Effects of UVB radiation on calcium metabolism in psittacine birds

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The effects of providing ultraviolet (UV) radiation (285 to 315 nm, ultraviolet B) on calcium metabolism in two groups of 20 healthy grey parrots (*Psittacus e erithacus*) fed either a seed or pellet-based diet were investigated. There was a significant increase in the concentration of ionised calcium in the plasma of both groups, independent of the calcium and vitamin D_3 content of the diets fed, and a significant increase in the plasma concentration of 25-hydroxycholecalciferol in only the seed-fed group. In a separate study there were no significant increases in plasma ionised calcium or 25-hydroxycholecalciferol between March and August in a group of 28 South American parrots (*Pionus* species) exposed to unfiltered natural sunlight.

CALCIUM and vitamin D_3 metabolism have been studied extensively in domestic poultry (Kumar 1984, Soares 1984, Taylor and Dacke 1984, Norman 1987, Hurwitz 1989), and it has been shown that they must be supplied with adequate levels of dietary calcium and vitamin D_3 to avoid disorders of calcium metabolism such as rickets in chicks or cage-layer osteoporosis in adults (Taylor and Dacke 1984).

Birds can obtain vitamin D_3 by endogenous synthesis and from their diet. Vitamin D_3 (cholecalciferol) is the form of vitamin D found throughout the animal kingdom but only very rarely in plants (Boland 1986) where it occurs as vitamin D_2 (ergocalciferol); birds cannot make use of the vitamin D_2 in their diet (Massengale and Nussmeier 1930), owing to the rapid renal clearance of the vitamin rather than to its poor intestinal absorption (Hoy and others 1988). In addition, the binding of vitamin D_2 to plasma proteins is less efficient in birds than in mammals (Deluca and others 1988).

As in mammals, it has been established that in birds the skin is the organ in which endogenous vitamin D_3 production occurs, and the route of synthesis is essentially the same in both groups. Poultry secrete 7-dehydrocholesterol (provitamin D_3) on to featherless areas of skin (Koch and Koch 1941), and there is 30 times more provitamin D_3 on the featherless leg skin than on the back skin, indicating the importance of this area for vitamin D_3 metabolism (Tian and others 1994b). Provitamin D_3 is converted to cholecalciferol (previtamin D_3) by an isomerisation reaction induced by ultraviolet (UV) B light (UVB) (285 to 315 nm). Cholecalciferol is a sterol prohormone that undergoes a temperature-dependent isomerisation reaction to form vitamin D_3 (Tian and others 1993). After translocation into the circulation vitamin D_3 is transported bound to a specific globulin-binding protein (Bouillon and others 1980). There is a time delay between the production of vitamin D_3 in the skin and its translocation into the circulation (Tian and others 1994a). Cholecalciferol can be stored in adipose tissue, but to be physiologically active it must be metabolised by a two-stage hydroxylation process (Holick 1995); it is initially metabolised in the liver to 25-hydroxycholecalciferol (Blunt and others 1968), which is transported by carrier proteins to the kidney where it is converted to either 1,25-dihydroxycholecalciferol or 24,25-dihydroxycholecalciferol, the normal active metabolites of cholecalciferol in the domestic fowl. The most significant active metabolite of vitamin D_3 in domestic chickens is 1,25-dihydroxycholecalciferol, which controls both bone development and intestinal calcium absorption (Brommage and Deluca 1985); it has a short half life, being rapidly degraded by its target organs (Holick 1999); 24,25-dihydroxycholecalciferol is also thought to have an active role in poultry (Henry and Norman 1978).

The production of 25-hydroxycholecalciferol is rapid and has no feedback mechanism from calcium metabolism in poultry; it is controlled purely by product inhibition (Omdahl and Deluca 1973). In practice the blood concentration of 25-hydroxycholecalciferol can vary, depending on recent dietary intake (Johnson and Ivey 2002). The synthesis of 1,25-dihydroxycholecalciferol is also rapid, but this conversion is tightly regulated by many factors, in particular parathyroid hormone (PTH), in response to the calcium status of the bird (Fraser and Kodicek 1973). There are no known cases of vitamin D_3 toxicity resulting from excessive exposure to UV radiation in any species, owing to the UVB-dependent photoisomerisation of previtamin D_3 to lumisterol and tachysterol, which are inert. This process is reversible and these photoisomers can act as a store for cholecalciferol in the skin (Holick 1994). Excess UVB can also degrade cholecalciferol to inert compounds (such as prasterol I, suprasterol II and 5,6-transvitamin D_3) that are also inert (Ferguson and others 2003). The main role of vitamin D_3 in mammals, birds and reptiles is to control bone metabolism by regulating calcium absorption and by controlling the differentiation of its cellular elements (Norman and Hurwitz 1993). Vitamin D_3 also has profound effects on the immune system, skin and cancer cells in both mammals and poultry (Abe and others 1983); for example, in broiler chicks vitamin D_3 deficiency depresses the cellular immune response (Aslam and others 1998). The importance of the vitamin's role in bone development and the requirement of UV light for its metabolism has been overcome by the commercial availability of dietary vitamin D_3 that is essential for the indoor production of poultry (Edwards and others 1994). If UV light is excluded and broiler chickens are fed diets containing less than 400 iu/kg cholecalciferol, their plasma calcium concentrations decrease and rickets may develop.

Three ranges of UV wavelengths are recognised: UVA (315 to 400 nm), UVB (285 to 315 nm) and UVC (100 to 285 nm). Although the spectrum of radiation from the sun reaching earth's atmosphere ranges from 100 to 3200 nm, wavelengths shorter than 290 nm are absorbed by the ozone layer thus removing all UVC radiation (Frederick and others 1989). Both UVA and UVB are responsible for skin erythema and the production of the skin tan, but only UVB is associated with the photobiology of vitamin D_3. The UV index (UVI) is an internationally recognised unitless system of measuring UV radiation. In human beings, the latitude, time of day and season of year greatly affect the production of cholecalciferol in the skin (Holick 1999). Studies of the behaviour of reptiles have shown that they adjust their basking behaviour according to the vitamin D_3 content of their diet (Ferguson and others 2003). The UV light required for endogenous vitamin D_3 synthesis in captive animals can either be supplied naturally from full spectrum sunlight or by using artificial lamps that provide UVB radiation. Exposure to direct unfiltered sunlight is the optimal way to provide UVB light depending on the local UVI (Adkins and others 2003). The maximum conversion of provitamin D_3 to previtamin D_3

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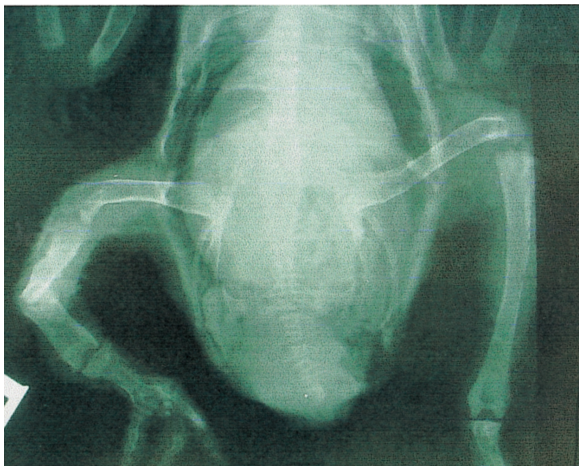

FIG 1: Radiograph of an eight-week-old grey parrot (*Psittacus e erithacus*) with osteodystrophy, showing a fracture of the tibiotarsus

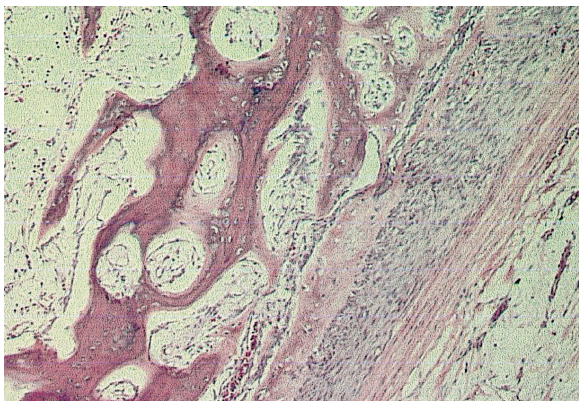

FIG 2: Section of cortical bone from the left tibiotarsus of the grey parrot (*Psittacus e erithacus*) in Fig 1, showing a loss of normal osteoid and its replacement with fibrous tissue, especially in the periosteal region of the bone. Haematoxylin and eosin. x 200

occurs at wavelengths between 294 and 300 nm (Holick and others 1982). Monochromatic light at 295 nm has been shown to convert 7-dehydrocholesterol in the skin to pro-vitamin D_3 with approximately 70 per cent efficiency, in contrast with exposure to full-spectrum sunlight that has a conversion efficiency of only 20 per cent (MacLaughlin and others 1982). Frequently, the greatest impediment to vitamin D_3 synthesis in poultry is the barrier to UVB provided by buildings. Even light-transmitting materials such as glass do not transmit light at wavelengths below 334 nm (Hess and others 1922). Chickens exposed daily to 30 minutes artificial UVB radiation while being fed a vitamin D_3-deficient diet developed significantly less skeletal problems than chickens denied supplementary UVB light (Edwards 2003). The provision of UV fluorescent lighting has been demonstrated to reduce the incidence of tibial dyschondroplasia in broilers (Edwards and others 1992). Rachitic chicks improve significantly after exposure to UVB light (Mac-Auliffe and McGinnis 1976). Continuous UV irradiation of broiler chickens does not affect their growth or food conversion efficiency but does lead to the loss of corneal structure (Barnett and Laursen-Jones 1976). In mature laying pullets additional UVB lighting has been shown to have no direct effect on the laying cycle but it does affect behaviour, in particular food intake (Lewis and others 2000). The plasma concentration of 25-hydroxycholecalciferol has been shown to be a useful indicator of the UVB exposure of an individual (Horst and others 1981).

Fluorescent tubes that provide some UVB radiation in addition to visible light, designed mainly for captive reptiles are available commercially (Logan 1969). The lights are lined with phosphorus and contain mercury that, when stimulated by an electric discharge, ionises and induces the phosphorus to emit UVB radiation. The success of these lamps to encourage vitamin D_3 metabolism depends on many factors; the glass used to construct the tube, the type or amount of phosphorus and the ambient temperature when the light is in use are important; in addition the phosphorus decays over time so that the lamps should be replaced regularly. The exposure of an individual animal to UVB radiation will also depend on its basking behaviour, its distance from the light and the presence of UVB filters in the enclosure (Gehrmann 1996). Many commercially available lamps produce both heat and UVB, and it has been shown that they induce significantly higher concentrations of 25-hydroxycholecalciferol in Chuckwallas than lamps that do not generate heat (Aucone and others 2003). The UVB output of most lamps is described in terms of percentage UVB production rather than absolute output. In one study none of the commercial lamps examined produced significant amounts of UVB at the wavelengths required for the synthesis of vitamin D, so care should be taken in selecting the lamps (Bernard 1995).

In captivity, grey parrots (*Psittacus e erithacus*) commonly suffer from disorders of calcium metabolism, such as osteodystrophy in chicks (Figs 1, 2) and neurological signs in adults (Hochleithner 1989, Harcourt-Brown 2003). They are considered to be due to a secondary nutritional hyperparathyroidism (Fig 3) caused by feeding seed-based diets containing little calcium or vitamin D_3 (Klasing 1998). In nature, grey parrots are indigenous to central Africa where they live in exposed areas with little shade and experience excellent levels of natural sunlight (May 1996). Captive grey parrots are usually kept indoors with limited access to natural UV light so that the lack of adequate UVB radiation may lead to a vitamin D_3 deficiency and associated problems with calcium metabolism. The conversion of previtamin D_3 to vitamin D_3 in the skin is also known to be temperature dependant (Tian and others 1993). The temperature at which a grey parrot is kept in captivity in the UK would be lower than its wild counterparts in central Africa and this might also contribute to a failure of vitamin D_3 metabolism. The role of UVB radiation in the control of vitamin D_3 metabolism in psittacine birds has not been investigated.

This study investigated the effects of artificial UV on the calcium metabolism of two groups of 20 grey parrots fed two different diets. In a separate study of 28 South American parrots (*Pionus* species) the effects of increasing UVB radiation from sunlight were investigated.

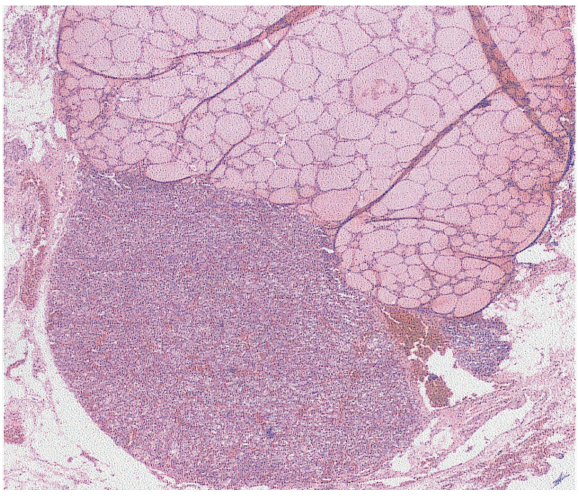

FIG 3: Section of the parathyroid gland from the grey parrot (*Psittacus e erithacus*) in Fig 1; the gland is enlarged and vacuolated, consistent with hyperparathyroidism. The adjacent thyroid gland is shown for comparison. Haematoxylin and eosin. x 20

TABLE 1: Nutritional composition of the seed-based diet and pellet-based diet fed to two groups of grey parrots (*Psittacus e erithacus*), used to investigate the effects of UV radiation on calcium metabolism

Ingredient	Seed diet	Pellet diet
Crude protein (%)	15·33	20
Crude fat (%)	17·39	12
Crude fibre (%)	5·25	5
Crude ash (%)	2·2	3·2
Moisture (%)	27·01	10
Calcium (%)	0·08	0·9
Phosphorus (%)	0·38	0·4
Calcium/ phosphorus ratio	0·21	2·25
Vitamin A (iu/kg)	1450	11,000
Vitamin D (iu/kg)	0	1650
Vitamin E (mg/kg)	599·9	450

TABLE 2: Pulse-based diet fed to 28 South American parrots (*Pionus* species) used to investigate the effects of UV radiation on calcium metabolism

Ingredient	Pulse mix
Crude protein (%)	23·05
Crude fat (%)	6·20
Crude fibre (%)	8·10
Crude ash (%)	4·20
Moisture (%)	23·46
Calcium (%)	0·29
Phosphorus (%)	0·33
Calcium/phosphorus ratio	0·89
Vitamin A (iu/kg)	3015
Vitamin D (iu/kg)	185
Vitamin E (mg/kg)	81·54

MATERIALS AND METHODS

One hundred healthy, sexually mature grey parrots were selected from 297 birds that had been caught in the wild, exported from Guyana, and purchased from a single source in 1999; each of them had been clinically examined by a standard protocol before it was purchased. Faecal samples were examined parasitologically, microbiologically and by Gram-stain. Blood samples were subjected to routine haematological and biochemical analyses, and tested for circovirus, polyomavirus and chlamydophilia by PCR testing. Each bird was examined by laparoscopy to confirm its sexual maturity and gender. On the basis of these tests, 50 pairs of healthy adult birds were selected.

Each pair was housed in an identical breezeblock and wire aviary, measuring 2 m x 2·5 m, which had a wooden shoebox-design nest box measuring 40 cm x 30 cm. All the aviaries were housed in a single-span windowless farm building built of brick with a slate roof. Forty of the 100 birds were selected by using a simple randomisation process and randomly allocated into two groups of 10 pairs (10 male and 10 female); they were kept in the same building under the same conditions. During the study one group was fed a seed-based diet (Tidymix Seed) and the other a pellet-based diet (Harrison's High Potency Course Pellett) (Table 1). During their annual health examination additional blood was taken from these 40 birds, with the informed consent of the owner, in order to investigate their calcium metabolism. The concentrations of total calcium, ionised calcium, parathyroid hormone and 25-hydroxycholecalciferol in plasma were measured.

Routine biochemical analysis of the heparinised serum sample was performed using a wet chemistry analyser (SPACE; Randox) within 12 hours of sample collection. The analyser uses standard spectrophotometer methodology and was calibrated every 24 hours. Albumin concentrations were measured using serum protein electrophoresis because of potential problems with avian protein evaluation (Lumeij and others 1989). Haematological analysis was performed manually. Ionised calcium, sodium and potassium concentrations were determined using an AVL 9181 analyser within 30 minutes of venepuncture in order to avoid potential problems associated with any delay on electrolyte assays. The methodology employed by the analyser is based on the ion selective electrode (ISE) measurement principle to precisely determine ion values. The IDS OCTEIA 25-hydroxycholecalciferol kit (IDS) was used for the quantitative determination of 25-hydroxycholecalciferol. The kit is an enzyme immunoassay for the quantitation of 25-hydroxycholecalciferol in serum and plasma. PTH was assayed using a PTH 1-34 (Peninsula Laboratories) research kit because of the problems associated with a commercial intact PTH 1-84 assay in grey parrots.

After 12 months all the birds were placed under artificial UVB light for 12 hours each day with the diets remaining unchanged. The UVB light was supplied by paired 1200 mm bird lamps (36 W FB36; Arcadia) suspended directly above each aviary. Perching birds would have been at most 0·5 m from the tubes. A reflector (ALR36; Arcadia) was mounted behind each tube to direct the light towards the birds and maximise the amount of UVB they received. A UVB light monitor (Elsec 763; Littlemore Scientific Engineering) was used to demonstrate that all the birds experienced an equal increase in UV light levels. The tubes were replaced after six months according to the manufacturer's instructions. Further blood samples were taken during the annual health examination after 12 months' exposure to UVB light, and the biochemical measurements were repeated.

Blood samples taken from a collection of 28 South American parrots (*Pionus* species) were also analysed for ionised calcium and 25-hydroxycholecalciferol for comparison with the grey parrots. These birds were fed a pulse-based diet with additional vitamin and mineral supplementation (Avimix; Vetark Products) (Table 2). The birds were kept in a variety of aviaries in North Yorkshire; 18 of them had no access to UV radiation and 10 of them were fully exposed to natural sunlight. The blood samples were taken under isoflurane anaesthesia in August 2003 during an endoscopic examination for sex determination. The birds were considered healthy on the basis of a clinical and endoscopic examination and the results of a blood analysis.

In 2004 blood samples were taken from the same birds in March and August in order to assess the effects of the seasonal increase of natural UV light on their vitamin D_3 metabolism, after concerns about their skeletal development in the previous year. All 28 birds were exposed to natural sunlight at this stage of the study.

RESULTS

Effects of UVB radiation on calcium metabolism in grey parrots

The results of providing UVB lighting for 12 months on the mean (sd) serum and median concentrations of serum ionised calcium, total calcium, 25-hydroxycholecalciferol and PTH in the two groups of parrots are shown in Table 3. In the seed-fed group there were significant increases in ionised calcium, total calcium and 25-hydroxycholecalciferol, and in the pellet-fed group there were significant increases in the serum ionised calcium and total calcium concentrations but there were no other significant differences.

There were no significant differences between the concentrations of the four variables in the groups fed the different diets after they had been exposed to UVB for 12 months.

TABLE 3: Mean (sd) [median] concentrations of ionised calcium, total calcium, 25-hydroxycholecalciferol and parathyroid hormone before and after exposure to ultraviolet B radiation of two groups of 20 grey parrots (*Psittacus e erithacus*) fed either a seed-based or pellet-based diet

Variable	Dietary group	No UVB	With UVB	P
Ionised calcium	Seed	1·11 (0·06) [1·10]	1·23 (0·05) [1·24]	0·0001
(mmol/l)	Pellet	1·20 (0·07) [1·19]	1·24 (0·06) [1·24]	0·0053
Total calcium	Seed	1·99 (0·13) [1·97]	2·22 (0·09) [2·22]	0·0001
(mmol/l)	Pellet	2·08 (0·12) [2·04]	2·22 (0·10) [2·22]	0·0055
25-hydroxycholecalciferol	Seed	71·5 (90·0) [35·3]	139·7 (69·2) [122·8]	0·0038
(nmol/l)	Pellet	130·8 (108·2) [118·4]	115·4 (16·6) [112·8]	NS
Parathyroid hormone	Seed	25·8 (21·52) [18·9]	20·7 (11·3) [16·8]	NS
(pg/ml)	Pellet	22·3 (15·3) [19·0]	19·2 (7·36) [16·0]	NS

NS Not significant

Effect of UVB on calcium metabolism in *Pionus* species

Table 4 shows the results of measurements of ionised calcium and 25-hydroxycholecalciferol in 10 parrots that had been exposed to full sunlight and 18 that had not. There were no significant differences between the two groups.

Table 5 shows the results of similar measurements made on blood samples taken in March and August 2004 from 27 mixed *Pionus* species birds that were exposed to unfiltered natural sunlight. The UVI (National Radiation Board) demonstrated that there had been an increase in UVB radiation received by the birds over this period. There were no significant differences between the concentrations of plasma 25-hydroxycholecalciferol or ionised calcium in the samples taken in March and August.

DISCUSSION

The provision of UV light significantly increased the concentrations of plasma ionised calcium and total calcium in both dietary groups of grey parrots. Plasma vitamin D_3 concentrations were significantly increased in the seed-fed group but not in the pellet-fed group. This suggests that the pellet-fed group may already have had adequate stores of vitamin D_3 from their diet in the form of 25-hydroxycholecalciferol. The additional UVB light would not have been expected to have caused vitamin D_3 toxicity in the pellet-fed group owing to the feedback mechanisms in vitamin D_3 metabolism. It would be reasonable to suggest that the 25-hydroxycholecalciferol concentrations recorded in these healthy birds exposed to UVB light and fed a pellet diet could be used to indicate a healthy normal range for the species; this would be clinically useful when investigating parrots with suspected hypocalcaemia or hypovitaminosis D_3. Although UV lighting has been widely used in reptile husbandry it has only recently been suggested as potentially useful in aviculture. The main function of supplementary full spectrum UV lighting in captive bird husbandry is to enable the birds to see the UV markings in their plumage, leading to increased fertility and fecundity, an effect that is thought to be due to UVA radiation, not UVB. The effect of artificial UVB lighting on vitamin D_3 metabolism in parrots has not been fully considered by the lamp manufacturers.

The majority of captive grey parrots are either kept indoors or live in northern latitudes where they do not receive adequate UV light, in comparison with birds living in equatorial Africa. The failure to provide adequate UV radiation in captivity may explain why grey parrots are so susceptible to the signs of hypocalcaemia. It is proposed by the author that grey parrots should be provided with UVB radiation as a standard part of their husbandry. Ideally it should come from solar radiation, because there are potential problems with supplying artificial UVB radiation, both from the performance of the lamps and the practicalities of keeping the bulbs close to the birds. Poultry have been shown to have no requirement for vitamin D_3 if they are supplied with adequate UV light and this may also be the case with grey parrots. Endogenous vitamin D_3 synthesis is known to be a temperature-dependent reaction and most captive grey parrots are kept at lower environmental temperatures than their wild counterparts. The provision of heat might be considered for grey parrots, to ensure adequate vitamin D metabolism.

The significant increase in plasma ionised calcium concentrations, combined with no significant decrease in parathyroid hormone levels, may be explained by the fact that all the birds were normocalcaemic throughout the study. There was no significant difference between the two dietary groups in the concentrations of plasma ionised calcium, total calcium and 25-hydroxycholecalciferol after the provision of UVB radiation.

The results of the initial study on South American (*Pionus* species) suggested that there was no difference in plasma ionised calcium and 25-hydroxycholecalciferol concentrations between the group of birds kept indoors with no exposure to natural UV light and the group kept outdoors. In a follow-up study on the same birds it was demonstrated that there were no significant changes in plasma ionised calcium and 25-hydroxycholecalciferol concentrations between March and August, when the birds were exposed to naturally increasing levels of UV light. These results suggest that this South American family may not be as dependent on UV radiation for vitamin D_3 metabolism as grey parrots. For economic and practical reasons this study involved small groups of birds and did not have the tight dietary controls observed in the main study. Controlled studies, involving larger numbers of South American species should be performed, because these results suggest that different psittacine species may have different husbandry requirements; the differences may explain why grey parrots are more susceptible to disturbances of calcium metabolism. The South American rainforest has a denser tree canopy than the majority of African rainforests and this could explain why African birds appear to be more dependent than South American birds on UV light for adequate vitamin D_3 metabolism. A breed difference has also been reported in vitamin D_3 concentrations in wild cockatoos. Major Mitchell cockatoos (*Cacatua leadbeateri*) appear to be susceptible to hypocalcaemia if they are kept in areas with cloud cover reducing the intensity of the UV light they receive (D. Macdonald, personal communication). New World monkeys have similar problems to grey parrots with their vitamin D_3 metabolism. The New World species have a higher requirement for UVB radiation than Old World mon-

TABLE 4: Mean (sd) [median] concentrations of ionised calcium and 25-hydroxycholecalciferol in 10 *Pionus* species parrots exposed to full sunlight and 18 that had no access to sunlight

Group	Number of birds	Ionised calcium	25-hydroxycholecalciferol
Exposed to full sunlight	10	1·14 (0·001) [1·19]	168·1 (190·2) [145·0]
Not exposed to full sunlight	18	1·10 (0·088) [1·12]	250·0 (126·6) [180·6]

TABLE 5: Mean (sd) [median] concentrations of ionised calcium and 25-hydroxycholecalciferol in blood samples taken in March and August 2004 from 27 *Pionus* species exposed to unfiltered natural sunlight

Time of sampling	Ionised calcium	25-hydroxycholecalciferol
March	1·16 (0·06) [1·15]	220·8 (96·6) [178·5]
August	1·13 (0·07) [1·14]	160·9 (90·6) [120·4]

keys. It has been suggested that the New World monkeys are less efficient in converting provitamin D_3 than the other species. An alternative theory is that in the New World monkeys vitamin D_3 does not bind as efficiently to carrier proteins. A further theory suggests that the problem may be a case of vitamin D_3 resistance, due to the failure of the vitamin D_3 receptors. As it becomes feasible to measure vitamin D_3 receptors in birds it should become possible to investigate whether grey parrots have a congenital vitamin D_3 resistance. In particular, the effect of UV light on vitamin D_3 metabolism in other parrot species might be investigated.

On the basis of the results of this study the author routinely recommends the provision of artificial unfiltered UVB light for captive grey parrots, in addition to a formulated diet with adequate levels of calcium and vitamin D_3, in order to prevent the clinical signs of disorders of calcium metabolism.

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