Summary of Research

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ABSTRACT

The exposure of the body to microgravity during space flight causes a series of well-documented changes in Ca\(^{2+}\) metabolism, yet the cellular/molecular mechanisms leading to these changes are poorly understood. There is some evidence for microgravity-induced alterations in the vitamin D endocrine system, which is known to be primarily involved in the regulation of Ca\(^{2+}\) metabolism. Vitamin D-dependent Ca\(^{2+}\) binding proteins, or calbindins, are believed to have a significant role in maintaining cellular Ca\(^{2+}\) homeostasis.

We used immunocytochemical, biochemical and molecular approaches to analyze the expression of calbindin-D\(_{28k}\) and calbindin-D\(_{9k}\) in kidneys and intestine of rats flown for 9 days aboard the Spacelab 3 mission. The effects of microgravity on calbindins in rats in space vs. "grounded" animals (synchronous Animal Enclosure Module controls and tail suspension controls) were compared. Exposure to microgravity resulted in a significant decrease in calbindin-D\(_{28k}\) content in kidneys and calbindin-D\(_{9k}\) in the intestine of flight and suspended animals, as measured by enzyme-linked immunosorbent assay (ELISA). Immunocytochemistry (ICC) in combination with quantitative computer image analysis was used to measure \textit{in situ} the expression of calbindins in kidneys and intestine, and insulin in pancreas. There was a large decrease in the distal tubular cell-associated calbindin-D\(_{28k}\) and absorptive cell-associated calbindin-D\(_{9k}\) immunoreactivity in the space and suspension kidneys and intestine, as compared with matched ground controls. No consistent differences in pancreatic insulin immunoreactivity between space, suspension and ground controls was observed. There were significant correlations between results by quantitative ICC and ELISA. Western blot analysis showed no consistent changes in the low levels of intestinal and renal vitamin D receptors.

These findings suggest that a decreased expression of calbindins after a short-term exposure to microgravity and modelled weightlessness, may affect cellular Ca\(^{2+}\) homeostasis and contribute to Ca\(^{2+}\) and bone metabolism disorders induced by space flight.
INTRODUCTION

Vitamin D is the precursor of the steroid hormone, 1,25-dihydroxyvitamin D₃ \([1,25(\text{OH})_2\text{D}_3]\) (Norman et al., 1982; Henry and Norman, 1984; Sergeev, 1989). 1,25(OH)₂D₃ produces a wide spectrum of biological effects via both receptor mediated regulation of nuclear events (Minghetti and Norman, 1988; Lowe et al., 1992) and rapid actions independent of the genomic pathway (Norman et al., 1992; Farach-Carson et al., 1991; Sergeev and Rhoten, 1995). The vitamin D receptor (VDR) regulates genes associated with Ca²⁺ homeostasis (e.g., calbindins; Christakos et al., 1984), with the proliferation pathway, the differentiation pathway and the developmental cascade (Lowe et al., 1992). Calbindins are believed to be essential, as intracellular Ca²⁺ sequestrants/buffers, to the process of intestinal Ca²⁺ absorption (Nemere et al., 1991; Norman et al., 1992) and renal Ca²⁺ reabsorption (Johnson and Kumar, 1994; Hemmingsen et al., 1995). Calbindin-D_{28k}, functioning as an intracellular Ca²⁺ buffer, is crucial for preventing accumulation of excessive levels of cytosolic free Ca²⁺ (Iacopino et al., 1992; Rhoten and Sergeev, 1994) and, thus, determining cell fate (Dowd, 1995).

There is the evidence that the integrated operation of the vitamin D endocrine system is affected by factors of space flight, including evidence from human and rat models of microgravity (Arnaud et al., 1991; Morey-Holton et al., 1988; Sergeev et al., 1982-1986; Spirichev and Sergeev, 1988). Serum 1,25(OH)₂D₃ concentration decreased in healthy volunteers after one year bed rest with the head lower than the feet (Sergeev and Morukov, unpublished observations). The 1,25(OH)₂D₃ production in the kidney and accumulation of the hormone in the bone and intestine markedly decreased in rats after long-term hypokinesia (Sergeev et al., 1983; 1984). These were accompanied by a decrease in the intestinal Ca²⁺ absorption (Sergeev and Spirichev, 1987) and osteopenia (Kabitskaya et al., 1984; Sergeev et al., 1987). Prophylactic treatment with vitamin D₃ active metabolites prevented bone loss to a significant extent in rats during long-term hypokinesia (Sergeev et al., 1982a,b, 1985, 1987; Ushakov et al., 1982, 1983a,b, 1984), indicating a crucial role for the vitamin D endocrine system in regulation of bone and Ca²⁺ metabolism in modeled weightlessness.
The phenomenology of possible changes in vitamin D-mediated biological responses (e.g., calbindins) during and after space flights remains currently unknown. It seems probable that the vitamin D hormone-mediated regulation, particularly that of calbindins, may be a critical factor in adaptational and readaptational changes of at least Ca\(^{2+}\) metabolism under the action of weightlessness/gravity.

We participated in the organ sharing program for tissues from rats flown aboard the Spacelab 3 mission and compared the effects of microgravity on calbindins in rats in space vs. "grounded" animals (synchronous Animal Enclosure Module, tail suspension, and vivarium controls). We hypothesized that exposure to microgravity might affect expression of vitamin D-dependent calcium binding proteins, calbindin-D\(_{28k}\) and calbindin-D\(_{9k}\). To test this hypothesis we evaluated the following parameters in the kidney and intestine: 1) calbindin-D\(_{28k}\) and calbindin-D\(_{9k}\) contents; 2) immunocytochemical expression of calbindin-D\(_{28k}\) and calbindin-D\(_{9k}\); 3) the level of vitamin D receptors.
MATERIALS AND METHODS

Sample handling

Male Sprague-Dawley rats (weighing ca. 150 g and aged 6 wk at launch) were flown for 9 days aboard the Spacelab 3 mission. Upon return to earth, the animals were dissected within "zero", 24 and 72 h post-flight (groups FR0, FR24, and FR72). Age- and sex-matched ground control animals were maintained in the Animal Enclosure Module (AEM) where factors of the space flight, except microgravity, were synchronously reproduced (groups FCR0, FCR24, and FCR72). Tail suspension rats were used as a model which mimics some effects of microgravity (groups SynSuspR0, SynSuspR24, and SynSuspR72); corresponding controls for these animals were rats kept in the vivarium (SynVivR0, SynVivR24, and SynVivR72). Moreover, the pre-flight, basal control group (VivL0) was dissected before launch.

The organs (right kidney and the washed upper portion of the small intestine ca. 10 cm in length) were snap-frozen in liquid nitrogen, stored at -70°C and shipped to the laboratory on dry ice. Left kidney, duodenum and pancreas were fixed in formalin.

Enzyme-linked immunosorbent assay

Calbindin-D_{28k} and calbindin-D_{9k} in kidneys and calbindin-D_{9k} in intestine were measured by means of an enzyme-linked immunosorbent assay (ELISA), as described previously (Miller and Norman, 1983; Rhoten and Sergeev, 1994). Diluted cytosol aliquots (50 µL; 0.5 mg/mL total protein of kidney cytosol for calbindin-D_{28k}, 0.1 mg/mL total protein of kidney cytosol for calbindin-D_{9k}, and 0.1 mg/mL total protein of intestinal cytosol for calbindin-D_{9k}) were assayed in calbindin-D_{28k}-coated (10 ng/well) or calbindin-D_{9k}-coated (2.5 ng/well) multiwell flat bottomed immunoassay plates. Chicken intestinal calbindin-D_{28k} was a gift from Dr. A.W. Norman (University of California-Riverside), and bovine intestinal calbindin-D_{9k} was purchased from Sigma (St. Louis, MO). Calbindin-coated plates were washed and preblocked with 1%
bovine serum albumin, 0.5% Tween 20 in phosphate buffered saline (PBS), and then incubated for 2 h at room temperature with unknowns (0 - 400 ng calbindin-D_{28k}/well or 0 - 12.5 ng calbindin-D_{9k}/well) and primary antibody (150 µL; mouse monoclonal anti-calbindin-D_{28k}, clone CL-300, Sigma, 1:140,000 dilution, or rabbit antiserum against calbindin-D_{9k}, 1:5,000 dilution, a gift from Dr. M.E. Bruns). The washed plates were then incubated for 2 h with a secondary antibody (alkaline phosphatase labeled goat anti-mouse or anti-rabbit IgG, 1:1,500 dilution, Sigma). Substrate, \( p \)-nitro-phenylphosphate (1 mg/mL in diethanolamine buffer, pH 9.8), was used to produce a chromogen which was quantitated at 405 nm in the microplate reader. Protein concentration in cytosols was measured with a Bio-Rad detergent-compatible protein microassay (Bio-Rad Laboratories, Hercules, CA), using a microplate format.

**Immunocytochemistry**

Cellular localization and quantification of calbindin-D_{28k} in kidneys, calbindin-D_{9k} in duodenum, and insulin in pancreas was carried out on the formalin-fixed tissues, as described previously (Rhoten et al., 1985; Rhoten, 1987; Rhoten and Christakos, 1990). Fixed tissues were embedded in Paraplast (Monoject Scientific, St. Louis, MO). Microtome sections were affixed to Superfrost/Plus microscope slides (Fisher Scientific, Pittsburgh, PA) and stored at room temperature until use. Paraffin was removed and sections rehydrated. Slides were treated with 3% \( H_2O_2 \) in PBS for 10 min, rinsed in PBS, and then incubated overnight in a humid chamber at 4°C with primary antibody. The antibodies used were mouse anti-calbindin-D_{28k}, clone CL-300, 1:200 dilution in Tris buffered saline, pH 7.6, containing 2% normal goat serum, and 1% albumin (SA-TBS); rabbit anti-rat intestinal calbindin-D_{9k}, 1:400 dilution in SA-TBS; and guinea pig anti-insulin, 1:200 dilution in SA-TBS. Slides were then washed with SA-TBS, and primary antibodies detected using goat peroxidase labeled anti-mouse IgG, 1:100 dilution (Sigma); goat peroxidase labelled anti-rabbit IgG, 1:100 dilution (Incstar, Stillwater, MN); and rat peroxidase labelled anti-guinea pig IgG, 1:200 dilution (Sigma). Slides were incubated with secondary antibodies for 45 min at room temperature. Chromogen used was 3,3'-diaminobenzidine.

*In situ* levels of the calbindins and insulin were quantified on the basis of the intensity
of the oxidized diaminobenzidine reaction product present in individual cells, using an Image-1 image acquisition, processing and analysis system (Universal Imaging, West Chester, PA). Labeling intensity (brightness) was measured on digitized images in arbitrary O.D. units based on a 255 tone gray scale, where the value of zero is completely black and 255 is completely white (transparent). The intensity value encompassed both the number of labeled cells and their individual brightness values, and was obtained by defining the outline of the cell cluster and determining the average brightness value over the entire area. The same defined area was placed on the image close to the measured positive (darker) area to determine the level of background non-specific staining. Data are expressed as relative labeling intensity in %, i.e., (brightness of the positive area)/(brightness of the background area) X 100, so that lower (i.e., more dense) numbers correspond to higher levels of calbindins. For each stained slide, 2 - 3 fields of cells were captured and at least three areas of labeled cells were counted.

Western blot analysis

Vitamin D receptors in the intestine and kidney were detected using Western blot technique. Tissues were thawed on ice, intestinal mucosa was scraped, and homogenates (20% wt/vol) in a high-ionic-strength buffer (KTED: 300 mM KCl, 10 mM Tris, pH 7.4, 1.5 mM EDTA, 5.0 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) were prepared (Sergeev and Norman, 1992; Norman et al., 1993). The cytosol fraction of homogenates (35,000 g, 1 h, 4°C) was used for Western blot analysis.

The cytosol aliquots (150 µg protein) were subjected to SDS-polyacrylamide (12%) gel electrophoresis and transferred to nitrocellulose sheets using a Bio-Rad transfer unit. The sheets were preblocked with 5% non-fat milk in PBS and then probed with rat monoclonal anti-VDR antibodies (1:500 dilution, 2 h at room temperature) (Affinity BioReagents, Golden, CO). The bands were visualized with alkaline phosphatase labeled secondary antibodies (anti-rat IgG, Sigma) for 1 h at room temperature. Chromogen used was BCIP/NBT.

For Western blot analysis of calbindin-D$_{28k}$, kidney cytosols were subjected to SDS-PAGE, transfer and blocking, as described for VDRs. The nitrocellulose sheets were probed with mouse
monoclonal anti-calbindin-D$_{28k}$ (1:200 dilution, 2 h at room temperature), and the bands were visualized with alkaline phosphatase labeled secondary antibodies (anti-mouse IgG, Sigma) (Mutema and Rhoten, 1994).

In Western blot analysis of $\beta$-actin, primary antibodies used were mouse monoclonal anti-$\beta$-actin (Clone Ac-15, 1:1000 dilution; Sigma) and secondary antibodies were alkaline phosphatase labeled anti-mouse IgG (1:1000 dilution; Sigma).

Statistics

Statistical analysis of the data was performed using Sigma Stat v. 1.0 software (Jandel Scientific, San Rafael, CA).
RESULTS

Measurement of calbindin-D_{28k} and calbindin-D_{9k} in kidneys and calbindin-D_{9k} in intestine by ELISA

ELISA was used to measure total calbindin contents in kidneys and the intestinal mucosa of space, suspension and ground animals. Calbindin contents varied among the individual animals (see Appendix). However, when data were pooled and normalized per mg protein, the calbindin-D_{28k} content in kidneys and calbindin-D_{9k} content in the intestine of space and suspension animals proved to be significantly reduced compared with ground control animals (Fig. 1). In terms of the flight groups, this decrease was 24.0, 23.9 and 23.5% for calbindin-D_{28k} in kidneys at "zero", 24 and 72 h post-flight, respectively (see also Fig. 1, left panel); for calbindin-D_{9k} in the intestine the decrease was 57.8, 19.2 and 47.3% at "zero", 24 and 72 h post-flight (see also Fig. 1, middle panel). Quantitatively similar decreases were revealed when synchronous suspension and vivarium groups were compared at "zero", 24 and 72 h post-flight (see Fig. 1).

The content of calbindin-D_{9k} in kidneys was very low, as compared with the calbindin-D_{28k} content (in ng/mg protein range vs. \mu g/mg protein range for calbindin-D_{28k}) (Fig. 1, right panel). An apparent decrease in the renal calbindin-D_{9k} of suspension animals at "zero" and 72 h, but not 24 h post-flight was observed. This trend was statistically significant for FR0 vs. FCR0 groups.

In situ quantification of calbindin-D_{28k} in kidneys, calbindin-D_{9k} in intestine and insulin in pancreas using immunocytochemistry

Immunocytochemistry (ICC) in combination with quantitative computer image analysis was used to measure in situ the expression of calbindins in kidneys and intestine, and insulin in pancreas. The predominate immunolocalization of renal calbindin-D_{28k} in all animals was similar to that first reported by us (Rhoten and Christakos, 1981), i.e., cells of distal convoluted tubules, connecting tubules and cortical collecting tubules. Some flight and grounded animals
had kidneys exhibiting a highly variable amount of immunoreactivity for calbindin-D$_{28k}$ in the medullary collecting ducts and papillary ducts. The absence of consistent immunolocalization in medullary collecting ducts and papillary ducts argued against quantifying the calbindin-D$_{28k}$ found in these sites. Intestinal localization of calbindin-D$_{9k}$ appeared to be confined to the absorptive cells. As seen in Fig. 2, there was a large decrease in the distal tubular cell-associated calbindin-D$_{28k}$ immunoreactivity and in the absorptive cell-associated calbindin-D$_{9k}$ immunoreactivity in the space kidneys and intestine, as compared with matched ground control animals. Insulin was localized to relatively large numbers of cells making up the core of pancreatic islets. A result consistent with the localization of insulin in $\beta$-cells of the rat. There was no consistent difference in pancreatic insulin immunoreactivity of space and ground animals (see Fig. 2). No specific immunoreactivity for calbindin-D$_{28k}$ was observed in the pancreatic islet cells of any of the flight or grounded animals.

Summary of quantitative image analysis of tissues from space, suspension and ground animals are presented in Fig. 3. Comparison of groups was done as described above for ELISA. Reductions in the calbindin-D$_{28k}$ level in kidneys and the calbindin-D$_{9k}$ level in the intestine were similar to those found with ELISA (see Fig. 3, left and middle panels). Linear regression analysis of data obtained using ELISA and quantitative ICC, showed a statistically significant correlation between two methods (Fig. 4). Insulin level in pancreas varied widely among animals within a group, and no apparent trend to the decreased insulin immunoreactivity was revealed in space and suspension animals (see Fig. 3, right panel).

Detection of vitamin D receptors in the intestine and kidneys using Western blot analysis

VDR levels in kidneys and the intestinal mucosa of flight and flight control animals were compared using Western blotting with rat monoclonal anti-VDR antibodies. As seen on representative immunoblots (Fig. 5), there were no consistent changes in the VDR level, based on the intensity of specific bands. Importantly, there was also no changes in the intestinal $\beta$-actin level. Calbindin-D$_{28k}$ levels, measured on some of the same membranes, varied in the same way as seen quantitatively by ELISA.
Fig. 1. Calbindin contents in the intestine and kidneys of space, suspension and ground animals. Calbindin-D_{2k} and calbindin-D_{28K} were measured by ELISA, as described in Materials and Methods. The data, analyzed by ANOVA, represent mean values ± SEM. Only significance of differences between F vs. FC and SynSusp vs. SynViv groups are presented. (*), *P < 0.05.
Fig. 2. Immunocytochemical localization and quantification of calbindins and insulin. Tissue sections were immunoreacted and calbindin-D_{28k} in kidney (p. 12), calbindin-D_{9k} in the intestine (p. 13), and insulin in pancreas (p. 14) were measured, as described in Materials and Methods. Scale marker = 50 μm (kidneys, pancreas) or 100 μm (intestine).
Fig. 3. Calbindin levels in the intestine and kidneys, and insulin level in pancreas of space, suspension and ground animals. Calbindin-D_28k and calbindin-D_9k were quantified by ICC, as described in Materials and Methods. Note that higher (i.e., less dense) values correspond to lower levels of calbindins and insulin (see also Materials and Methods). The data, analyzed by ANOVA, represent mean values ± SEM. Only significance of differences between F vs. FC and SynSusp vs. SynViv groups are presented. (*), $P < 0.05$; N.D., not determined.
Fig. 4. Correlation between enzyme-linked immunosorbent and immunocytochemical quantification of renal calbindin-D$_{28k}$ and intestinal calbindin-D$_{9k}$. Linear regression analysis revealed for two methods, a correlation coefficient of $\geq 0.7$ and $P < 0.05$. A 95% confidence interval is shown by short-dashed lines; long-dashed lines fit values predicted by the regression model.
Fig. 5. Western blot analysis of VDRs and calbindins in the intestine and kidneys of space and ground animals. The cytosol extracts of the intestinal mucosa and kidneys were subjected to SDS-PAGE and immunoblotting, as described in Materials and Methods. Protein standards of the indicated molecular masses (K) were run in a parallel lane. CNTR = cytosol extract of MDBK cells which have lower levels of the VDR expression, as compared with rat tissues.
DISCUSSION

Calcium metabolism and its regulation change promptly (within days) with exposure of the body to microgravity or simulated weightlessness (hypokinesia, suspension) (Arnaud and Morey-Holton, 1989; Spirichev and Sergeev, 1988). The vitamin D hormone, 1,25(OH)\textsubscript{2}D\textsubscript{3}, is the critical component of the Ca\textsuperscript{2+}-regulating endocrine system (Norman et al., 1982, 1992). Vitamin D-dependent Ca\textsuperscript{2+}-binding proteins, calbindin-D\textsubscript{28k} and calbindin-D\textsubscript{9k}, play the essential roles in the regulation of Ca\textsuperscript{2+} metabolism and maintenance of cellular Ca\textsuperscript{2+} homeostasis. Calbindins are involved in the intestinal Ca\textsuperscript{2+} absorption, renal Ca\textsuperscript{2+} reabsorption, and intracellular Ca\textsuperscript{2+} buffering (Christakos et al., 1989; Rhoten and Sergeev, 1994; Johnson and Kumar, 1994).

In this study, we have demonstrated, using two independent approaches, a significant decrease in the renal calbindin-D\textsubscript{28k} and intestinal calbindin-D\textsubscript{9k} content and immunocytochemical expression in rats exposed for 9 days to microgravity on board the Spacelab 3 mission. Importantly, suspension animals demonstrated virtually identical changes in calbindins. These findings strongly suggest that factors of modelled weightlessness (suspension) can mimic the effects of microgravity on calbindins.

Because calbindins are vitamin D-regulated proteins and because VDRs seem to be not affected by the factors of space flight, the decreased circulating concentration of the hormonal form of vitamin D, 1,25(OH)\textsubscript{2}D\textsubscript{3}, might be primarily responsible for the reduction in calbindin contents and levels of expression in kidneys and intestine of flight and suspension animals. Decreased production of 1,25(OH)\textsubscript{2}D\textsubscript{3} in kidneys may determine, to a large extent, such reduction in the serum 1,25(OH)\textsubscript{2}D\textsubscript{3} concentration. As we have shown earlier, this is the case for hypokinetic model of weightlessness in rats (Sergeev et al., 1984; Spirichev and Sergeev, 1988).

The depression of calbindin-D\textsubscript{28k} in kidneys and calbindin-D\textsubscript{9k} in the intestine appears to be relatively selective, because β-actin level in the intestine and insulin level in the pancreas, evaluated by Western blot analysis and ICC respectively, were not changed in space and
suspension animals.

It is also noteworthy, that the decrease in calbindins was independent of the time of harvesting the tissues after reentry. This suggests that the reduction in amounts of calbindins and gene expression for these calcium-binding proteins is a long-lasting effect of microgravity and suspension per se, rather than a rapid, transient stress response.

Functional consequences of decreased calbindin expression, at the organismal level, might be the decreased absorption of Ca\textsuperscript{2+} in the intestine, increased excretion of Ca\textsuperscript{2+} in the urine, and, at the cellular level, the sustained increase in the concentration of cytosolic free Ca\textsuperscript{2+}, which may interfere with Ca\textsuperscript{2+} signaling and cause an increase in the rate of cell death.

Thus, our study implies that the decrease of calbindin contents and immunocytochemical expressions in kidneys and intestine from rats exposed to microgravity and modelled weightlessness (tail suspension) may be directly related to changes in Ca\textsuperscript{2+} metabolism under the effects of these conditions. Changes in calbindins may be attributed to an interference of microgravity and suspension with functioning of the vitamin D-endocrine system. Future space and ground-based experiments are necessary to test this hypothesis.
CONCLUSIONS

Content and immunocytochemical expression of calbindin-D_{28k} in kidney and calbindin-D_{9k} in the intestine were decreased in flight vs. flight control animals and in tail suspension vs. vivarium controls at "zero", 24 and 72 h post-flight. Decrease in renal calbindin-D_{28k} and intestinal calbindin-D_{9k} may affect Ca^{2+} handling in these organs; namely, it may be partly responsible for the increased Ca^{2+} excretion in the urine and decreased Ca^{2+} absorption in the small intestine observed after a short-term space flight and modelled weightlessness.

No consistent changes in the low levels of intestinal and renal VDRs of flight animals were found with Western blot analysis, implying that decreased circulating concentration of 1,25(OH)_{2}D_{3} in space flight and modelled weightlessness might reduce the calbindin expression in these tissues.

Factors of space flight and modelled weightlessness had no apparent effect on the immunocytochemical expression of insulin in pancreas.

Our findings suggest that the decreased expression of calbindins after a short-term exposure to microgravity and modelled weightlessness may affect cellular Ca^{2+} homeostasis and contribute to Ca^{2+} and bone metabolism disorders induced by space flight.
SIGNIFICANCE AND FUTURE GOALS

This investigation has allowed us to assess the effects of short-term space flight on calbindins and their regulation by the vitamin D endocrine system. Assessment included the application of a new method for quantifying calbindins at the single cell level. This technically significant advance should be utilized in future studies on cells, tissues and organs in altered gravity states. The results contribute to our understanding of the genesis of space flight-induced disorders of Ca\(^{2+}\) metabolism.

Future space and ground-based experiments are necessary to study functional consequences of the decreased expression of calbindins at the cellular level and to investigate whether up-regulation of calbindins, e.g., with analogs of the vitamin D hormone will be useful for prevention of disorders of Ca\(^{2+}\) metabolism induced by space flight.

ACKNOWLEDGEMENTS

We are grateful to Dr. Anthony W. Norman and Dr. Elizabeth M. Bruns for a generous gift of calbindin-D\(_{28k}\) and anti-calbindin-D\(_{9k}\) antiserum. We thank Michelle Carney and Aslam Chaudhry for technical assistance. We also greatly appreciate assistance provided by Dr. Willy Hinds of NASA-Ames Research Center and support from this agency.

Part of this study was presented at the 26th Congress of the Anatomical Society of Southern Africa, South Africa, 22 April, 1996. Some of the results will also be presented at the Annual Scientific Congress of the Zimbabwe Association of Clinical Pathologists and Medical Scientists, 6 July, 1996.
REFERENCES


## Calbindin-D28k, Kidney, ELISA

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A1
Calbindin-D9k, Intestine, ELISA

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Calbindin-D9k, Kidney, ELISA

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Calbindin-D28k, Kidney, ICC

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