# INFUSION OF CHICKENS WITH MAMMALIAN OR AVIAN CALCITONIN

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INFUSION OF CHICKENS WITH MAMMALIAN

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# CHAPTER I

## INTRODUCTION

It is generally agreed that calcitonin is a hypocalcemic factor produced in the C cells of the thyroid gland of mammals and the ultimobranchial bodies of birds, reptiles, and amphibians.

Major studies have been made of the effects of calcitonin in rats. In addition, porcine, ovine, and bovine calcitonin as well as calcitonin from chickens, skinks, and dogfish sharks have all been injected into rats and the effects studied. However, little work has been done in studying the effect of this relatively new hormone in species other than mammals. In view of this lack of information concerning the effects of calcitonin in birds, these experiments were designed to determine if the infusion of calcitonin into chickens could be shown to have any effect on the blood calcium or inorganic phosphorus.

## CHAPTER II

## LITERATURE REVIEW

The primary function of the parathyroid gland is the maintenance of a relatively constant level of calcium in the body fluids. MacCallum and Voegtlin in 1909 (45) observed that the surgical removal of these glands from dogs was followed by a profound fall in blood calcium accompanied by tetany and convulsions. Most investigators (36, 27) are of the opinion that the secretion of the parathyroid is controlled by the level of calcium in the blood with hypocalcemia stimulating the secretion of parathormone and hypercalcemia supressing it. McLean and Urist (46) have proposed that the relatively constant level of plasma calcium (Ca) is achieved by alterations in the secretion rate of parathormone (PTH). This hormone increases the plasma Ca level by increasing the rate of bone breakdown. This interaction of PTH and bone was described by Bates *et al.* (5). Bates studied the effects of PTH on the release of hydroxyproline from the collagen in the bone matrix, into the plasma. A decrease in the level of Ca in the blood stimulates the parathyroid glands to secrete more PTH; a rising plasma Ca would produce inhibition of secretion (46). This hypothesis was first tested by Copp *et al.* (14) in perfusion studies of the dog thyroid and parathyroid glands. Copp perfused hypercalcemic blood (12-14 mg%) through the parathyroid-thyroid complex for two hours. The blood was recirculated

continuously through a pump and oxygenator. The plasma was separated and injected into a test dog. A fall in plasma Ca of up to 1 mg/100 ml. within 20 minutes was noted. Their results were used to postulate the existence of a new hormone which was called calcitonin. This name was used since the hormone was apparently involved in the regulation of the normal level or "tone" of Ca in the body fluids. The studies in dogs did not indicate the source of calcitonin. The parathyroid glands of the dog are imbedded in the thyroid gland, and it was impossible to distinguish which tissue was responsible for the release of calcitonin. It was thought, however, that calcitonin was secreted by the parathyroid glands (15, 17).

The existence of calcitonin was soon confirmed by Kumar et al. (42). In experiments on the dog, the thyroid and parathyroid glands were perfused together in situ, with blood of high or low Ca content. This was done without net addition or removal of Ca from the circulation. There was a marked fall in systemic plasma Ca whenever the thyro-parathyroid glands were perfused with high Ca blood. This result could have been due to the inhibition of PTH secretion, but this explanation was excluded by control experiments. These workers also observed that the complete removal of the thyroid and parathyroid glands produced little change in plasma Ca in two and a half hours in contrast to a marked hypocalcemia accompanying thyro-parathyroid perfusion. A calcium-lowering substance must have been liberated from the thyroid or parathyroid glands during high calcium perfusion (16).

However, these experiments did not indicate whether calcitonin came from the thyroid or parathyroid glands. While studying parathyroidectomized rats, Hirsch et al. (33) discovered a Ca-lowering

principle in rat thyroid glands. They called this principle thyrocalcitonin in deference to the original name and adding the prefix to indicate the hormone's origin.

Perfusion of the parathyroid gland alone in the goat was next done to determine the origin of this Ca-lowering principle. (21) The goat was used because the superior pair of parathyroid glands is completely separate both in blood supply as well as by space from the thyroid and can thus be perfused separately. The findings showed that no effect was obtained by perfusing the parathyroid glands while a marked hypocalcemic response followed perfusion of the thyroid. This indicated that calcitonin was a thyroid hormone and substantiated the use of the term thyrocalcitonin.

Immunofluorscence techniques were employed in an effort to discover where in the thyroid gland thyrocalcitonin was synthesized. Nonidez (68) was the first to differentiate the two different cell types in the thyroid gland of mammals which had been described by Baber in 1876 (60). Hargis et al. (31) detected thyrocalcitonin (TCT) in the cytoplasm of all epithelial cells of the thyroid gland of pigs by the use of antibody fluorescence. TCT was present in those cells which normally elaborate the follicular colloid. However, TCT was not present in the follicular colloid. Foster (22) proposed, on the basis of cytochemical tests, that the mitochondria-rich cells of the thyroid rather than the regular follicular cells were the source of TCT. Pearse (56), using cytochemical techniques, suggested that the C cells in the thyroids of both dogs and pigs as well as cells tentatively called pericytes were the source of the hormone. Roher et al. (68) using an electron microscopic examination, found a strong functional

relation between the C cells of the thyroid and TCT. They found that an increase in Ca increased the number of parafollicular cells. Pearse reported that the C cells responded to high blood Ca levels by increased secretion of the product contained in their spherical vesicles (57). Further support for the C cells as the origin of TCT was provided by Bussolati and Pearse (7, 60). They used guinea-pig anti-calcitonin and an indirect technique to obtain immunofluorsecent evidence for the origin of TCT. The findings showed that calcitonin was manufactured in the C cells in both the pig and dog thyroid glands. They also found calcitonin in pericytes. The function of these cells was postulated to be a transfer mechanism of TCT from the C cells to the blood stream.

The next advance concerning the ultimate origin of TCT was made by Pearse and Carvalheria (58, 10). These workers produced cytochemical evidence showing that C cells of the thyroid are derived from the ultimobranchial bodies (UBB) and that ultimobranchial cells are the source of calcitonin. Mosely et al. (50) found that the UBBs developed from the hind part of the pharyngeal endoderm immediately behind the last pair of gill pouches. They also found that UBBs are present in most all vertebrates, but the UBBs in mammals have fused with the thyroid where they are represented by the C cells. Copp et al., Tauber, and Kraintz and Puil (19, 71, 40) have demonstrated that the site of calcitonin synthesis in chickens is the UBB. This was demonstrated by injecting UBB extracts into rats. Upon injection of the UBB extracts, the blood Ca dropped significantly. It was also found that the UBBs of skinks and the dogfish, Squalus suckeyi, contained calcitonin (19). Many other workers (50, 58, 71) have confirmed the

UBB origin of calcitonin. Copp has stated that "calcitonin is a fundamental calcium-regulating hormone present in all higher verbebrates and is an ultimobranchial hormone rather than a thyroid hormone" (20).

In the course of all these investigations, it has been necessary to purify calcitonin in order to have the hormone in a usable form. One of the most popular methods of purification was proposed by Hirsch (35). The first step involved the centrifugation of the extract obtained by homogenizing porcine thyroid glands in 0.1 N HCl at 100,000g for 18 hours at 4°C. This gave a ten fold increase in purity. Additional purity was obtained by five to ten gel filtrations with lyophilized supernate in 8 M urea on Sephadex G-50 equilibrated with 0.05 M sodium acetate buffer at pH of 3.8. Many other systems were worked out (3,71,29). However, the latest improved simplified separation was described by MacIntyre (48). This new method uses a much more efficient extraction with a new column partition chromatographic stage and gel filtration. The procedure is summarized below:

Acetone dried pig thyroid
  extract with 3, 1, 5, 0.6 l. n. butanol:
  acetic acid: water, 75:7.5:21(v/v) at room
  temperature for 12, 4, 0.5 hours
combined extract
  add 23 l. acetone, stand 72 hours at -10°C
  wash precipitate with cold acetone and ethanol
precipitate
  extract with 0.5 and 0.25 l. of 70% ethanol
  pH 5.5 for 2 or 3 hours
  concentrate in vacuo
combined extract
  add trichloroacetic acid (TCA) to make 12g./100 ml.
  stand for 12 hours at 4°C
  wash precipitate with 10% TCA (w/v) then
  four times with 80 ml. acetone: ether,
  50:50 (v/v) at -10°C
  dry in vacuo

precipitate
column partition chromatography — activity zones
beta activity
gel filtration
highly purified beta-calcitonin

These purification studies have also given rise to studies on the chemical nature of calcitonin. Tenenhouse (72) reported a molecular weight of 8700 or 8666. Later the molecular weight was reported to be between 5000 and 6000 with one fraction at 3000. MacIntyre reported a molecular weight of 3600 (48). Calcitonin has now been synthesized and was found to consist of a chain of 32 amino acid residues with a molecular weight of 3600 (69).

As mentioned previously, it was found that when calcitonin was injected into the rat, plasma Ca and phosphate started to decrease within minutes and reached a minimum in approximately one hour. It was observed by Robinson et al. (66) that the hormone increased the phosphate excretion in the urine of parathyroidectomized rats. However this phenomenon was found to be a minor one and not attributable to the hormone (67).

Many workers tried to identify the target organ of calcitonin. The first possible target organ studied was the kidney. Hirsch et al. (34) found that the injection of calcitonin exhibited its usual hypocalcemic effect in nephrectomized rats. He concluded that this activity persumably ruled out the kidney being involved in the action of calcitonin. Aliapoulis et al. (2) excluded the gut as playing a role in the action of calcitonin. This was done by showing that calcitonin caused hypocalcemia even when the entire gut had been removed from the experimental animals. Wase et al. (75) used nephrectomized rats which has received 25 uCi of $^{45}CaCl_2$ and 23 units of Hirsch type

calcitonin. Their experiment showed an increased incorporation of $^{45}Ca$ in the tibia and femur of the calcitonin-treated nephrectomized rats. They concluded from this observation that calcitonin enhanced deposition of Ca into bone.

Several studies of bones maintained in tissue cultures followed this work. Friedman and Raisz (25) found that calcitonin inhibited the release of Ca from the long bones of embryo rats in tissue cultures; Aliapoulis (2) confirmed these results. He used calvaria of Swiss albino mice and noted that the addition of calcitonin to the tissue culture media counteracted the resorption of bone stimulated by parathyroid extract. Harris and Sjoerdsma (32) showed that the rate of excretion of hydroxyproline in rat urine reflected the rate of bone destruction. Martin (49) reported that urinary hydroxproline dropped when animals were infused with calcitonin. On the basis of the work of Harris and Sjoerdsma and the work of Martin, it was concluded that bone resorption and collagen breakdown had been slowed or stopped after injection of calcitonin. These findings were confirmed by Aer (1) and Klein (39). Both Aer and Klein have reported that calcitonin reduced Ca and hydroxyproline excretion. More evidence showing calcitonin's effect on bone was reported by MacIntyre and Parsons (47). They noted that the action of calcitonin seemed to be directly on the bone since the hormone lowered the Ca content in the effluent blood from isolated perfused cat tibias. Using roentgenologic and histologic techniques, Foster (23) made some interesting observations in parathyroidectomized rats treated with calcitonin:

1. an increase in metaphyseal bone

2. a reduced number of osteoclasts in affected areas

3. an increase in the proportion of partially mineralized and unmineralized osteoid to the total trabecular bone volume.

The metaphyseal bone had increased in the vertebrae of the rats which has been given calcitonin. The amount of bone mineral in the proximal metaphysis of the treated animals was 50% greater than the controls. Foster used a microdensitometer with an aluminum standard for this measurement. Histological staining with toluidine blue of the tenth caudal vertebra and the use of integrating eyepieces showed that the amount of bone present in the trabeculae was about twice that in the normal controls. Foster concluded that calcitonin either increased the rate of bone formation, reduced the rate of bone mineralization, or interfered with the resorptive phase of unminerialized collagen turnover.

Recent work by Lee et al. (44) using radioimmuncassay has shown that calcitonin is continuously secreted into the peripheral blood. They believe that calcitonin plays an important metabolic role in mammals by acting as a continuous influence on bone remodeling and rapidly suppressing bone resorption in a hypocalcemic challenge.

Baylink et al. (6) have studied the effects of calcitonin on bone formation in rats. They found that moderate doses significantly decreased the rate of osteoclastic bone resorption even in the absence of endogenous PTH. Calcitonin decreased the resorptive activity of individual osteoclasts and this contributes to a decreased rate of bone resorption. They also found that calcitonin decreased resorption of matrix as well as mineral.

Urist (73) studied the effects of porcine and avian calcitonin

in chickens. Avian calcitonin was obtained by homogenizing the UBBs of White Leghorn roosters, laying hens, or resting hens in 0.1 N HCl. The calcitonin was assayed in new-born chicks. After taking a blood sample, a single injection of the calcitonin being assayed was administered. After 1 hour, a final blood sample was taken and analyzed. Urist found that the response of the chick to xenogenic calcitonin was insignificant. Extracts of avian allogenic ultimobranchial tissue had no effect on the chick, but the same extracts had a potent hypocalcemic action in rats.

Ziegler et al. (76) observed that the perfusion of the ultimobranchial glands of the hen with hypercalcemic blood stimulated a distinct hypocalcemic activity in the out-flowing blood. Neither high levels of magnesium or potassium nor hormones such as PTH, thyroid stimulating hormone, glucagon, or cortisone produced any effect.

Kraintz and Intscher (41) performed further studies of the effects of calcitonin in chickens. These workers infused avian or porcine calcitonin into intact roosters for 15 min. Blood samples were taken every 15 min. for 3 hours and the plasma Ca concentration noted. Neither the avian calcitonin nor the porcine calcitonin produced any marked changes in the plasma Ca concentration when compared with the control birds. The workers next eliminated the effect of endogenous PTH which might have masked the hypocalcemic action of the calcitonins studied by removing the superior pair of the parathyroid glands. The infusion of porcine or avian catcitonin into the parathyroidectomized chickens resulted in a marked drop in the plasma Ca concentration within 1 hour. The carrier failed to produce any change. Kraintz and Intscher concluded that the UBBs of chickens

were involved in plasma Ca homeostasis and that the failure to obtain a hypocalcemic response in intact chickens by the infusion of calcitonin was due to rapid adjustments of the plasma Ca concentration by the parathyroid glands.

Thus, it can be seen that calcitonin is a hormone released from the UBB in birds, reptiles, and sharks and from the C cells of the mammalian thyroid. Its principal site of action appears to be bone. The presence of this hormone causes a decrease in the rate of bone resorption. It has been postulated that the presence of this hormone is important in the fine regulation of Ca levels of the blood.

## CHAPTER III

## MATERIALS AND METHODS

White Leghorn cockerels were grown in batteries from day-old until they attained a weight of between 1200 to 1500 grams. The chickens were maintained on a broiler ration provided by the Oklahoma State University Poultry Department. The birds were given tap water ad libitum.

Two groups of chickens were infused in this experiment. The first group received mammalian calcitonin; the second group received avian calcitonin. The birds were infused in pairs and were randomly selected from one of the batteries. One of the birds was administered either the avian calcitonin or mammalian calcitonin while the other bird received the appropriate carrier.

After weighing the birds, they were anesthetized with sodium pentabarbitol* by injecting the anesthetic to effect into the brachialis profundus vein in the left wing.

Urine samples were collected from the first experimental group. The ureters were canulated** immediately after the birds were anesthetized. The urine was collected until enough urine was present for

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*Sixty-five mg. of sodium pentabarbitol/cc prepared in 0.2 volume ethanol and 0.8 volume distilled water.

**P. E. 90 Intramedic polyethylene tubing.

analysis. This was done as the urine flow was found to be rather erratic and no set time interval proved to be fully adequate. The urine samples were frozen immediately after collection.

The first group of chickens was infused with mammalian calcitonin*. The hormone was infused into the brachialis profundus vein in the right wing. The device used for this operation consisted of a 22 guage needle less the screw lock, attached to a piece of polyethylene tubing 31 cm. long. The other end of the tubing was slipped tightly over a standard 22 guage needle. The needle was placed on a 5 ml. glass syringe, which was then mounted on the infusion pump**. The pump was allowed to run for 30 min. prior to use in order for the machine to gear down to the low gear used--28. After obtaining an initial blood sample via cardiac puncture, the needle delivering the calcitonin, or 0.001 N HCl for control birds, was inserted into the brachialis profundus vein of the right wing. The infusion pump then delivered 0.000912 ml./min. for 3 hours. Blood samples were taken hourly for three hours. These samples were immediately heparinized, and centrifuged; and the plasma was frozen after collection.

The next experimental group was infused with avian calcitonin which was extracted from the UBBs of mature roosters. The roosters were decapitated. The UBBs were then removed and weighed. Between 75 to 90 mg. of UBB tissue was used. The UBBs were homogenized in 1.5 ml. of 0.1 N HCl and placed in a water bath for 1.5 hours at

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*Three mg. of calcitonin was dissolved in 0.75 ml. of 0.001 N HCl. Calcitonin purchased from Wilson Laboratories, 4221 Southwestern Blvd., Chicago, Illinois. Lot No. 135609-TCA Powder.

**Model 955 by Harvard Apparatus Company, Inc.

60°C. The extract was then centrifuged and the supernate collected.

This supernate was then infused into the brachialis profundus vein of the right wing for 3 hours according to the same procedure used in infusing the first group of chickens. Blood samples were taken every hour for 3 hours. They were immediately heparinized, centrifuged, and the plasma frozen and saved for analysis.

The birds receiving UBB extracts were run in three groups. The extract used for the first group contained 47 mg. of tissue per ml.; that of the second group had 52 mg. of tissue per ml.; and that of the third group 48 mg. of tissue per ml. The total amount of extract infused into each bird contained an equivalent of 7.7 mg. of tissue for group I, 8.55 mg. of tissue for group II, and 7.88 mg. of tissue for group III.

The plasma samples were analyzed for Ca using a fluorometric technique* by G. K. Turner Associates method**. Ca ion combines with 3,6 dihydroxy 2,4 bis (N,N'-carboxymethyl)-aminomethyl flouran, one of the condensation products of fluorescein with formaldehyde and iminodiacetic acid, to form a fluorescent complex. Flourscence is generated only by calcium, barium, strontium--the latter two being insignificant in serum. Sodium, potassium, magnesium, chlorine, and phosphate do not interfere with complex formation nor does protein. Hemoglobin up to 500 mg/100 ml. and bilirubin to 20 mg./100 ml. do not interfere.

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*Aminco Bowman Spectrofluorometer was used.

**Manual of Fluorometric Clinical Procedures, G. K. Turner, Associates.

The procedure consisted of placing 4 ml. of working reagent* into a cuvette. Twenty microliters of plasma or standard solution was added. The tubes were then inverted 10 times. The mixtures were incubated in a water bath at any temperature between 20° and 30°C for 10 to 30 min. The samples were then read on the flurometer**.

The plasma samples were also checked for phosphorus content by a modification of the Fiske-Subbarow method***. One-half milliliter of plasma was placed in a 12 ml. conical centrifuge tube. Two and one-half milliliters of distilled water and 2.0 ml. of 20% TCA were added. The tube was shaken and allowed to stand for 5 to 10 min. The tubes were then centrifuged for 5 min. and 2 ml. of the supernate were mixed with 3.0 ml. of distilled water in a spectrophotometric tube. One milliliter of 1.25% acid molybdate in 2.5N sulfuric acid was added to the tube. The contents of the tubes were thoroughly mixed and 0.25 ml. of Fiske-Subbarow reducer solution**** was added. The tubes were mixed by inversion and allowed to stand at room temperature for 10 min. before measuring the absorbance at 660 mu in a spectrophotometer*****. A water blank was run with the samples.

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*.8N K OH Stock reaction mixture--10 mg/ml. calcein in 0.8 N KOH. Working reagent--7.00 ml. stock to 1 liter with 0.8 N KOH.

**Excitation wavelength 484u microns, emission wavelength 516u microns (mu).

***Sigma Chemical Company, 3500 DeKalb Street, St. Louis, Missouri 63118. Sigma Tentative Technical Bulletin No. 670 December, 1965.

****1.0.g. in 6.3 ml. distilled water.

*****Coleman Junior Spectrophotometer, Coleman Instruments, Inc.

Urine samples were also analyzed for phosphorus (P). The above procedure was followed except that 1 ml. of urine was diluted to 10 ml. of distilled water and treated as plasma.

The urine was also analyzed for hydroxyproline.* One volume of 7% solution (w/v) of chloamine T was mixed with 4 volumes of acetate-citrate buffer** just before a series of determinations was run. A solution consisting of 2 g. of Erlich's reagent mixed with 3 ml. of 60% perchloric acid was made. This amount was good for one determination, thus multiples of this ratio were required for many samples. The Erlich's solution was then mixed with isopropanol in the ratio of 3 volumes of Erlich's solution to 13 volumes isopropanol. One milliliter of neutral or slightly acid solution was pipetted into a test tube. Two milliliters of isopropanol were added and mixed. One milliliter of the oxidant solution was added and thoroughly mixed. The tubes were allowed to stand for 4 minutes at room temperature. Thirteen milliliters of Erlich reaction solution were added and mixed. The tubes were heated for 25 minutes at 60°C in a water bath.*** The tubes were cooled for 2 to 3 minutes under tap water and then diluted to 50 ml. with isopropanol. The absorbance was measured at 558 mu. The color was stable for 4 hours. This technique was accurate for 20 to 35 parts per million.

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*Bergman, Imanuel and R. Loxley, "Two Improved and Simplified Methods for Spectrographic Determination of Hydroxyproline," Analytical Chemistry, 35 (November, 1963), pp. 1961-1965.

**57 g. Na acetate.3($H_2O$), 37.5 g. trisodium citrate, 5.5 g. citric acid ($H_2O$) and 385 ml. isopropanol made to 1 l. with $H_2O$.

***Precision Scientific Company--Water Constant Temperature Bath.

The final dilution was usually temporarily omitted and the tubes were read before dilution. This reading was 3 times as sensitive although the color was stable for only a half hour. It was found that best results were obtained when the final dilution was omitted.

The mammalian calcitonin and the UBB extracts were qualitatively assayed for hypocalcemic activity according to the procedure of Cooper et al. (13). Male Holtzman rats 5 weeks old and weighing 125 to 145 g. were used. All the animals were fed a commercial ration and given tap water ad libitum. The animals were fasted 18 to 24 hours before use.

The animals were lightly anesthetized with ether and a blood sample taken via cardiac puncture. The rats were then given a dose of either mammalian or avian calcitonin subcutaneously equal to that which the chickens received during the 3 hours of infusion. Control rats received the carrier. After 70 min., the rats were again lightly anesthetized with ether and another blood sample was taken via cardiac puncture. The blood samples were immediately heparinized after collection, centrifuged, and the plasma collected. The plasma was frozen until analyzed. The plasma was analyzed for Ca in the same manner as was the chicken plasma.

# CHAPTER IV

## RESULTS AND DISCUSSION

The mean plasma Ca and P levels obtained during the infusion of chickens with mammalian calcitonin are shown in Table I. The plasma Ca and P levels of the experimental birds at 0, 1, 2, and 3 hours were compared with their respective controls, i.e., the plasma Ca and P levels at zero hour for the experimental birds was compared with the plasma Ca and P levels at zero hour for the controls. Student's t test was used in the comparisons. No statistically significant difference was noted between the plasma Ca and P levels of the experimental birds at 0, 1, 2, and 3 hours and the plasma Ca and P levels of the control birds at the corresponding hour. A t test was also used to compare the body weights of the animals; no significant difference was noted between the body weights of the control and experimental birds.

The mean plasma Ca and P levels noted after infusing chickens with extracts of avian UBBs (avian calcitonin) are also shown in Table I. Similar comparisons were made between the experimental birds and the control birds as was done with the first group. No statistically significant difference was noted between any of the plasma Ca or inorganic P levels of samples taken at the end of the same hour. The comparison of the body weights yielded no significant difference.

TABLE I

MEAN PLASMA CALCIUM AND PHOSPHORUS LEVELS

| Group | | Hours After Infusion 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| **Mammalian Calcitonin** | | | | | |
| Experimental | CA(mg%) | 6.858±.348 | 6.439±.437 | 6.234±.414 | 6.525±.386 |
| | P (mg%) | 5.242±.243 | 5.079±.352 | 5.484±.376 | 6.155±.488 |
| Controls | Ca | 7.197±.364 | 6.525±.422 | 6.840±.369 | 6.709±.440 |
| | P | 4.867±.205 | 4.808±.181 | 5.638±.242 | 5.972±.383 |
| **Avian Calcitonin** | | | | | |
| Experimental | Ca(mg%) | 6.940±.573 | 5.602±.476 | 6.006±.449 | 5.775±.385 |
| | P (mg%) | 5.426±.415 | 5.509±.284 | 6.516±.306 | 5.882±.658 |
| Controls | Ca | 5.668±1.05 | 5.355±1.090 | 4.857±1.100 | 5.407±1.260 |
| | P | 4.486±.828 | 4.850±.985 | 4.701±1.030 | 4.663±1.010 |

The rate of urine flow was very erratic. A sample of 15 ml. was collected from one chicken in 1 hour, but none was collected during the next 2 hours. This erratic flow made the collection of samples at regular intervals impossible. The collection was continued, discounting time, until enough urine was collected to analyze. The lack of time coordination made the valid comparison of one set of data with another impossible. It was for this reason that no attempt was made to collect urine in the second part of the experiment.

The lack of a significant lowering of the plasma Ca level and an effect on the plasma P level in the chickens could have been due to inactive hormonal preparations. In order to determine if the hormonal preparations were active, similar mammalian and avian calcitonin preparations were assayed in rats. The mean plasma Ca levels observed in these rats are shown in Table II.

The assay of the mammalian calcitonin preparation showed that it was active. There was a significant drop ($P<.05$) in the plasma Ca after 70 min. Comparison of the Ca levels of rats injected with carrier, 0.001 N HCl, with those given the mammalian calcitonin was significant ($P<.05$).

The assay of an avian calcitonin preparation in rats showed that it was also active. A significant drop ($P<.01$) in the plasma Ca level occured 70 min. after the injection of the hormone. Comparison of the Ca levels of rats injected with carrier, 0.1 N HCl, showed a significant difference ($P<.05$).

The results of this experiment were similar to the results of Kraintz and Intscher (41). In this experiment and in the experiment of Kraintz and Intscher, intact roosters were infused with mammalian

TABLE II

MEAN PLASMA CALCIUM LEVELS (in mg.%)
FROM ASSAY OF MAMMALIAN AND
AVIAN CALCITONIN

| | Zero Hour | Termination Time |
|---|---|---|
| **Mammalian Calcitonin** | | |
| Experimental | 6.401±.502 | 4.764±.494 |
| Control | 6.862±.332 | 5.712±.827 |
| **Avian Calcitonin** | | |
| Experimental | 5.817±.519 | 2.460±.852 |
| Control | 7.559±.456 | 5.988±.637 |

calcitonin or avian calcitonin. The infusion of the avian or mammalian calcitonin into the intact birds failed to elict a usual hypocalcemic response. It was concluded from this experiment that intact chickens were relatively unresponsive to calcitonin.

Kraintz and Intscher also studied the effects of infusing avian or mammalian calcitonin into chickens which had had the superior pair of parathyroid glands removed. These workers found that the infusion of an avian or mammalian calcitonin preparation into these chickens resulted in a marked hypocalcemia. Kraintz and Intscher concluded from their experiment that the unresponsiveness of chickens to the infusion of a calcitonin preparation was due to the rapid mobilization of the parathyroid glands.

# CHAPTER V

## SUMMARY

White Leghorn cockerels weighing between 1200 and 1500 grams were infused for three hours. One group of birds received a solution containing 3.0 mg. of mammalian calcitonin in 0.75 ml. of 0.001 N HCl; the controls received 0.001 N HCl alone. Another group received UBB extracts of approximately 49 mg. of tissue in 1.5 ml. of 0.1 N HCl. The extracts were obtained by homogenizing the UBBs and placing the mixture in a water bath set at 60°C for 1.5 hours. The mixture was centrifuged and the supernate collected and used in the infusion. Controls received the 0.1 N HCl alone.

Hourly blood samples were taken via cardiac puncture. The plasma was collected and frozen until analyzed for inorganic P with a modified Fiske-Subbarow method and for Ca with a flourometric method.

The results of this experiment indicated no statistically significant difference between the plasma Ca and P levels of the experimental birds and the control birds at any one time of sampling. The lack of a significant lowering of the plasma Ca and an effect on the plasma P in the chickens could not have been due to inactive hormonal preparations. Similar hormonal preparations of both the avian and mammalian calcitonin were assayed in rats. Both the avian and mammalian calcitonin preparations were found to be

active hypocalcemic factors in the rats. It was concluded that chickens were relatively unresponsive to calcitonin.

On the basis of the work of Kraintz and Intscher, it was found that the unresponsiveness of the chickens to the infusion of calcitonin was due to the rapid mobilization of the parathyroid glands in the presence of a hypocalcemic challenge.

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# APPENDIX

APPENDIX A

CALCIUM AND PHOSPHORUS LEVELS (in mg%) IN CHICKENS PERFUSED WITH MAMMALIAN CALCITONIN

| Experimental | Bird Number | Body Weight | Zero Hour | First Hour | Second Hour | Third Hour |
|---|---|---|---|---|---|---|
| | 1 | 1200 | 7.3 | 7.3 | 4.15 | ---- |
| Ca | | | 6.1 | 5.5 | 3.5 | ---- |
| | Avg. | | 6.7 | 6.4 | 3.8 | ---- |
| P | 1 | | 7.1 | 7.3 | 5.5 | 5.75 |
| | | | 5.4 | 5.5 | 5.6 | 5.7 |
| | Avg. | | 6.25 | 6.4 | 5.55 | 5.73 |
| | 2 | 1738 | 6.6 | 5.0 | 5.2 | 6.1 |
| Ca | | | 6.6 | 4.0 | 5.2 | 5.9 |
| | Avg. | | 6.6 | 4.5 | 5.2 | 6.0 |
| | 2 | | 3.8 | 4.15 | 4.77 | 3.85 |
| P | | | 3.75 | 4.0 | ---- | 3.80 |
| | Avg. | | 3.78 | 4.07 | 4.17 | 383 |
| | 3 | 1322 | 8.2 | 7.6 | 7.0 | 5.4 |
| Ca | | | 7.8 | 7.6 | 7.0 | 5.0 |
| | Avg. | | 8.0 | 7.6 | 7.0 | 5.2 |
| | 3 | | 5.35 | 5.76 | 7.8 | 9.0 |
| P | | | 6.25 | 6.5 | 8.45 | 9.0 |
| | Avg. | | 5.8 | 6.13 | 8.12 | 9.0 |
| | 4 | 1683 | 6.1 | 5.5 | 4.7 | 5.8 |
| Ca | | | 6.1 | 5.8 | 4.3 | 5.9 |
| | Avg. | | 6.1 | 5.65 | 4.5 | 5.85 |
| | 4 | | 2.2 | 4.1 | 4.4 | ---- |
| P | | | 6.75 | 4.25 | 4.6 | ---- |
| | Avg. | | 4.47 | 4.17 | 4.5 | ---- |
| | 5 | 1340 | 7.9 | 9.1 | 6.3 | 8.0 |
| Ca | | | 7.7 | 9.1 | 6.5 | 8.0 |
| | Avg. | | 7.8 | 9.1 | 6.4 | 8.0 |
| | 5 | | 5.7 | 6.5 | 5.9 | 4.5 |
| P | | | 5.0 | 5.0 | 6.1 | 8.6 |
| | Avg. | | 5.35 | 5.75 | 6.0 | 6.43 |
| | 6 | 1288 | 9.26 | 6.77 | 7.8 | 6.2 |
| Ca | | | 8.75 | 7.00 | 7.4 | 6.0 |
| | Avg. | | 9.0 | 6.88 | 7.6 | 6.1 |
| | 6 | | 4.7 | 5.75 | 3.75 | 6.99 |
| P | | | 5.0 | 3.9 | ---- | 6.6 |
| | Avg. | | 4.85 | 4.8 | 3.75 | 6.79 |

| Experimental | Bird Number | Body Weight | Zero Hour | First Hour | Second Hour | Third Hour |
|---|---|---|---|---|---|---|
| | 7 | 1350 | 4.75 | 6.01 | 10.00 | 10.00 |
| Ca | | | 5.1 | 5.76 | 6.8 | 7.6 |
| Avg. | | | 4.93 | 5.9 | 8.4 | 8.8 |
| | 7 | | 6.5 | 7.1 | 6.9 | 6.3 |
| P | | | ---- | ---- | ---- | ---- |
| Avg. | | | 6.5 | 7.1 | 6.9 | 6.3 |
| | 8 | 1410 | 8.2 | 8.0 | ---- | ---- |
| Ca | | | 8.0 | 8.2 | ---- | ---- |
| Avg. | | | 8.1 | 8.2 | ---- | ---- |
| | 8 | | 6.25 | 3.9 | ---- | ---- |
| P | | | 5.5 | 4.6 | ---- | ---- |
| Avg. | | | 5.88 | 4.25 | ---- | ---- |
| | 9 | 1300 | 6.2 | 7.1 | 6.6 | 5.0 |
| Ca | | | 6.6 | 8.0 | 6.5 | 5.4 |
| Avg. | | | 6.4 | 7.5 | 6.55 | 5.2 |
| | 9 | | 5.5 | 4.75 | 3.91 | 5.3 |
| P | | | 3.9 | 6.1 | 4.49 | 5.7 |
| Avg. | | | 4.7 | 5.42 | 4.2 | 5.5 |
| | 10 | 1366 | 5.98 | 3.5 | 5.0 | 7.5 |
| Ca | | | 6.3 | 4.1 | 5.62 | 7.2 |
| Avg. | | | 6.14 | 3.72 | 5.27 | 7.3 |
| | 10 | | 4.25 | 3.2 | 3.7 | 4.25 |
| P | | | 4.25 | 2.75 | 3.9 | 4.48 |
| Avg. | | | 4.25 | 2.97 | 3.8 | 4.36 |
| | 11 | 1202 | 7.5 | 6.2 | 7.0 | 7.2 |
| Ca | | | 7.3 | 6.5 | 7.7 | 6.8 |
| Avg. | | | 7.25 | 6.32 | 7.37 | 7.1 |
| | 11 | | 5.2 | 5.2 | 5.32 | 5.2 |
| P | | | 6.5 | 5.4 | 5.4 | 6.9 |
| Avg. | | | 5.85 | 5.3 | 5.36 | 6.05 |
| | 12 | 1140 | 5.0 | 5.6 | 6.2 | 5.2 |
| Ca | | | 5.6 | ---- | 6.8 | 6.2 |
| Avg. | | | 5.3 | 5.6 | 6.5 | 5.7 |
| | 12 | | 5.4 | 5.75 | 6.2 | 8.0 |
| P | | | 5.0 | 5.5 | 5.75 | 8.25 |
| Avg. | | | 5.2 | 5.62 | 5.98 | 8.18 |
| Mean Ca Level | | | 6.86 | 6.44 | 6.23 | 6.52 |
| Mean P Level | | | 5.24 | 5.08 | 5.48 | 6.16 |
| Controls | | | | | | |
| | 1 | 1360 | 5.0 | 3.7 | 4.0 | 4.1 |
| Ca | | | 6.0 | 5.5 | 3.6 | 4.8 |
| Avg. | | | 5.5 | 4.6 | 3.8 | 4.45 |
| | 1 | | 3.75 | 3.25 | 6.3 | 5.90 |
| P | | | 4.00 | 4.00 | 5.6 | 6.15 |
| Avg. | | | 3.83 | 3.62 | 5.95 | 6.02 |

| Controls | Bird Number | Body Weight | Zero Hour | First Hour | Second Hour | Third Hour |
|---|---|---|---|---|---|---|
| | 2 | 1318 | 6.3 | 7.4 | 7.2 | 6.9 |
| Ca | | | 7.0 | 6.8 | 7.5 | 5.2 |
| Avg. | | | 6.85 | 7.1 | 7.35 | 6.05 |
| | 2 | | 4.30 | 4.5 | 5.00 | 5.75 |
| P | | | 5.75 | 4.8 | 5.51 | 6.25 |
| Avg. | | | 5.02 | 4.65 | 5.25 | 6.00 |
| | 3 | 1403 | 5.8 | 5.3 | 5.6 | 6.3 |
| Ca | | | 6.0 | 5.2 | 5.9 | 10.1 |
| Avg. | | | 5.9 | 5.25 | 6.25 | 8.2 |
| | 4 | 1410 | 6.5 | 6.3 | 8.0 | 6.3 |
| Ca | | | 9.5 | 8.4 | 9.7 | 4.2 |
| Avg. | | | 8.0 | 7.35 | 8.85 | 5.25 |
| | 4 | | 4.6 | 4.49 | 5.75 | 5.9 |
| P | | | ---- | 4.25 | 4.76 | 6.5 |
| Avg. | | | 4.6 | 4.37 | 5.25 | 6.2 |
| | 5 | 1388 | 7.6 | 5.5 | 5.74 | 5.0 |
| Ca | | | 7.25 | 5.01 | 5.1 | 4.75 |
| Avg. | | | 7.43 | 5.25 | 5.42 | 4.87 |
| | 5 | | 5.2 | 3.75 | 5.3 | 5.0 |
| P | | | 4.25 | 3.8 | 5.01 | 7.3 |
| Avg. | | | 4.87 | 3.77 | 5.15 | 6.15 |
| | 6 | 1372 | 8.7 | 7.02 | 6.75 | 8.25 |
| Ca | | | 8.75 | 7.15 | 7.25 | 7.8 |
| Avg. | | | 8.73 | 7.08 | 7.0 | 8.03 |
| | 6 | | 5.2 | 5.5 | 4.4 | 11.0 |
| P | | | 4.7 | ---- | 4.75 | 8.5 |
| Avg. | | | 4.9 | 5.5 | 4.57 | 9.75 |
| | 7 | 1370 | 5.1 | 6.5 | 5.3 | 6.3 |
| Ca | | | 7.4 | 12.9 | 11.1 | 9.5 |
| Avg. | | | 6.25 | 9.7 | 8.2 | 7.9 |
| | 7 | | 3.8 | 5.51 | 6.9 | 6.5 |
| P | | | 3.81 | 5.18 | 6.95 | 6.5 |
| Avg. | | | 3.8 | 5.4 | 6.92 | 6.5 |
| | 8 | 1260 | 6.7 | 5.75 | 6.75 | 7.8 |
| Ca | | | 7.02 | 3.75 | 7.02 | 8.75 |
| Avg. | | | 6.86 | 4.75 | 6.88 | 8.28 |
| | 8 | | 5.5 | 6.2 | 3.0 | 3.2 |
| P | | | 5.5 | 5.4 | 4.8 | 6.25 |
| Avg. | | | 5.5 | 5.9 | 4.9 | 5.7 |
| | 9 | 1520 | 8.48 | 7.75 | 7.50 | 5.23 |
| Ca | | | 8.50 | 7.46 | 9.00 | 5.20 |
| Avg. | | | 8.49 | 7.60 | 8.25 | 5.22 |
| | 9 | | 5.25 | 5.30 | 6.75 | 5.75 |
| P | | | 4.76 | 4.60 | 6.10 | 5.90 |
| Avg. | | | 5.00 | 4.95 | 6.42 | 5.82 |
| | 10 | 1356 | 4.6 | 4.6 | 7.0 | 8.2 |
| Ca | | | 4.2 | 7.0 | 7.0 | 8.0 |
| Avg. | | | 4.4 | 5.8 | 7.0 | 8.1 |
| | 10 | | 6.25 | 5.0 | 6.2 | 5.25 |
| P | | | 7.2 | 5.75 | 5.9 | 6.25 |
| Avg. | | | 6.72 | 5.37 | 6.05 | 5.75 |

| Controls | Bird Number | Body Weight | Zero Hour | First Hour | Second Hour | Third Hour |
|---|---|---|---|---|---|---|
| | 11 | 1428 | 7.2 | 4.9 | ---- | ---- |
| Ca | | | 9.4 | 5.3 | ---- | ---- |
| Avg. | | | 8.3 | 5.1 | ---- | ---- |
| | 11 | | 4.75 | 4.48 | ---- | ---- |
| P | | | ---- | ---- | ---- | ---- |
| Avg. | | | 4.75 | 4.48 | ---- | ---- |
| | 12 | 1500 | 7.1 | 7.4 | 5.65 | 8.45 |
| Ca | | | 10.0 | 7.5 | 6.35 | 8.10 |
| Avg. | | | 8.5 | 7.45 | 6.00 | 8.27 |
| | 12 | | 3.90 | 3.5 | 4.0 | 4.48 |
| P | | | 4.00 | 4.0 | 5.2 | 4.75 |
| Avg. | | | 3.95 | 3.75 | 5.6 | 4.62 |
| | 13 | 1462 | 6.5 | 8.3 | 5.9 | 6.5 |
| Ca | | | 6.9 | 8.3 | 7.1 | 9.2 |
| Avg. | | | 6.7 | 8.3 | 6.5 | 7.8 |
| | 13 | | 5.20 | 4.75 | 5.5 | 3.8 |
| P | | | 5.25 | 4.48 | 7.45 | 4.0 |
| Avg. | | | 5.22 | 4.62 | 6.48 | 3.9 |
| | 14 | 1412 | 7.40 | 6.20 | 8.90 | 7.4 |
| Ca | | | 8.30 | 7.10 | 6.00 | 7.2 |
| Avg. | | | 8.85 | 6.65 | 7.45 | 7.3 |
| | 14 | | 5.3 | 5.3 | 6.0 | 5.0 |
| P | | | 5.75 | 5.75 | 5.75 | 4.25 |
| Avg. | | | 5.52 | 5.52 | 6.18 | 4.62 |
| Mean Ca Level | | | 7.19 | 6.52 | 6.84 | 6.71 |
| Mean P Level | | | 4.87 | 4.81 | 5.64 | 5.97 |

## APPENDIX B

### CALCIUM AND PHOSPHORUS LEVELS (in mg%) IN CHICKENS PERFUSED WITH ULTIMOBRANCHIAL EXTRACT

| Experimental | | Bird Number | Body Weight | Zero Hour | First Hour | Second Hour | Third Hour |
|---|---|---|---|---|---|---|---|
| | | 1 | 1266 | 6.2 | 5.62 | 4.8 | ---- |
| Ca | | | | 6.5 | 5.18 | 5.3 | ---- |
| | Avg. | | | 6.35 | 5.40 | 5.05 | ---- |
| | | 1 | | 5.45 | 5.5 | 8.49 | ---- |
| P | | | | 5.3 | 4.75 | 5.25 | ---- |
| | Avg. | | | 5.39 | 5.12 | 6.87 | ---- |
| | | 2 | 1410 | 8.8 | 6.82 | 6.2 | 5.8 |
| Ca | | | | 8.8 | 7.0 | 6.82 | 7.9 |
| | Avg. | | | 8.8 | 6.91 | 6.51 | 6.85 |
| | | 2 | | 3.8 | 4.0 | 4.75 | 4.48 |
| P | | | | 4.1 | 5.65 | 4.75 | 4.7 |
| | Avg. | | | 3.95 | 4.82 | 4.75 | 4.59 |
| | | 3 | 1376 | 6.7 | 7.0 | 6.2 | 5.8 |
| Ca | | | | 6.8 | 7.2 | 6.0 | 5.8 |
| | Avg. | | | 6.75 | 7.1 | 6.1 | 5.8 |
| | | 3 | | 6.25 | 5.3 | 7.0 | 5.75 |
| P | | | | 4.25 | 5.75 | 5.9 | 5.5 |
| | Avg. | | | 5.25 | 5.52 | 6.45 | 5.62 |
| | | 4 | 1202 | 7.0 | 5.2 | ---- | ---- |
| Ca | | | | 7.4 | 4.82 | ---- | ---- |
| | Avg. | | | 7.2 | 5.01 | ---- | ---- |
| | | 4 | | 6.52 | 6.51 | ---- | ---- |
| P | | | | 5.25 | 7.2 | ---- | ---- |
| | Avg. | | | 5.88 | 6.85 | ---- | ---- |
| | | 5 | 1508 | 8.3 | 6.2 | 5.75 | 5.15 |
| Ca | | | | 7.6 | 5.8 | 5.0 | ---- |
| | Avg. | | | 7.95 | 6.0 | 5.38 | 5.15 |
| | | 5 | | 4.15 | 5.0 | 7.0 | 7.72 |
| P | | | | 4.2 | 4.75 | 6.75 | 7.7 |
| | Avg. | | | 4.17 | 4.87 | 6.87 | 7.71 |
| | | 6 | 1188 | 7.15 | 7.0 | 6.0 | 4.8 |
| Ca | | | | 6.7 | 6.7 | 5.7 | 5.8 |
| | Avg. | | | 6.92 | 6.85 | 5.85 | 5.2 |
| | | 6 | | 5.25 | 5.33 | 7.25 | 5.48 |
| P | | | | 5.7 | 6.5 | 6.2 | 5.74 |
| | Avg. | | | 5.47 | 5.91 | 6.72 | 5.61 |

| Experimental | Bird Number | Body Weight | Zero Hour | First Hour | Second Hour | Third Hour |
|---|---|---|---|---|---|---|
| | 7 | 1053 | 7.1 | 3.0 | 9.1 | ---- |
| Ca | | | 9.1 | 3.9 | 9.0 | ---- |
| Avg. | | | 8.1 | 3.45 | 9.05 | ---- |
| | 7 | | 6.5 | 4.6 | 6.25 | ---- |
| P | | | 4.5 | 4.62 | 6.5 | ---- |
| Avg. | | | 5.5 | 4.61 | 6.38 | ---- |
| | 8 | 1600 | 1.7 | 4.0 | 3.8 | ---- |
| Ca | | | 5.2 | 4.2 | 4.4 | ---- |
| Avg. | | | 3.45 | 4.1 | 4.1 | ---- |
| | 8 | | 8.6 | 7.45 | 7.25 | ---- |
| P | | | 7.0 | 5.3 | 7.2 | ---- |
| Avg. | | | 7.8 | 6.37 | 7.23 | ---- |
| Mean Ca Level | | | 6.94 | 5.60 | 6.01 | 5.77 |
| Mean P Level | | | 5.43 | 5.51 | 6.52 | 5.88 |
| Controls | | | | | | |
| | 1 | 1496 | 7.2 | 4.8 | 4.8 | 8.8 |
| Ca | | | 7.9 | 5.8 | 4.8 | 8.4 |
| Avg. | | | 7.55 | 5.3 | 4.8 | 8.6 |
| | 1 | | 5.25 | 5.25 | 7.0 | 6.2 |
| P | | | 5.26 | 6.8 | 6.75 | ---- |
| Avg. | | | 5.26 | 6.02 | 6.87 | 6.2 |
| | 2 | 1208 | 5.22 | 5.0 | 3.8 | 7.9 |
| Ca | | | 4.8 | 5.58 | 4.3 | 7.0 |
| Avg. | | | 5.01 | 5.29 | 4.05 | 7.45 |
| | 2 | | 4.0 | 6.2 | 6.75 | 4.75 |
| P | | | 4.2 | 5.8 | 7.15 | 4.66 |
| Avg. | | | 4.6 | 5.0 | 5.5 | 6.5 |
| | 3 | 1378 | 5.8 | 6.7 | 7.0 | 8.8 |
| Ca | | | 6.2 | 7.4 | 7.68 | 8.3 |
| Avg. | | | 6.0 | 7.05 | 7.34 | 8.55 |
| | 3 | | 4.6 | 5.0 | 5.5 | 6.5 |
| P | | | 5.3 | 4.25 | 5.3 | 7.3 |
| Avg. | | | 4.95 | 4.62 | 5.4 | 6.9 |
| | 4 | 1236 | 5.7 | 4.8 | ---- | ---- |
| Ca | | | 4.8 | 4.8 | ---- | ---- |
| Avg. | | | 5.25 | 4.8 | ---- | ---- |
| | 4 | | 4.9 | 8.6 | ---- | ---- |
| P | | | 7.2 | ---- | ---- | ---- |
| Avg. | | | 6.05 | 8.6 | ---- | ---- |
| | 5 | 1532 | 4.2 | 4.6 | 4.0 | 3.7 |
| Ca | | | 8.0 | 4.6 | 4.3 | ---- |
| Avg. | | | 6.1 | 4.6 | 4.25 | 3.7 |
| | 5 | | 4.15 | 4.15 | 5.0 | 5.0 |
| P | | | 4.75 | 5.25 | 4.5 | 4.75 |
| Avg. | | | 4.45 | 4.70 | 4.75 | 4.87 |

| Controls | Bird Number | Body Weight | Zero Hour | First Hour | Second Hour | Third Hour |
|---|---|---|---|---|---|---|
| | 6 | 1462 | 6.83 | 9.3 | 4.8 | 2.5 |
| Ca | | | 6.9 | 9.6 | 6.42 | 7.0 |
| Avg. | | | 6.83 | 9.45 | 5.61 | 4.75 |
| | 6 | | 6.5 | 4.11 | 5.25 | 4.75 |
| P | | | 7.25 | 4.75 | 5.0 | 3.7 |
| Avg. | | | 6.87 | 4.43 | 5.12 | 4.22 |
| | 7 | 1286 | 8.58 | 5.8 | 8.0 | 4.8 |
| Ca | | | 8.56 | 6.9 | 7.9 | 4.8 |
| Avg. | | | 8.57 | 6.35 | 7.95 | 4.8 |
| | 7 | | 3.7 | 4.48 | 4.25 | 5.75 |
| P | | | 4.72 | 4.0 | 4.0 | 5.75 |
| Avg. | | | 4.21 | 4.24 | 4.12 | 5.75 |
| Mean Ca Level | | | 5.67 | 5.35 | 4.86 | 5.41 |
| Mean P Level | | | 4.49 | 4.85 | 4.70 | 4.66 |

## APPENDIX C

## ASSAY OF MAMMALIAN CALCITONIN

| Rat Number | Body Weight | Calcium Level at Zero Hour | Mean | Calcium Level at Termination Time | Mean |
|---|---|---|---|---|---|
| 1 | 126 g | 5.0 mg%<br>4.9 | 4.95 | 5.8 mg%<br>5.6 | 5.7 |
| 2 | 126 | 6.8<br>6.75 | 6.78 | 4.6<br>4.5 | 4.55 |
| 3 | 136 | 6.4<br>6.37 | 6.38 | 5.0<br>4.6 | 4.8 |
| 4 | 120 | 5.0<br>5.0 | 5.0 | 2.3<br>2.3 | 2.3 |
| 5 | 125 | 6.0<br>6.0 | 6.0 | 3.9<br>4.3 | 4.1 |
| 6 | 127 | 6.8<br>6.9 | 6.85 | 5.8<br>6.0 | 5.9 |
| 7 | 126 | 8.6<br>9.0 | 8.85 | 6.0<br>6.0 | 6.0 |
| | Means of the Means | | 6.37 | | 4.76 |

## APPENDIX D

## ASSAY OF AVIAN CALCITONIN

| Rat Number | Body Weight | Calcium Level at Zero Hour | Mean | Calcium Level at Termination Time | Mean |
|---|---|---|---|---|---|
| 1 | 124 g | 5.0 mg%<br>5.0 | | 3.8 mg%<br>3.2 | |
| | | | 5.0 | | 3.5 |
| 2 | 124 | 7.2<br>6.9 | | ----<br>---- | |
| | | | 7.05 | | ---- |
| 3 | 143 | 7.2<br>6.8 | | 2.0<br>2.8 | |
| | | | 7.0 | | 2.4 |
| 4 | 129 | 5.0<br>5.0 | | 2.3<br>2.3 | |
| | | | 5.0 | | 2.3 |
| 5 | 134 | 6.9<br>6.6 | | 2.2<br>2.0 | |
| | | | 6.75 | | 2.1 |
| 6 | 135 | 4.1<br>4.1 | | 2.0<br>2.0 | |
| | | | 4.1 | | 2.0 |
| | Means of the Means | | 5.81 | | 2.46 |

## APPENDIX E

### TEST OF EFFECT OF CARRIERS ON ASSAY

#### 0.1 N HCl

| Rat Number | Body Weight | Calcium Level at Zero Hour | Mean | Calcium Level at Termination Time | Mean |
|---|---|---|---|---|---|
| 1 | 153g | 6.6mg%<br>6.62 | | 7.2mg%<br>6.8 | |
| | | | 6.61 | | 7.0 |
| 2 | 142 | 8.0<br>8.2 | | 4.0<br>3.9 | |
| | | | 8.1 | | 3.95 |
| 3 | 136 | 7.5<br>7.3 | | 6.0<br>6.0 | |
| | | | 7.4 | | 6.0 |
| 4 | 134 | 8.0<br>7.8 | | 5.8<br>6.0 | |
| | | | 7.9 | | 5.9 |
| | Means of the Means | | 7.50 | | 7.46 |

#### 0.001 N HCl

| | | | | | |
|---|---|---|---|---|---|
| 1 | 146 | 6.4<br>7.1 | | 7.8<br>7.5 | |
| | | | 6.75 | | 7.65 |
| 2 | 146 | 7.2<br>7.0 | | 3.8<br>3.6 | |
| | | | 7.1 | | 3.7 |
| 3 | 144 | 5.6<br>5.8 | | 6.2<br>6.2 | |
| | | | 5.7 | | 6.2 |
| 4 | 131 | 8.0<br>7.8 | | 6.2<br>6.6 | |
| | | | 7.9 | | 6.4 |
| | Mean of the Means | | 6.86 | | 5.99 |

VITA 2

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