is very efficient during egg production, with PTH levels increasing due to the greater demand for calcium. A recent study in African grey parrots investigating hypocalcemia has used a mammalian 1-34 PTH enzyme-linked immunosorbent assay with consistent results.

PARATHYROID HORMONE-RELATED PEPTIDE (PTHrP)

PTHrP is a second member of the PTH family, originally discovered as a cause of hypercalcemia in malignancy in man and now the subject of much clinical research. PTHrP has three isoforms of 139, 141 and 173 amino acids, all with identical sequences through to amino acid 139. The hormone has distinct structural homology with PTH, suggesting a common ancestral gene. There is distinct homology between the structure of mammalian and avian PTHrP in the 1-34 segment, as has been found with PTH. PTH and PTHrP share a common receptor. Many tissues in the chicken embryo contain levels of PTHrP. In chickens as in man, PTHrP is thought to play many regulatory and developmental roles in a variety of tissues. Concentrations of PTHrP have been shown to rise in the shell gland of the chicken during the calcification cycle, affecting smooth muscle activity in the gland. Levels of PTHrP return to normal once the egg has been laid. PTHrP has been shown to have effects on bone resorption in the chicken embryo.

ULTIMOBANCHIAL GLANDS

The ultimobranchial gland in birds produces calcitonin. It is a 32-amino acid hormone that exerts an essentially hypocalcemic effect in response to rising serum ionized calcium levels. The levels of circulating calcitonin in birds, amphibians and possibly sub-mammals are high and readily detectable compared with PTH. The bioactivity of calcitonin also varies among phylectic groups, with fish calcitonin exhibiting the most potent effect. In the bird, calcitonin levels increase following injections of calcium. There is a direct correlation between calcitonin levels and dietary calcium concentrations (and, hence, serum calcium levels). Although calcitonin has been shown in man to exert its hypocalcemic effects mainly by inhibiting osteoclastic bone resorption, its biological action in the bird remains surprisingly unclear despite the high circulating levels of the hormone in this group.
PROSTAGLANDIN
Prostaglandins are powerful facilitators of bone resorption, with hypercalcemic effects similar to PTH and vitamin D\(_3\) metabolites. The osteoclast appears to be the site of action for prostaglandin. Injections of prostaglandin into chickens will produce hypercalcemia, and the use of prostaglandin antagonists will produce hypocalcemia.\(^2\)

ESTROGEN
Estrogens promote the formation of the vitellogenins from the liver. These are lipoproteins that are incorporated into the egg yolk. They bind calcium, and their production is followed by a rise in serum calcium levels. This estrogen-controlled hypercalcemic effect is not seen in mammals and is thought to be due to the need to produce large calcified eggs, requiring a rapidly mobilized source of calcium. Estrogens also influence the mobilization of medullary bone during the egg-laying cycle (and also during the nocturnal fast). The effect of estrogen on avian medullary bone is a large research area due to the importance of estrogen in maintaining bone mass in postmenopausal women.\(^3\)

Investigating Abnormalities of Calcium Metabolism

CALCIUM
The measurement of serum ionized calcium provides a more precise estimate of an individual’s calcium status than does total serum calcium, especially in the diseased patient.\(^8,26\) Unfortunately, the majority of veterinary pathology laboratories at the present time report only a total calcium value measured by spectrophotometer, reflecting the total combined levels of ionized calcium, protein-bound calcium and complexed calcium. This can lead to misinterpretation of calcium results in birds, as any change in protein-bound calcium levels is not thought to have any pathophysiological significance.\(^22,26\)

Measurement of total calcium in an avian patient with abnormal protein levels or acid-base abnormalities would not truly reflect the calcium status of the animal. Any state affecting serum albumin values in the blood will affect the total calcium concentration, leading to an imprecise result. In laying female birds, the serum albumin levels may rise by up to 100%. This would produce an inflated total calcium concentration due to an increased protein-bound calcium fraction, while not affecting the ionized calcium level. The binding reaction between the calcium ion and albumin is strongly pH-dependent, so acid-base imbalances will affect ionized calcium levels. Therefore, a patient with metabolic acidosis would be expected to show an ionized hypercalcemia level due to decreased protein binding. With an alkalotic patient, an ionized hypocalcemia would occur as the protein-binding reaction increases. Ionized calcium levels are therefore considered a far more accurate reflection of the patient’s calcium status, especially in a diseased animal. In mammals, positive correlations have been found between albumin and total calcium levels.\(^8,26\) This enables formulae to be developed that “correct” total calcium levels for fluctuations in albumin levels.

Recent research has suggested these corrected estimates of free calcium are inaccurate in 20 to 30% of cases in mammals.\(^8\) In mammals, the relationship between total calcium and albumin diminishes with the severity of the disease, and the correction formulae are now thought to be less useful. Previous research in psittacine birds found a positive correlation between albumin and total protein in African grey parrots, but not Amazons (\textit{Amazona} spp.);\(^12,15\) more recent research in healthy African grey parrots has not indicated a significant relationship.\(^21,22\) In the majority of cases, the measurement of ionized calcium is preferred in both mammals and birds.

Ionized calcium levels are measured by ion-specific electrodes. Analyzers using ion-selective electrodes are increasingly available for use in veterinary clinics. The methodology employed by the analyzers is based on the ion-selective electrode (ISE) measurement principle to precisely determine the ion values. The analyzers are normally fitted with ISE electrodes to assay the electrolytes sodium, potassium and ionized calcium. Each electrode has an ion-selective membrane that undergoes a specific reaction with the corresponding ions contained within a particular sample. The membrane is an ion exchanger reacting to the electrical charge of the ion, causing change in the membrane potential or measuring voltage, which is built up in the film between the sample and the membrane. A galvanic measuring chain within the electrode determines the difference between the two potential values on either side of the membrane of the active electrode. The potential is conducted to an amplifier by a highly conductive inner electrode. The ion concentration in the sample is then determined by using a calibration curve produced by measuring the potentials of standard solutions with a precisely known ion concentration. Blood samples for ionized calcium assays should be drawn into heparin and analyzed as soon as possible after venipuncture, as changes in the blood pH of the sample will affect the accuracy of the ionized calcium levels (ie, contact with carbon dioxide in the air will reduce the pH of the sample). Despite this, a study in dogs suggests that samples will not be adversely affected if not assayed for up to 72 hours, so it is possible to use external laboratories.\(^17\) This has been the
Fig 5.1 | Effect of sample handling delay on ionized calcium concentrations. There was no significant difference between ionized calcium concentrations in the same sample despite delays of up to 48 hours. The samples were stored at 3-5° C prior to analysis.

Fig 5.2 | Serum ionized calcium concentrations in 80 healthy captive African grey parrots. The ionized calcium concentrations are all within a tight range. The normal range was found to be 0.96-1.22 mmol/L.

The measurement of 25-hydroxycholecalciferol is considered the best assessment of vitamin D₃ status in the avian subject due to a longer half-life than other vitamin D₃ metabolites. There are assays available for the other metabolites of vitamin D₃ and it is important to ensure that any assay has been optimized for the metabolite of interest. Until recently, radioimmunoassays were the
Fig 5.3 | Serum total calcium levels in the same 80 healthy captive African grey parrots as Fig 5.2. The total calcium concentrations were outside the normal range (2.0-3.0 mmol/L) in several birds despite normal ionized calcium levels. This fluctuation in the total calcium concentration was due to protein variations in individual birds and has no pathological significance. This demonstrates the importance of measuring ionized calcium wherever feasible rather than total calcium.

Fig 5.4 | 25-hydroxycholecalciferol concentrations in seed-fed African grey parrots. There was a wide variation in vitamin D levels. It is postulated that this is due to both fluctuations in UV-B levels received by individual birds and low dietary vitamin D. Only commercial assays available for both 25-hydroxycholecalciferol and 1,25-hydroxycholecalciferol. An ELISA test for 25-hydroxycholecalciferol has recently been developed with the advantages of both convenience and economy. This assay has been shown to correlate well with the radioimmunoassays. This has allowed research to be carried out in the Psittacus species. A recent study in African grey parrots (a genus known to suffer from hypocalcemia) indicated a wide variation in 25-hydroxycholecalciferol levels in seed-fed birds (Fig 5.4). Any 25-hydroxycholecalciferol results should be interpreted within the context of the diet and the levels of UV light received by the bird. Recent research in psittacine birds has suggested that serum vitamin D₃ levels can be affected by both varying levels of dietary vitamin D₃ and UV-B light exposure. Blood should be drawn into heparin and preferably frozen immediately after venipuncture until the assay can be performed. The use of vitamin D₃ assays will have an increasing use in avian medicine due to the common presentation of calcium disorders, both in terms of clinical disease and poor reproductive results. Vitamin D₃ assays could possibly be useful for reptiles — another group that suffers problems with calcium metabolism, in many cases due to poor husbandry.
ASSAY FOR PTH

PTH is very labile and any assay requires exacting sample handling to produce good results. Blood should be drawn into EDTA and either assayed immediately or frozen to -70°C within 1 hour of venipuncture. The majority of human measurements involve a two-site radioimmunoassay in an attempt to reduce the interfering effect of the many cleavage products of PTH that have long half-lives. Most human assays concentrate on the middle and terminal segments of the PTH molecule due to the very short half-life of the biologically active 1-34 section. A human research kit for 1-34N section PTH has been used to assay PTH in African grey parrots with consistent results. This study indicated that as ionized calcium levels rose in a group of 40 African grey parrots, PTH levels fell. Unfortunately, the assay for PTH 1-34N is too intricate for routine use at the present time and, in the author’s opinion, intact 1-84 PTH assays are not useful in psittacine birds.8,13,18

Effects of Husbandry on Calcium Metabolism in Psittacine Birds

The effects of altering dietary vitamin D3, calcium or exposure to different levels of UV light have been well researched in poultry.3,4,10,14,21 Deficiencies in any of these parameters will lead to poor production and skeletal disorders such as tibial dyschondroplasia.3 Extensive research has produced published results, enabling the poultry industry to select varying levels of dietary vitamin D3 and calcium in accordance with the amounts of UV supplied. If domestic poultry are receiving no UV light, then all the cholecalciferol must come from the diet.10 The domestic fowl does not have a dietary requirement for vitamin D3 if it receives adequate UV-B radiation.10

Unfortunately, caged birds have not been extensively researched, but most would be expected to receive a poor diet with inadequate UV light. Seed-based mixes traditionally used for feeding captive parrots have low levels of both vitamin D3 and calcium.5,16,20,21 In addition, they can contain high levels of phosphorus that can bind the calcium in phytate complexes.10 Chronic deficiency of vitamin D would be expected to lead to hypocalemia and secondary hyperparathyroidism. Vitamin D is obtained by birds directly from the diet and by the action of UV-B on vitamin D precursors in the cutaneous tissues. Either a deficiency in dietary vitamin D or lack of available UV-B light would be expected to produce a vitamin D-deficient bird, potentially leading to subsequent problems with calcium metabolism.3,10 Hypocalcemia is a common syndrome in avian subjects, with African grey parrots (Psittacus erithacus) in captivity appearing particularly susceptible, although the etiology is still unconfirmed.5,6,7,10 Affected birds present clinically with a variety of neurological signs, ranging from slight ataxia to seizures, which respond to calcium therapy.21,22 In adult females, egg binding or poor reproductive performance is a common presentation. In young African grey parrots, osteodystrophy also is a common presenting clinical sign (Figs 5.5-5.8). The growing birds can be affected by bony deformities that range from subtle radiographic changes to obvious gross deformities in both the long bones and spinal column. Radiography has been routinely used to demonstrate juvenile osteodystrophy in many other species of birds. In a recent study,
Fig 5.7 | An adult African grey with osteodystrophy. The bird has characteristic bend- ing in the tibiotarsi. The condition was successfully corrected by osteotomy and fixation of both legs.

Fig 5.8 | Ventral dorsal radiograph of the bird in Fig 5.7. There is severe deviation in both tibiotarsi with folding pathological fractures. There also is evidence of abnormal skeletal development in both wings.

16 of 34 feather-plucking birds examined radiographically as part of a normal work-up revealed evidence of osteodystrophy. The condition will be permanent and deteriorates as the birds increase in weight, which puts increased pressure on the deformed bones and frequently requires corrective surgery.

A recent study in African grey parrots has shown that it is possible to produce changes in calcium parameters by varying husbandry conditions as in poultry. The study has demonstrated the effects of feeding a formulated nugget diet with increased levels of vitamin D₃ and calcium compared with a seed-fed control group. The nugget diet produced a statistically significant increase in both the ionized calcium levels and 25-hydroxycholecalciferol levels over the seed-fed group (Figs 5.9, 5.10). This was reflected in improved breeding performance in the nugget-fed group. These young birds developed without radiographic evidence of osteodystrophy.

In the same study, both the seed- and nugget-fed groups revealed a significant increase in 25-hydroxycholecalciferol and ionized calcium concentrations when they were exposed to artificial UV-B radiation (Figs 5.11, 5.12). The nugget-fed group under the same UV levels revealed significantly higher concentrations of vitamin D₃ and ionized calcium over the seed control group. The initial observations from this study suggest that UV light levels also are important factors in the vitamin D metabolism of captive birds in addition to diet. In the future, it will be possible to supply diets with varying amounts of vitamin D depending on the ambient supply of UV-B light available to captive birds. Initial work by the author with South American species has shown that they do not appear to be as dependent as African grey parrots on UV-B light for maintaining adequate vitamin D levels. This might explain the prevalence of disorders of calcium metabolism in African grey parrots compared with other psittacine birds. South American rain forest has a dense canopy compared with African forests, reducing the UV-B light levels reaching the birds, so their vitamin D metabolism would be expected to be different from those of African birds.

These studies show that it is important that breeders feed their birds a diet that contains adequate calcium and vitamin D. It also is important to supply adequate UV-B radiation to captive birds kept indoors. This would be expected to help prevent the many expressions of hypocalcemia seen specifically in African grey parrots and other susceptible species. This includes the parent birds, because the female birds lay down medullary bone starting about 6 weeks prior to laying eggs. The nutrition of female birds appears to affect the early development of the chicks. In a recent study in African grey parrots, parent-reared juveniles showed radiographic evidence of osteodystrophy at 8 weeks if the parents were fed seed-based diets. The young from nugget-fed parents had no radiographic signs of osteodystrophy at 12 weeks (Figs 5.13, 5.14). See Chapter 4, Nutritional Considerations, Section I Nutrition and Dietary Supplementation.
Fig 5.9 | Effect of diet on ionized calcium concentrations in African grey parrots. There was a significant increase in the serum ionized calcium concentrations in birds fed a formulated nugget diet compared with the same birds fed a seed-based diet.

Fig 5.10 | Effect of diet on vitamin D concentrations in African grey parrots. There was a significant increase in the 25-hydroxycholecalciferol concentrations in birds fed a nugget diet compared with the same birds fed a seed-based diet.

Fig 5.11 | Effect of UV-B supplementation on ionized calcium concentrations independent of diet fed. There was a significant increase in ionized calcium concentrations in both the seed- and nugget-fed groups exposed to 12 hours of UV-B light in every 24 hours.
**Fig 5.12** | Effect of UV-B supplementation on 25-hydroxycholecalciferol concentrations in African grey parrots independent of diet fed. There was a significant increase in 25-hydroxycholecalciferol concentrations in both seed- and nugget-fed groups exposed to 12 hours of UV-B light in every 24 hours.

**Fig 5.13** | Lateral radiograph of a 12-week-old African grey parrot with normal skeletal development. The bird was parent-reared until weaning by adults fed a formulated parrot food until weaning.

**Fig 5.14** | Ventral dorsal radiograph of a 12-week-old African grey parrot with normal skeletal development. The bird was parent reared until weaning by adults fed a formulated parrot food.
References and Suggested Reading
