Comparison of Whole-Body Liquid Scintillometry, Radiography, and Clinical Chemical Tests in the Evaluation of the Effect of Chronic Corticoid Dosing on Calcium in Beagles

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Abstract □ Chronic doses of 6α-methylprednisolone were administered to beagles for 106 days to bring about changes in calcium balance. During the initial 64 days of corticoid treatment, no outstanding changes were observed in the blood levels of calcium, phosphorus, or alkaline phosphatase. Radiographic changes in the skeleton were not seen after 64 days of treatment. Significant changes in ^{47}Ca retention were detected by whole-body liquid scintillometry after only 13 and 28 days of corticoid administration. On day 64 of the study, the dosage of 6α-methylprednisolone was increased. Decreased levels of calcium in blood plasma and increased levels of plasma alkaline phosphatase were observed after the dose of corticoid was increased. A great reduction in whole-body ^{47}Ca retention was also observed, and thinning of the cortices of the long bones of the legs was apparent. The results of the investigation indicate that whole-body liquid scintillation counting of ^{47}Ca is a sensitive indicator of alterations of calcium metabolism in relation to other diagnostic methodology.

Keyphrases □ Corticoid administration, chronic—Ca effect evaluation □ ^{47}Ca retention—chronic corticoid administration □ Scintillometry—analysis □ Atomic absorpion spectroscopy—analysis

The diagnosis of pathologic conditions affecting the skeleton is difficult. Clinical chemical methods and radiography can rarely detect skeletal disease until a critical stage has developed. Routine radiographic methods are sensitive to rarefying osteopathies only after a 30–50% loss of skeletal mineral (1, 2). There is a definite need for superior diagnostic methodology.

Experimentation in this laboratory (3) and that conducted by others (4–10) have demonstrated that ^{47}Ca and whole-body counting techniques show great promise for the evaluation of skeletal calcification. The purpose of the present investigation was to compare the abilities of whole-body liquid scintillation counting of ^{47}Ca, routine clinical chemical methods, radiography, and ^{45}Ca specific activity determinations of bone sample for the detection of changes in calcium balance brought about in beagle dogs by chronic dosing with the corticoid, 6α-methylprednisolone. Chronic administration of corticoid was chosen because one of the most serious complications of sustained glucocorticoid therapy is the development of osteoporosis (11). The development of osteoporosis from glucocorticoid administration is an insidious process, taking many months to become severe enough to be capable of clear radiological recognition (12, 13). Skeletal alterations resulting from corticoid treatment have been well documented in animals (14–16).

EXPERIMENTAL

Five pure-bred male beagles[1] were used for the investigation. The dogs were housed together in a large indoor kennel which permitted them to exercise freely. The dogs were maintained on a balanced commercial diet.[2] Tap water was allowed *ad libitum*. At 10 weeks of age, each dog received 100 μc. of ^{45}Ca in two subcutaneous doses 2 days apart. Approximately 50 days were allowed for equilibration of the isotope with the stable skeletal calcium pool. At various intervals throughout the study, bone samples were obtained from each dog by amputation of the third digit of a foot. The first phalanx of each digit was analyzed for ^{45}Ca and total calcium.

The bone was air dried, weighed, and ashed in a muffle furnace. The ash was dissolved in 1 *N* HCl. Calcium determinations were carried out in triplicate by atomic absorption spectroscopy[3] according to the methodology developed for the clinical use of the spectrophotometer (17, 18). Determinations of ^{45}Ca were made in triplicate by placing aliquots of the dissolved bone ash in a toluene modified XDC scintillator solution (19) and counting the samples with a Packard Tri-Carb scintillation spectrometer.[4] An internal standardization procedure was utilized to correct the counting data for quenching (20).

Following the ^{45}Ca equilibration period, corticoid administration was initiated. Four of the dogs received a daily oral dose of 2 mg./kg. of body weight of 6α-methylprednisolone for 63 days. On the 64th day, the corticoid dose for these four dogs was raised to 10 mg./kg. of body weight daily and maintained until day 106. The fifth dog served as a control and received doses of lactose in place of corticoid during the entire study.

Before and during corticoid administration, the dogs were radiographed at approximately 2-week intervals. The areas radiographed were the lumbar spine, foreleg, and mandible. All radiographs were made using regular-speed film and ultradetail intensifying screens.

Clinical chemical determinations were performed prior to and at various times during corticoid dosing. After 24 hr. of fasting, a 10-ml. sample of whole blood was obtained from the external jugular vein of each dog in heparinized syringes. Plasma alkaline phosphatase determinations were made using Phosphatabs-Alkaline Quantitative.[5] This simplified test for alkaline phosphate makes use of reagent tablets containing phenolphthalein phosphate substrate (21). The reagent tablets have been reported to produce results which are in excellent agreement with more sophisticated techniques for alkaline phosphatase determination (22–24). An analysis using a standardized enzyme solution[6] was run with each series of alkaline phosphatase determinations as a check against errors in methodology. Serum inorganic phosphorus was determined with Hycel phosphorus reagent.[7] A standardized serum[8] was analyzed with each series of phosphorus determinations to assure against technical errors. Serum calcium was determined by adding 0.2 ml. of plasma to 5 ml. of a solution of 1% lanthanum as $LaCl_3 \cdot 7H_2O$ and 5% trichloroacetic acid in double distilled water. The mixture was centrifuged to precipitate the plasma proteins. The calcium contained in the supernatant was determined by atomic absorption spectroscopy. All clinical chemical determinations were made in triplicate. Each time clinical chemical analyses were conducted, the average value obtained for the corticoid-treated dogs was compared to the values for the control dog.

[1] American Animal Industries, Indianapolis, Ind.

[2] Wayne Dog Krumettes, Allied Mills, Inc., Chicago, Ill.

[3] Perkin-Elmer Atomic Absorption Spectrophotometer, model 303, Perkin-Elmer, Norwalk, Conn.

[4] Model 574, Packard Instrument Company, Inc., Downers Grove, Ill.

[5] General Diagnostic Division, Warner-Chilcott Laboratories, Morris Plains, N. J.

[6] Versatol-E-N, General Diagnostic Division, Warner-Chilcott Laboratories, Morris Plains, N. J.

[7] Hycel Incorporated, Houston, Tex.

[8] Versatol-A, General Diagnostic Division, Warner-Chilcott Laboratories, Morris Plains, N. J.

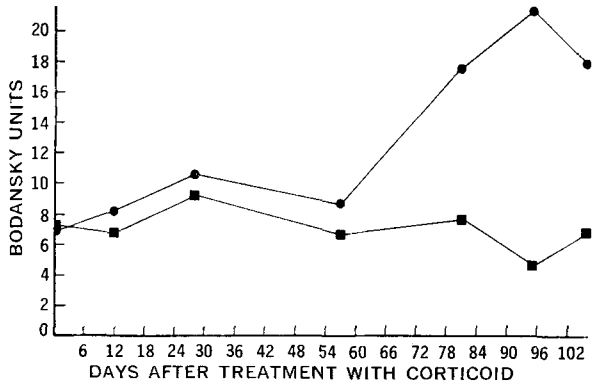

Figure 1—*Plasma alkaline phosphatase levels. Key: ●, 6α-methylprednisolone-treated dogs; and ■, control dog.*

A 4-pi whole-body liquid scintillation counter (3, 25) was used to determine whole-body retention of ^{47}Ca. Retention of ^{47}Ca was determined before corticoid administration and at four intervals during corticoid dosing. For each whole-body counting study, each dog was administered 0.2 μc. of ^{47}Ca intravenously into the external jugular vein. Fifteen minutes after isotope administration, ^{47}Ca retention was determined in each dog. This was considered to be time 0 and the count rate to be 100% retention of ^{47}Ca. Each dog was counted at given time intervals for 144 hr. after time 0. The ^{47}Ca retention values for the four corticoid-treated dogs were averaged at each time interval. Comparisons were made between the average retention values after various periods of corticoid dosing and the average retention values before corticoid dosing. Whole-body counting data were examined statistically using an analysis of variance and a Newman–Keuls Sequential Range test. At the termination of the study, the dogs were euthanitized with pentobarbital, and necropsies were performed.

RESULTS AND DISCUSSION

During the period of corticoid dosing at a level of 2 mg./kg. of body weight, no outstanding changes were observed in the plasma alkaline phosphatase (Fig. 1), serum calcium (Fig. 2), or serum inorganic phosphorus (Fig. 3) levels of the treated dogs as compared to the control dog. No apparent radiographic changes in the treated dogs in relation to the control dog were observed at this lower dosage level of corticoid. However, by whole-body liquid scintillometry, it was possible to detect significant ($p = 0.01$) changes in ^{47}Ca whole-body retention in the dogs after 13 and 28 days of corticoid administration as compared to ^{47}Ca retention in the same dogs previous to the initiation of drug treatment (Fig. 4). At each time period investigated, statistical differences between whole-body ^{47}Ca retention curves occurred 60 hr. and thereon after ^{47}Ca administration. The results of the investigation during the period of corticoid dosing at a level of 2 mg./kg. illustrate the ability of whole-body liquid scintillation counting of ^{47}Ca to detect alterations of calcium

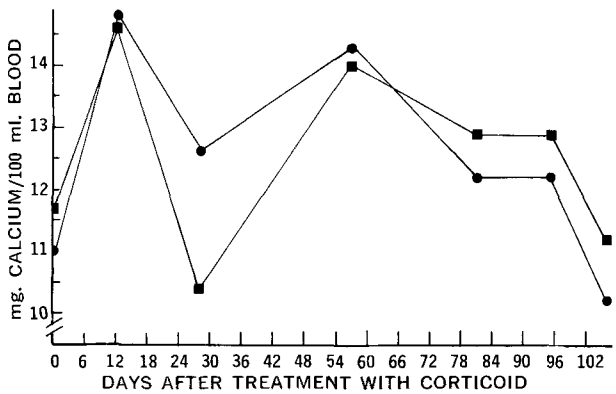

Figure 2—*Serum calcium levels. Key: ●, 6α-methylprednisolone-treated dogs; and ■, control dog.*

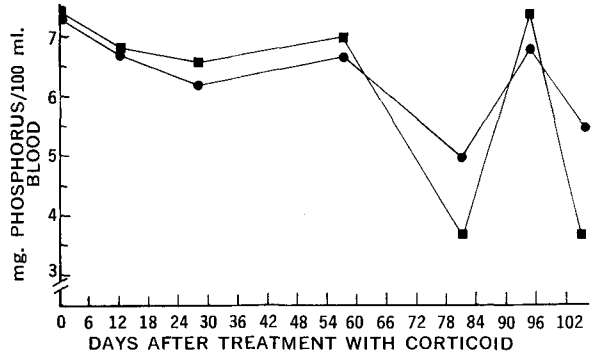

Figure 3—*Serum inorganic phosphorus levels. Key: ●, 6α-methylprednisolone-treated dogs; and ■, control dog.*

metabolism in the absence of radiographic and clinical chemical changes.

As may be seen in Fig. 4, after 54 days of corticoid administration at the lower dosage level the whole-body retention of ^{47}Ca began to approach the values observed before drug treatment. It appeared that the treated dogs were adapting to the pharmacologic effect of the drug. When the dosage level of 6α-methylprednisolone was raised to 10 mg./kg. of body weight, the whole-body retention of ^{47}Ca was found to be greatly decreased in the treated dogs in relation to ^{47}Ca retention in the dogs before corticoid treatment. Following the increase in dosage level the serum calcium in the treated dogs was decreased in relation to the control dog. Also, plasma alkaline phosphatase was elevated in the treated dogs as compared to the control dog. No conspicuous changes were observed in serum inorganic phosphorus during the entire study.

Radiographs taken at termination of the study (after 42 days of corticoid at a dose of 10 mg./kg. of body weight) showed thinning of the cortices of the long bones in the forelegs of the corticoid-treated dogs. Figure 5 illustrates the difference between the radiographic appearance of the long bones of the forelegs of the corticoid-treated dogs as compared to the control dog. It was also noted that the dogs treated with 6α-methylprednisolone developed curvature of the forelegs with the convex side anterior. Periosteal thickening of both the radius and the ulna on the concave side (posterior) occurred. In the middle third of the ulna there was an increased transverse trabecular pattern.

The ^{45}Ca specific activity of bone samples was expressed as c.p.m. ^{45}Ca/mg. Ca/g. bone. For each dog, the bone specific activity at the initiation of corticoid or control treatment was considered

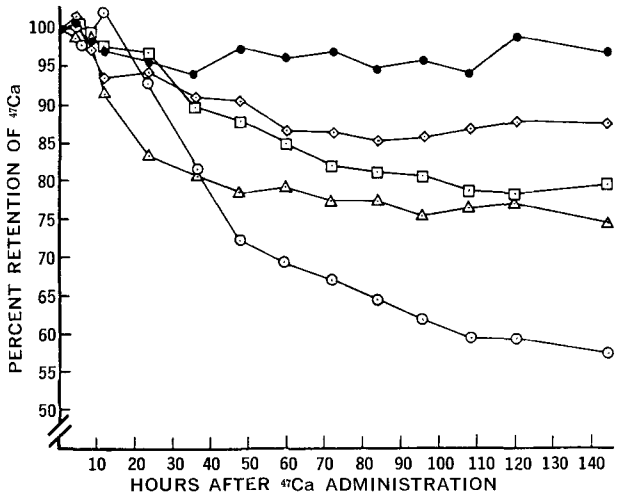

Figure 4—*Whole-body liquid scintillation determination of ^{47}Ca retention by dogs receiving chronic doses of 6α-methylprednisolone. Key: ●, dogs before corticoid treatment; ◇, dogs after 13 days of corticoid; □, dogs after 54 days of corticoid; △, dogs after 28 days of corticoid; and ○, dogs after 98 days of corticoid.*

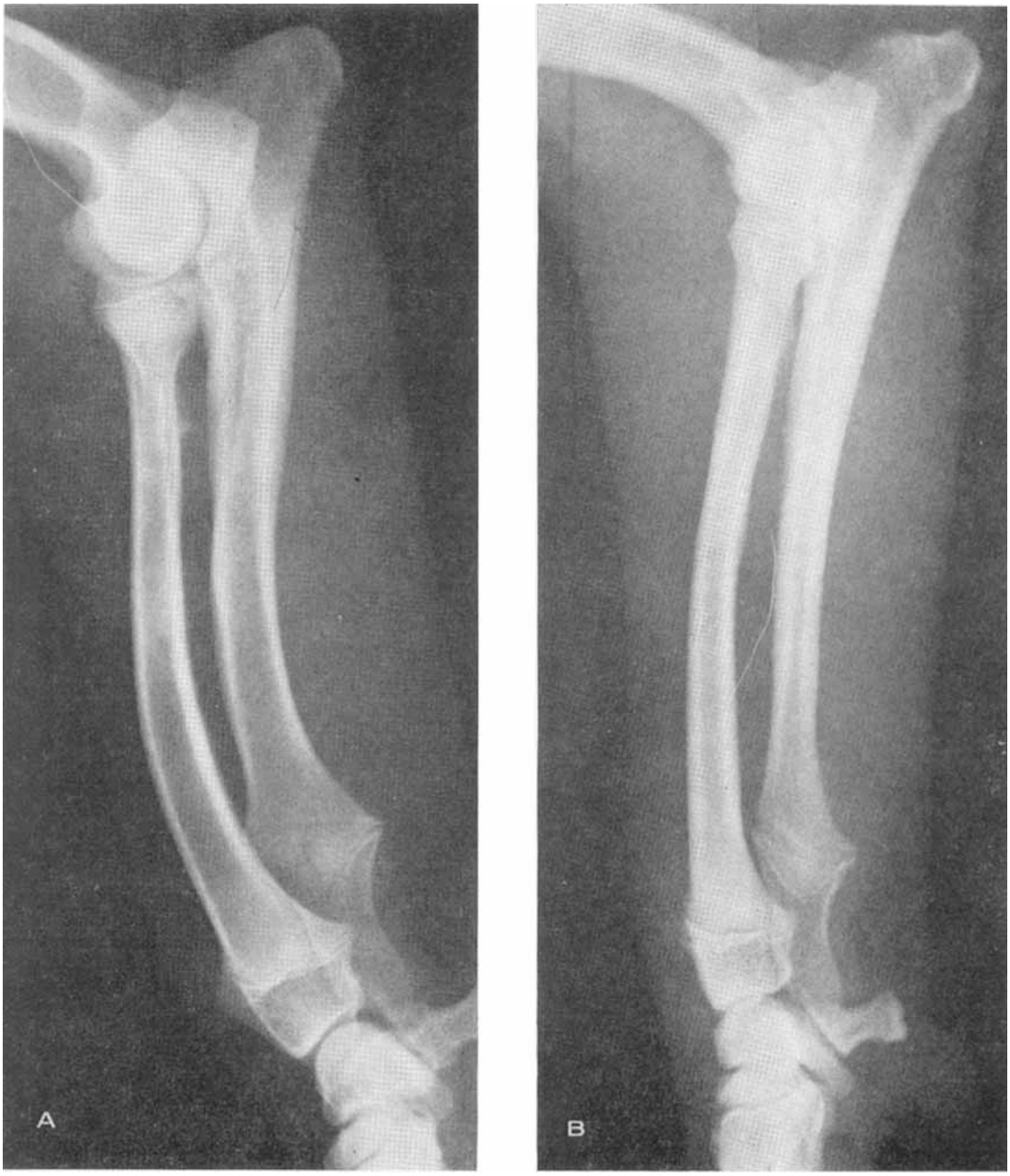

Figure 5—*Radiograph of the foreleg. Key:* A, *dog after chronic doses of 6α-methylprednisolone for 106 days; and* B, *control dog.*

as 100% and subsequent samples were expressed as the percent of the initial specific activity remaining. After 59 days of corticoid dosing at a level of 2 mg./kg. of body weight, the specific activity of the bone samples from the treated dogs was 64% of the initial specific activity while the specific activity of the control dog was 59%. After a total of 106 days of corticoid dosing the specific activity of the bone samples from the treated dogs was 63% of the specific activity found previous to the initiation of corticoid administration while the specific activity of the control dog was 49%. Necropsy examinations at the termination of the study revealed no gross pathology in any of the dogs. However, gonadal atrophy was evident in the corticoid-treated dogs.

Whole-body liquid scintillation detection of ^{47}Ca retention in this investigation indicated that corticoid administration results in a decreased calcium retention. Collins *et al.* (26, 27) and Garrett *et al.* (28, 29) observed that chronic corticoid administration to beagle dogs resulted in decreased ^{47}Ca uptake by bone and an increased fecal excretion of ^{47}Ca. These workers concluded that decreased bone formation rather than increased destruction was the major effect of administered corticoids.

It would appear from the whole-body counting studies that either osteoblastic activity was depressed so that osteoclastic activity, normally held in balance, could show a greater net effect of resorption on bone, or that osteoclastic activity was enhanced resulting in increased bone resorption. Since osteoclasts have been shown to be associated with phosphatase enzymes (30), increased osteoclastic activity may account for the increased alkaline phosphatase levels observed in the corticoid-treated dogs. Conversely, the increased alkaline phosphatase levels may have been due to an increased osteoblastic activity attempting to heal bone damaged caused by the 6α-methylprednisolone (31). However, Frost and Vilaneuva (32) examined the bones of a group of 21 human patients who had received cortisone or ACTH for more than 2 weeks. Only two cases showed normal osteoblastic activity. The remaining 19 cases showed depression or cessation of osteoblastic activity. Frost (32, 33) has concluded that human patients receiving phar-

macodynamic doses of corticoids go into negative calcium balance as a result of an unequal inhibition of both bone formation and bone resorption. The inhibition of formation is greater than the inhibition of resorption with the result that a negative skeletal balance ensues, leading to osteoporosis. In the present investigation, it is possible that an inhibition of bone formation by the corticoid may account for the decreased whole-body retention of ^{47}Ca and that inhibition of resorption may account for the increased retention of ^{45}Ca by the bone samples analyzed. The foreleg curvature developed by the dogs treated with 6α-methylprednisolone presents further evidence of inhibited bone formation. It has been shown in these laboratories that retardation of the distal ulnar epiphysis in dogs will result in curvature of the bones of the foreleg accompanied by periosteal thickening of these bones on the concave area.

SUMMARY

From these studies it can be seen that whole-body liquid scintillation counting of ^{47}Ca is a sensitive indicator of alterations of calcium metabolism in relation to other diagnostic methodology. During the first 64 days of corticoid treatment, no outstanding changes were observed in the blood levels of calcium, phosphorus, or alkaline phosphatase. Radiographic changes in the skeleton were not apparent after 64 days of treatment. However, it was possible to detect significant changes in ^{47}Ca retention by whole-body liquid scintillometry after only 13 days of corticoid administration. Significant radiographic and clinical chemical changes occurred only after elevated doses of corticoid were administered.

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