6-1971

The role of the ultimobranchial glands in calcium metabolism of the laying hen

Abdul Karim Gaidan Al-Khazraji

To the Graduate Council:

I am submitting herewith a dissertation written by Abdul Karim Gaidan Al-Khazraji entitled "The role of the ultimobranchial glands in calcium metabolism of the laying hen." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

H. V. Shirley, Major Professor

We have read this dissertation and recommend its acceptance:

O. E. Goff, R. C. Fraser, R. L. Murphree

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

June 10, 1971

159

To the Graduate Council:

I am submitting herewith a dissertation written by Abdul Karim Gaidan Al-Khazraji entitled "The Role of the Ultimobranchial Glands in Calcium Metabolism of the Laying Hen." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

H. V. Shirley
Major Professor

We have read this dissertation
and recommend its acceptance:

R. L. Murphree

O. E. Goff

R. C. Fraser

Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

THE ROLE OF THE ULTIMOBRANCHIAL GLANDS IN CALCIUM METABOLISM OF THE LAYING HEN

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Abdul Karim Gaidan Al-Khazraji

June 1971

ACKNOWLEDGMENTS

The author wishes to acknowledge the assistance of and to express appreciation to:

Dr. H. V. Shirley, Poultry Department, for serving as Committee Chairman, for his supervision and guidance during the course of graduate study, the planning and conducting of the research, and in the preparation of the manuscript.

Dr. O. E. Goff, Head of the Poultry Department, for serving on the Graduate Committee, for counsel and guidance during the course of graduate study and for guidance in the planning of the research and preparation of the manuscript.

Dr. R. C. Fraser, Zoology Department, for serving on the Graduate Committee, for counsel during the course of graduate study and for reviewing the manuscript.

Dr. R. L. Murphree, Animal Husbandry Department, for serving on the Graduate Committee, for counsel during the course of graduate study and for reviewing the manuscript.

Mrs. R. L. Mason, Nutrition Department, for her consultation and extremely valuable technical assistance in bone densitometry.

Mr. G. C. McGhee, Poultry Department for his advice and technical assistance.

Dr. J. K. Bletner, Poultry Department, who offered suggestions and gave encouragement.

Dr. E. W. Swanson, Dairy Department; Dr. S. L. Hansard, Animal Husbandry Department; and Dr. J. M. Stewart, Agronomy Department, for their technical assistance.

Miss Patricia Goodman, for typing the rough draft of this manuscript, and for advice and assistance.

Mrs. Jennifer Marshall, for typing the rough draft of this manuscript.

Mrs. Kamillo Szathmary, secretary to the Poultry Department, for advice and assistance and for typing the final copy of this manuscript.

ABSTRACT

An experiment was conducted in two trials involving 113 Single Comb White Leghorn pullets to study the effect of ultimobranchialectomy and confinement on calcium metabolism of the laying hens.

Ultimobranchialectomy and sham operations were performed on chicks at one week of age. They were then reared to maturity in floor pens and housed through the laying period in 6 x 8 and 10 x 16 inch cages and in floor pens.

In trial 1, ultimobranchialectomy combined with stress of confinement resulted in a significant reduction in bone density. In addition, it also resulted in significant increases in weights of the thyroid and adrenal glands. In trial 2, however, the operation resulted in no significant decrease in bone density but did result in non-significant increases in the weights of the thyroid or the adrenal glands.

In trial 1, ultimobranchialectomy combined with the stress of confinenemt in cages resulted in a non-significant reduction in bone breaking strength; whereas, in trial 2, a significant reduction was found as a result of the operation combined with the stress of confinement.

In both trials, the response of the ultimobranchialectomized (UBX) pullets to bovine parathyroid extract as measured by serum calcium level was found to be greater as compared to the sham operated controls. Ultimobranchialectomy resulted in a non-significant increase in the incidence of cage layer fatigue. In

addition, the operation resulted in non-significant increases in specific gravity scores of eggs, parathyroid weights, serum calcium, and hematocrit values.

Confinement of pullets to laying cages resulted in a significant decrease in bone density and bone breaking strength in one of the two trials. In both trials, confinement of laying pullets to small cages resulted in significant changes in the ultimobranchial glands of these birds which were: (1) increased weight, (2) hypertrophy of cells, and (3) a decrease in calcitonin content.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

TABLE PAGE

LIST OF FIGURES

I. INTRODUCTION

Osteoporosis is defined as an abnormal conditions of the skeleton characterized by a decrease in the amount of the hard tissue without any change in volume or external configuration of the bone. It is now recognized as a heterogeneous group of disorders that may develop at any rate of bone resorption from high to low in animals as well as in man.

Osteoporosis is a significant problem of laying hens maintained in cages. Laying hens bred for heavy egg production and kept in cages may develop either a severe form of osteoporosis commony referred to as cage layer fatigue, or a lesser form of the disorder known as avian osteoporosis.

Birds with cage layer fatigue generally show leg weakness and are unable to reach their feed and water. Sometimes, they fall on their side and are unable to regain their stance; if left in cages, the severly affected birds die. Hens with cage layer fatigue are characterized by brittle bones. The femurs show thin cortices and enlarged haversian canals, however, the serum calcium, phosphorus, magnesium and alkaline phosphatase are within normal limits (Urist, 1960, and Bell and Siller, 1962).

Cage layer fatigue has been attributed to many causes among which are disuse, confinement and nutritional deficiencies. The metabolic factors responsible for this disorder are obscure.

Cage layer fatigue has been produced experimentally by confining laying hens to 6 x 10 inch cages (King, 1965), by feeding

hens diets low in calcium (Urist, 1959) and phosphorus (Simpson, 1963).

Recently there is growing evidence suggesting the involvement of thyrocalcitonin in osteoporosis of mammals. Foster (1968) found that porcine calcitonin prevented osteoporosis from developing in rats receiving a toxic dose of vitamin A.

In birds calcitonin is produced by the ultimobranchial glands, however, very little information is available pertaining to the significance of this hormone in bone metabolism of chickens.

As it has been shown that close confinement of layers can result in cage layer fatigue and that calcitonin influences calcium metabolism, there exists the possibility that stress of confinement influences the function of the ultimobranchial gland, either directly or indirectly.

II. OBJECTIVE

The objective of the experiment reported here was to investigate the role of the ultimobranchial glands in calcium metabolism of laying pullets as related to the incidence of cage layer osteoporosis.

III. REVIEW OF LITERATURE

Cage Layer Osteoporosis

Couch (1955) described cage layer fatigue in White Leghorn hens kept in cages as an abnormal condition which occurs in birds of high production and excellent feed efficiency. The affected birds developed leg weakness. There was no difference in the percentage bone ash of the affected birds as contrasted with those from the normals. A study of the calcium and phosphorus levels in the blood likewise failed to reveal differences. Affected birds recovered when they were removed from the cages and placed on the floor. The rate of egg production was decreased greatly or ceased during the period of recovery.

Grumbles (1959) noted that the incidence of cage layer fatigue has increased with the increase in the practice of keeping layers in individual cages. According to Grumbles, cage layer fatigue has become a problem in cage layer operations in the South and Southwest.

Francis (1957) noted a variation in the incidence of cage layer fatigue ranging from 0.65 percent to 3.95 percent in different strains of birds.

Bell et al. (1959) described two forms of cage layer fatigue, one, characterized by extreme leg weakness and another, associated with sudden death of healthy birds immediately after laying. In both cases the bones were considerably decalcified and flexible, especially the sternum, ribs, skull and sometimes the pelvis; decalcification of the long bones was not as obvious. No abnormal

values were found for blood plasma Ca, P and Mg. Alkaline phosphatase activity was extremely high in extreme leg weakness cases.

Gardiner (1965) stated that cage layer fatigue is a condition found only in caged pullets during their first three months of production. The onset of the symptoms is described as very sudden and the first warning may be the sudden collapse of a bird, often just after laying, or the finding of a dead bird. The bones of the affected bird were said to be thinner and more flexible and that the initial symptoms may be mistaken for neural fowl paralysis.

Urist (1960) found severe osteoporosis in 50 percent to 75 percent of the culls from a flock of heavy laying White Leghorns in Southern California. The long bones, especially the femur and the tibia, had a thin cortex, enlarged haversian canals, low osteoblastic activity and few or no osteoclasts.

Urist and Deutsch (1959) reported some endocrine organ changes associated with a condition of avian osteoporosis which were atrophy of the adrenal cortices and a decrease in the size and weight of the gonads.

The influence of adrenocorticotrophic-stimulating hormone (ACTH) upon avian osteoporosis was studied also by Urist and Deutsch in 1960. Black Minorca roosters and White Leghorn hens were treated with 40 mg. injection of lyophilized ACTH at intervals and periods of 14 to 49 days. The hens treated with ACTH had enlarged adrenal glands and the ovaries became progressively smaller 2, 4, and 6 weeks after treatment. The cortex of the long bones was thinner in hens than in roosters. No changes were found in serum total lipids, phosphorus, total nitrogen, albumin, globulin or alkaline

phosphatase. The marrow cavities were filled with poorly calcified bone. Total calcium was higher than 35 mg./100 ml. Osteoporosis was extremely high in hens treated with ACTH for six weeks. This disorder did not develop in roosters injected with ACTH.

King (1965) observed a 60 percent incidence of cage layer fatigue in 23-week-old hens kept for 4 months in small cages, 6 x 12 inches, in one experiment and 120 percent incidence in the second experiment. In contrast, no cases of cage layer fatigue were observed in hens confined to cages 8 x 12 inches or larger. He ascribed the occurrence of cage layer fatigue in his experiments to the osteoporosis of disuse. No evidence of bone mass loss, however, was presented in King's report. The rate of egg production in each experiment did not show any relationship to cage size.

Norris and Kratzer (1968) conducted two experiments, one for six 28-day periods and one for four 28-day periods, to study the effect of restricted activity and adrenal hormones on egg production and the status of bone in Single Comb White Leghorn mature hens. In the first experiment hens were confined in 12 x 18 and 6 x 12 inch cages. They found that the average percent egg production of hens in large cages was 54.5 percent and that of the hens in the small cages, 35.4 percent. No difference was observed, however, in egg shell thickness. The average bone density, as measured by X-rays of the left tibiae of the hens in large cages was 0.747 grams per square centimeter compared to 0.635 grams for the hens in small cages. In the later stages of the experiment four of the hens in small cages developed symptoms of cage layer fatigue. Three of the hens recovered after oral

administration of 0.25 grams of $CaCO_3$ and one died.

In the second experiment reported by these investigators, hydrocortisone and epinephrine supplied in the diets reduced bone mineral, calcium and phosphorus. Restricting the activity of hens by confinement in small cages gave results similar to those obtained with adrenal hormones. They also indicated that these observations imply that the stress of restricted activity promoted increased production of cortical hormones, perhaps by indirectly stimulating a greater synthesis of epinephrine.

Urist (1959) reported that within 10 days after laying hens were deprived of calcium and vitamin D the serum calcium fell from as high as 21.4 to as low as 10.3 mg./100 ml. and egg production ceased abruptly. The weight of the ovary decreased from 28.8 to 1.3 grams and that of the adrenal glands increased from 105 to 220 milligrams. The loss of ovarian weight in the hens was thought to be due to a failure to form complexes of phosphoprotein and calcium in the liver and to transport and deposit yolk protein in the ovarian follicles while the gain in weight of the adrenal gland was probably due to the secretion of ACTH by the anterior pituitary and enlargement of the adrenal cortex. Under these conditions, the skeleton showed deposits of intramedullary bone as large or larger than in the control hens, but with extensive thinning of the cortex due to bone resorption or osteoporosis. Calcium deprivation produced over activity of the parathyroid glands.

Simpson et al. (1963) found that pullets fed low phosphorus diets developed weakness and had histological evidence of bone damage. They pointed out that phosphorus deficiency might be

implicated in the cage layer disease syndrome. Pullets that received 3.0 percent Ca and 0.7 percent P had significantly higher bone ash than those receiving the other experimental diets of 2.0 percent Ca and 0.7 percent P and, 3.0 percent Ca and 0.34 percent P. There were no significant differences in bone phosphorus or calcium among the three experimental groups although the group fed low P had a lower percentage of Ca and P in the bone. However, differences were observed when tibial bones of the birds fed the three diets were examined histologically. Sections of tibia from birds on Ca deficient diets were similar to those from control birds in that the trabeculae were mature bone. However, trabeculae from Ca deficient birds were thicker and more irregularly shaped and had a slightly wider coating of osteoid. The diaphysis of the tibia of birds fed a phosphorus-deficient diet contained large, broad trabeculae. In a bone breaking test for fragility ratings, bones from birds fed a phosphorus deficient diet were more fragile than bones from birds fed the other two diets.

Ultimobranchial Glands and Calcitonin

The name ultimobranchial body (ultimobranchiale Korper) was first suggested by Greil in 1905 as reported by Copp, 1969. The name was chosen for the glands which arise consistently from the last branchial pouch, during embryological development. Kingsbury (1935 a,b) stated that in many lower vertebrates only the left gland develops and the one on the right remains vestigial. In birds the ultimobranchial glands are paired and in most mammals they become embedded in the thyroid glands.

Dudly (1942) summarized a study on chicken ultimobranchial glands as follows:

1. The branchial pouches of the chick are six in number. The fifth and sixth are vestigial and early included with the fourth pouch in a caudal pharyngeal complex. The fifth pouch is transitory; the sixth becomes the ultimobranchial body.
2. With the descent of the heart and aortic arches, the caudal pharyngeal complex is separated from the median part of the pharynx and from the ectoderm. The connecting strand between the ultimobranchial anlage and the more laterally located parathyroid and thymus anlagen is broken. With the disappearance of the fourth aortic arch on the left and its shifting on the right, the ultimobranchial anlagen reach their definitive position caudal and dorsal to the third aortic arch near the division of the brachiocephalic into common carotid and subclavian arteries. The one on the right is usually more caudally located. Either may be close to, or partly surround parathyroid IV and the glomus caroticum.
3. The growth of the ultimobranchial body becomes accelerated in about the seventh day of incubation. Buds push forth, branch and enclose some of the surrounding mesenchyme. Until about the thirteenth day, a central denser core can be distinguished around the original lumen. The parenchyma of the matured ultimobranchial body is of abundant fine branching strands of polygonial cells. The stroma consists of a reticular network between the strands containing numerous blood vessels.
4. The blood comes to the ultimobranchial body by a branch of the common carotid artery and leaves by branches to the jugular.
5. The nerve supply is largely from the vagus nerve. Branches of the sympathetic system and recurrent nerve also enter. Bundles of nerve fibers from the posterior part of the ganglion nodosum often contain ganglion cells which may even be found carried into the ultimobranchial body.
6. From one to three accessory parathyroids may arise from the ultimobranchial parenchyma in any part of the organ.
7. The production of eosinophiles is a characteristic feature of the ultimobranchial body from the tenth day of incubation through at least the first months after hatching.
8. Lymphocytes may be found in the ultimobranchial body during the latter half of incubation but only become numerous 10 days or more after hatching. They tend to increase with age and areas where they are abundant may become thymus-like. Portions of the ultimobranchial body, however, remain almost entirely free of lymphocytes.

Copp et al. (1967) found that in chickens the ultimobranchial

gland is located in the chest near the bifurcation of the common carotid and axillary artery and that they are paired follicular glands which arise from the ventral floor of the last branchial pouch in a similar manner to the origin of the parathyroids from the floor of the third and fourth branchial pouches.

Van Dyke (1943) found that in young sheep the ultimobranchial bodies consisted of large, multiple cysts lined by stratified squamous epithelium in the thyroid gland. The epithelium of these cysts frequently produces cords or clumps of gland like cells which invade the neighboring thyroid parenchyma.

Copp (1969) reported that in sharks and amphibians the ultimobranchial glands contain prominent follicles, but in most other vertebrates they consist of sheets of polygonal cells superficially resembling the parathyroids.

The ultrastructure of the ultimobranchial was studied by Stoeckel and Porte (1969) who reported that the ultimobranchial body of the chick consists of glandular cords made up of main secretory cells and supporting cells. According to the diameter of the secretory granules, two main cell types can be distinguished: cells with small granules (200 mμ) and cells with large granules (250-300 mμ).

Hodges (1969) found that the ultimobranchial body of the fowl is extermely well innervated. It is supplied mainly from the vagus, but also from the recurrent nerve and the sympathetic system. This, together with the presence of numerous fine nerve fibers and some ganglion cells within the gland, has suggested the possibility of some measure of nervous control of its endocrine function.

Copp et al. (1962) presented evidence for a hypocalcemic hormone released by high calcium perfusion of the thyroid and parathyroid glands of the dog. They named this new hormone calcitonin. Although it was originally thought to come from the parathyroids, it soon became evident that the hormone was released from cells in the mammalian thyroid glands.

Hirsch et al. (1963) reported the extraction of a potent hypocalcemic material from rat thyroid which they named thyrocalcitonin to indicate the source of the material and the possible relationship to calcitonin.

Copp (1967) indicated that calcitonin is not derived from the regular colloid containing thyroid cells, but is in fact an ultimobranchial hormone which can be extracted from the mammalian thyroid only because of the presence of ultimobranchial tissue within that organ.

Hirsch et al. (1964) described the first bioassay for the hormone based on the fall in plasma calcium one hour after injection of the extract into male rats which had previously been fed a low calcium diet for four days. The Hirsch bioassay was modified and improved by Cooper et al. (1967).

Copp et al. (1967) found no detectable hypocalcemic response from injecting acid extract from thyroids of small shark and chickens while very potent hypocalcemic responses were obtained in rats with similar extracts from the ultimobranchial glands of these two species.

Copp and Parkes (1968) studied the calcitonin activity from ultimobranchial tissues of representative species from all classes

of vertebrates. They observed that the highest activity was with the chicken ultimobranchial extract.

MacIntyre (1967) summarized a review on calcitonin as follows:

1. Calcitonin (thyrocalcitonin) is a powerful polypeptide hormone which directly inhibits bone breakdown and so lowers plasma calcium.
2. The hormone is secreted by a separate and previously unrecognized endocrine system. In mammals this is represented by the C cells within the thyroid.
3. The ultimobranchial body in birds secretes calcitonin. The C cells in mammalian thyroids are derived from the ultimobranchial body.
4. The hormone can be purified from acetone dried pig thyroid. The most highly purified fraction have an activity exceeding 250 MRC units per mg.
5. The molecular weight of calcitonin is about 3,600.
6. In addition to acting on the skeleton, calcitonin also produces increased excretion of phosphate in the urine.
7. Calcitonin is active in man. It may play a role in human bone disease.

Urist (1967) found that ultimobranchial preparations from chickens had no activity when administered into normal intact birds.

Calcitonin concentration of ultimobranchial glands of chickens aged 3 weeks, 3 months and 9 months was studied by Wittermann et al. (1969). They found that nine month old, hypercalcemic hens showed similar concentrations of calcitonin in their ultimobranchial bodies when compared with the two younger age groups of chickens which had been normocalcemic. They concluded that hypercalcemia of egg laying does not lead to a measurable depletion of calcitonin from the ultimobranchial bodies.

Copp et al. (1969) carried out calcium infusion studies in young turkeys and determined the effect of ultimobranchialectomy during the infusion. When the glands were intact and the birds were infused with 10 mg. calcium as $CaCl_2$/Kg./hour, the calcium rose 10 to 13 mg./100 ml., leveled off for a few hours and then

fell even though the infusion was continued. The experiment was repeated and the ultimobranchial glands were removed after four hours of calcium infusion. The plasma calcium rose rapidly to 19 mg./100 ml. and the plasma calcitonin level fell to a point where it could not be detected. They concluded that these experiments indicate that the ultimobranchial and calcitonin do play a role in controlling hypercalcemia in birds.

Brown et al. (1969) studied the effect of ultimobranchialectomy upon the maintenance of normal skeletal structure in three-month-old male chickens. Mortality, body growth, serum calcium phosphorous, alkaline phosphate, X-ray appearance and micro-radiographs of the tibia of calcitonin deficient animals were not different from sham operated birds.

Hurst and Newcomer (1969) found that plasma calcium dropped in cockerels 6 to 10 weeks of age whose parathyroid glands, ultimobranchial glands, or both, had been removed 11 hours previously as compared with those of sham operated controls. By 50 hours after surgery, the plasma calcium returned to near control values in parathyroid or ultimobranchial removed birds, but remained low when parathyroid and ultimobranchial were removed. They pointed out that these data along with histological evidence consituted direct evidence for the secretion of parathormone by the accessory parathyroid tissue in the ultimobranchial glands.

Perfusion of the ultimobranchial gland of pullets with hypercalcemic blood was done by Ziegler et al. (1969). They found that hypercalcemic blood resulted in increased calcitonin secretion.

The maximal calcitonin secretion rates were reported to be 1.25, 1.30 mU./min. per organ.

Chan et al. (1969) reported that hypercalcemia was found to cause hyperplasia and hypertrophy of the ultimobranchial glands in four week old chicks fed a high calcium diet (4 percent $CaCO_3$). They added that the results of their study seem to suggest the involvement of the ultimobranchial glands in calcium metabolism.

Copp et al. (1958) reported that young cockerels fed diets low (0.1 percent), normal (1.0 percent) or high (5 percent) in calcium for periods of one to eight weeks resulted in many large granules in the ultimobranchial cells of birds fed the low or normal calcium diets. Very few granules were seen in glands from the high calcium group and the calcitonin concentration was lower.

Gittes et al. (1968) found that hypercalcemia induced by injecting $CaCl_2$, gavage feeding of calcium lactate or high calcium diet reduced thyrocalcitonin content of the thyroid glands of male Holtzman rats as measured by biological assay. The reduction in the hormone was 35 percent of the control rats.

Robertson (1968) found that injecting frogs with 200,000 I.U. of vitamin D_2 and exposing the frogs to water containing 0.8 percent $CaCl_2$ resulted in hypertrophy of ultimobranchial cells and depletion of their secretaroy granules.

Young and Capen (1969) reported that feeding cows 30 million I.U. of vitamin D_2 per day for 30 days resulted in hypertrophy and degranulation of the parafollicular cells. The thyrocalcitonin content of these cells was reduced. They concluded that the reduction in the thyrocalcitonin content was due to vitamin D

induced hypercalcemia. They also reported that the reduction in the thyroid store of thyrocalcitonin suggested that the rate of thyrocalcitonin release exceeded that of synthesis.

Gaillard (1963) observed enhanced bone resorption when parathormone was added to the medium of cultures of explanted limb bone rudiments from new born mice. The resorption was inhibited when 0.5 to 1.0 mU./ml. of calcitonin was added to the medium.

Foster et al. (1966) found that calcitonin reduced bone resorption when parathyroidectomized rats were given four daily subcutaneous injections of 40 mU. of calcitonin per rat. After 28 days of treatment, the rats were killed and the bones were examined roentgenologically and histologically. Compared with the controls, the animals receiving calcitonin had heavier bones containing more trabeculae. There was an increase in fully mineralized bone and in partially mineralized and unmineralized osteoid. The osteoclast count was also significantly reduced.

Foster et al. (1968) studied the effect of calcitonin on rats that developed osteoporosis by receiving a toxic level of vitamin A (2,500 I.U. three times a week for four weeks). They found a daily dose of 100 mU. porcine calcitonin prevented osteoporosis which developed in animals receiving vitamin A.

Baud et al. (1969) reported that 160 MRC units daily of porcine thyrocalcitonin for one month resulted in increased bone mineralization in osteoporotic patients. After one month of treatment, as reported by the investigators, the pain of these patients dieappeared, allowing normal physical activity to be resumed.

IV. EXPERIMENTAL PROCEDURES

Experimental Animals

A total of 113 Single Comb White Leghorn pullets (Babcock B-300) was employed in two trials designed to investigate the role of the ultimobranchial glands in bone calcium metabolism of chickens as it relates to the disorder of avian osteoporosis.

Operative Technique

One-day-old White Leghorn female chicks obtained from a local hatchery were wing banded and vaccinated with a combination Newcastle-infectious bronchitis vaccine. The chicks were brooded on the floor employing standard feeding and management practices.

At one week of age, the chicks were fasted over night prior to surgery. They were lightly anesthetized with sodium pentobarbital injected intraperitoneally and the ultimobranchial glands removed under a dissecting microscope. Sham operations were performed by exposure of the glands without further dissection. The sham operated group served as a control. Bleeding was minimal during the operation with no interference with blood to nearby vital structures.

Housing

At 20 weeks of age, the ultimobranchialectomized (UBX) and the sham operated (control) pullets were housed in individual laying cages of two different sizes, one bird per cage, and in floor pens. The floor areas of the laying cages were 6 x 8 and 10 x 16 inches. All cages were 18 inches high with slanting wire

floors. Feed and water troughs were in front of the laying cages. The floor pens and the laying cages were located in four 8 x 10 feet ventilated rooms allowing 14 hours of artificial light per day.

Composition of Diets

The composition of the diets fed during the starting, growing and laying periods is shown in Table I. They were considered to be adequate in all known nutrients and to meet the requirements as given by the National Research Council.

Densitometry

At two months of age and every 28 days thereafter, bone density measurements were made across the left tibia, three centimeters from the hock joint, of each bird in the different treatment groups. A bone densitometer was used to make these measurements. The densitometer was developed under the direction of the Department of Nutrition, College of Home Economics, through The University of Tennessee Agricultural Experiment Station.

The bone densitometer uses a highly collimated, low energy X-ray beam for the source of energy. An absorption curve was recorded on graph paper as the chosen bone site was passed through the X-ray beam. The instrument was designed to scan the human little finger, however, with a special stand was designed to restrain chickens for scanning the tibia. The feathers were removed from the left tibia of the chicken so that the absorption curve would represent only flesh and bone.

A reference wedge of known density was traced following bone

TABLE I

COMPOSITION AND CALCULATED ANALYSES OF STARTER, GROWER, AND LAYER DIETS

Ingredients	Starter	Grower	Layer
		percent	
Yellow corn	63.800	71.875	66.975
Alfalfa meal, 17% protein	2.500	5.000	5.000
Fish meal	2.500	2.500	2.500
Vitamin mix	0.600[1]	0.600[1]	0.500[2]
Defluorinated rock phosphate	1.500	1.500	1.500
Ground limestone	0.600	1.000	6.000
Salt	0.480	0.500	0.500
Manganese sulfate, 75%	0.020	0.025	0.025
Soybean oil meal, 50% protein	25.500	14.500	17.000
Coccidiostat premix	2.500	2.500	--
Total	100.000	100.000	100.000
Calculated analysis:			
Crude protein, %	22.04	17.68	17.19
Productive energy, C./kg.	2108	2128	2006
Calcium, %	0.894	1.057	2.957
Phosphorus, %	0.711	0.636	0.621
Available phosphorus, %	0.414	0.422	0.417

[1]Vitamin mix supplied the following amounts per kilogram of diet: 4167 I.U. of Vitamin A; 749 I.C.U. of Vitamin D_3; 474 mg. of Riboflavin; 11.0 mcg. of Vitamin B_{12}; 406 mg. of Choline; 40.3 mg. of Niacin; 7.00 mg. of Bantothenic Acid, and 11.9 mg. of Aureomycin.

[2]Vitamin mix supplied the following amounts per kilogram of diet: 2463 I.U. of Vitamin A; 2956 I.C.U. of Vitamin D_3; 4.43 mg. of Riboflavin; 6.50 mcg. of Vitamin B_{12}; 440 mg. of Choline; 24.2 mg. of Niacin and 4.76 mg. of Pantothenic Acid.

and flesh tracing. The wedge was a homogeneous alloy of 92.8 percent aluminum and 7.2 percent zinc. Its atomic number approximates that of hydroxyapatite. Its mass absorption coefficient is closely equivalent to that of bone mineral.

The area under the recorded absorption curve was measured by use of a planimeter and the average height of the flesh and bone areas were transferred to the standard curve. Measurements from these were used for computer calculation of bone density. The bone density value was expressed as X-ray equivalent grams of alloy per cubic centimeter of bone.

Chemical Analysis

Plasma and serum calcium levels were determined by use of an atomic absorption spectrophotometer (model SP90), and phosphorus by a modification of the method of Fiske and Subbarow (1925).

Extraction of Calcitonin

At 40 weeks of age, three sham operated pullets from the floor pen group and three from the small cages, 6 x 8 inches, were sacrificed and the ultimobranchial glands of each pullet were immediately removed, trimmed of adherent tissue, and weighed on a torsion balance. The ultimobranchial glands were extracted according to the method of Copp et al. (1967). The two glands were then defatted with acetone and the dried material was homogenized with 0.1 N HCl and extracted at room temperature for one hour. The homogenate was centrifuged for 10 minutes to remove cell debris. The extract was brought to pH 4 by the addition of 0.1 N NaOH and

suitable dilutions were made with 0.9 percent NaCl so that the final volume injected was 0.5 ml.

Bioassay of Calcitonin

The ultimobranchial extract was injected intraperitoneally into a total of 108 female rats 50 days of age, weighing 185 to 200 grams each. The dose levels given were 0.25, 0.5 and 1.0 milligrams of fresh wet extract. Three rats were employed for each dose level and were fasted 24 hours prior to injection of the extract. Six rats were used to serve as controls and were injected intreperitoneally with 0.5 ml. of saline. Samples of blood obtained by heart puncture were collected at 0, 1, 3 and 6 hours post injection and analyzed for calcium.

Hematocrit Reading

Blood samples from the wing vein were collected in heparinized capillary tubes. The tubes were centrifuged for five minutes in an Adams-Readacrit microhematocrit centrifuge and the hematocrit reading in percent was determined using the hematocrit reading chart.

Gross Observations

Each day every bird was observed for symptoms of leg weakness, broken bones and diseases.

Histological Studies

The ultimobranchial, adrenal, parathyroid and the thyroid glands were removed, trimmed of adherent tissue and fixed with AFA fixative and dehydrated in a graded series of alcohol solutions and embedded in paraffin. Tissue sections 10 microns thick were

prepared and stained for a detailed microscopic study.

Breaking Strength of Bones

The force needed to break the left and the right tibiae removed from each pullet was measured by the use of the Allo-Kramer shear press (Model No. S-2HE). The tibia was supported near the two ends approximately five to six centimeters from the midpoint at which the force was applied. The press traveled at the rate of one centimeter each 2.4 seconds. The force required to break each tibia was read from the shear press dial.

Trial 1

Thirty-three UBX and thirty-three sham operated Single Comb White Leghorn pullets, 20 weeks of age, were each divided into six groups and assigned at random to 6 x 8 and 10 x 16 inch cages and floor pens.

In each of two cage rooms, four UBX and four sham operated birds were housed in 6 x 8 inch cages. Similar groups were housed in 10 x 16 inch cages.

Seven UBX and seven sham operated birds were housed in floor pens in each of two rooms. An additional six birds, three UBX and three sham operated, were housed in one of the two rooms for parathormone injection.

The experimental groups were fed a standard laying diet containing 17.2 percent crude protein, 3.0 percent calcium and 0.6 percent phosphorus. Feed and water were available to the pullets ad libitum. Data was collected during a laying period of 140 days.

Trial 2

Trial 2 was conducted in the same manner as was Trial 1. At 20 weeks of age, a total of twenty-seven UBX and twenty-seven sham operated pullets was housed in this trial. Six birds of each treatment were placed in 6 x 8 inch laying cages and six birds of each treatment went into 10 x 16 inch cages. Fifteen pullets of each group were placed in floor pens among which three UBX and three sham operated pullets were used only for parathormone injection. The laying period consisted of 140 days.

Method of Data Collection

Information was recorded on egg production, egg weight, shell thickness, bone density, body weight, blood serum and plasma calcium, serum phosphorus, hematocrit reading and bone breaking strength of the chicken tibiae.

Egg production was recorded daily for each individual bird throughout the laying period and was calculated as number and percent egg production.

Eggs were weighed one day during each twenty-eight day period. The specific gravity of these eggs was determined by the use of a series of salt solutions, ranging in specific gravity from 1.068 to 1.100, by increments of 0.004. A score of zero was given to eggs having a specific gravity of less than 1.068. This score increased by one as the specific gravity of the solutions increased by 0.004. Eggs having a specific gravity higher than 1.100 were given a score of nine.

Individual body weights of pullets were obtained at 8 weeks

of age and at 4 week intervals thereafter.

The bone density measurement of the left tibia of each bird was taken at 8 weeks of age, at 28 day intervals thereafter, and at 40 weeks of age.

At 28 weeks of age, bovine parathyroid extract 100 USP/Kg. (Eli-Lilly Co., Indianapolis) was injected intramuscularly into three UBX and three sham operated pullets from the floor pen group. Blood samples from each bird were taken by heart puncture at 0, 3, and 6, and 9 hours post injection and analyzed for calcium and phosphorus levels.

At 40 weeks of age, blood samples were taken from a sample of five birds from each experimental group for calcium and hematocrit determinations. Concluding these tests, all the experimental groups were sacrificed. The ultimobranchial, parathyroid, thyroid and the adrenal glands were removed, trimmed and weighed on a torsion balance. Extracts of ultimobranchial glands from three sham operated pullets from the 6 x 8 inch cages and three sham operated pullets from the floor pen were bioassayed for calcitonin content. The remaining ultimobranchial glands along with the thyroid, parathyroid and the adrenal glands were used for histological study.

Breaking strength of the left and right tibia of each bird was obtained using the Allo-Kramer shear press.

Analysis of Data

Data were statistically analyzed by analysis of variance (Snedecor, 1956); differences between treatments were determined

by Duncan's Multiple Range Test (1955) and Student's "t" Test (Snedecor, 1956). Data of some observations were transformed to Arcsin (Snedecor, 1956) and comparisons of means were made on the transformed scale. The means were reconverted to the original scale of measures for presentation in the tables.

V. RESULTS

Trial 1

Bone density. In Table II is shown the effect of ultimobranchialectomy of birds maintained under the three housing conditions. Significant differences became evident by the end of the fourth laying period. Both sham operated and ultimobranchialectomized birds in the floor pens had higher bone density indices than birds in the two other cage sizes. Sham operated birds in 10 x 16 inch cages had greater bone density indices than UBX birds maintained in the same cage size. By the fifth laying period, the average bone density of the UBX birds kept in 6 x 8 inch cages was significantly lower than that of the other five groups.

In Table III, the effect of ultimobranchialectomy is shown with pooled data pertaining to housing conditions. No significant differences were found between the operated and the non-operated birds at any time during the five periods. However, bone density tended to become greater in both groups as the trial progressed.

Table IV contains data on the effect of the three housing conditions on bone density with data of UBX and sham operated birds for each condition pooled together.

At the end of the fourth laying period and continuing through the fifth, the birds kept in 6 x 8 inch cages had significantly lower bone density indices as compared to birds of the other two treatments.

At the end of the fourth 28 day laying period, when the

TABLE II

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON BONE DENSITY OF LAYING PULLETS, TRIAL 1

Treatments	Pre-laying period[6]	Bone density index[1,2,3,4] Laying periods[5] 1	2	3	4	5
Ultimobranchialectomized						
6" x 8" cages (7)[7]	1.20±0.27	1.29±0.07^{a}	1.39±0.04^{a}	1.44±0.07^{a}	1.50±0.02^{ab}	1.34±0.07^{a}
10" x 16" cages (8)	1.17±0.04	1.34±0.03^{ab}	1.43±0.04^{b}	1.45±0.06^{a}	1.47±0.05^{a}	1.60±0.05^{b}
Floor pens (12)	1.20±0.04	1.49±0.06^{b}	1.57±0.04^{b}	1.52±0.03^{a}	1.57±0.06^{bc}	1.56±0.08^{b}
Sham operated						
6" x 8" cages (7)	1.13±0.05	1.30±0.05^{a}	1.46±0.04^{ab}	1.35±0.06^{a}	1.46±0.03^{a}	1.50±0.06^{b}
10" x 16" cages (8)	1.15±0.02	1.31±0.04^{a}	1.41±0.05^{a}	1.38±0.09^{a}	1.57±0.05^{bc}	1.53±0.06^{b}
Floor pens (14)	1.11±0.10	1.50±0.03^{b}	1.56±0.03^{b}	1.46±0.05^{a}	1.60±0.05^{c}	1.62±0.05^{b}

[1] X-ray equivalent grams of alloy per cubic centimeter of bone.

[2] Mean ± S. E.

[3] Left tibia.

[4] Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[5] At 24 weeks of age and 28 day intervals thereafter.

[6] At 20 weeks of age.

[7] Number of birds per group.

TABLE III

THE EFFECT OF ULTIMOBRANCHIALECTOMY ON BONE DENSITY OF LAYING PULLETS, TRIAL 1

Treatments[6]	Bone density index[1,2,3,4] Laying periods[5] 1	2	3	4	5
Ultimobranchialectomized (27)[7]	1.38±0.03	1.46±0.03	1.47±0.03	1.51±0.04	1.50±0.04
Sham operated (29)	1.36±0.03	1.48±0.02	1.40±0.03	1.54±0.04	1.55±0.03

[1]X-ray equivalent grams of alloy per cubic centimeter of bone.

[2]Left tibia.

[3]Mean ± S. E.

[4]Means in each column did not differ significantly ($P > 0.05$) when tested by analysis of variance.

[5]At 28-day intervals.

[6]Pullets were kept in 6" x 8", 10" x 16" cages, and in floor pens.

[7]Number of birds per group.

TABLE IV

THE EFFECT OF HOUSING CONDITIONS ON BONE DENSITY OF LAYING PULLETS, TRIAL 1

Housing conditions[6]	Bone density index[1,2,3,4] Laying periods[5] 1	2	3	4	5
6" x 8" cages (14)[7]	1.30 ± 0.04^{a}	1.43 ± 0.04^{a}	1.39 ± 0.04^{a}	1.48 ± 0.04^{a}	1.42 ± 0.05^{a}
10" x 16" cages (16)	1.33 ± 0.02^{a}	1.42 ± 0.03^{a}	1.42 ± 0.05^{a}	1.52 ± 0.03^{b}	1.57 ± 0.04^{b}
Floor pens (26)	1.48 ± 0.03^{b}	1.56 ± 0.03^{b}	1.49 ± 0.02^{a}	1.58 ± 0.04^{b}	1.59 ± 0.04^{b}

[1] X-ray equivalent grams of alloy per cubic centimeter of bone.

[2] Left tibia.

[3] Mean ± S. E.

[4] Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[5] At 28-day intervals.

[6] Ultimobranchialectomized and sham operated pullets combined.

[7] Number of birds per group.

pullets were 36 weeks of age, and had been in production for three months, three of the sham operated and three of the UBX pullets from 6 x 8 inch cages began to show symptoms of leg weakness, however, they continued to lay eggs, eat and drink. By the end of the fifth laying period these birds were prostrate, but continued to lay, eat and drink when water and feed were within reach. The rib bones were hard and brittle, and the long bones of the wings could be easily broken by applying a small amount of pressure.

A comparison of pullets evidencing symptoms of cage layer fatigue was made with those pullets with no apparent symptoms. These results are shown in Table V. Of those parameters studied, bone density index is the only one in which the "normal" and the cage fatigued birds differed significantly. There were no significant differences found in respect to number of eggs laid, blood calcium level, hematocrit values and the weight of the ultimobranchial and the adrenal glands between the two groups of birds.

No cases of cage layer fatigue were observed in laying pullets confined to large cages or in the floor pens.

Specific gravity score. The effect of ultimobranchialectomy and cage size on specific gravity score of eggs is shown in Table VI.

Over the four 28 day laying periods, eggs from UBX pullets maintained in 6 x 8 inch and 10 x 16 inch cages tended to have thicker shells as determined by specific gravity, than those of the sham operated pullets maintained in similar cage sizes. At the end of the second 28 day laying period, eggs from the UBX pullets maintained in 6 x 8 inch cages had significantly higher specific gravity scores than eggs from the control pullets kept

TABLE V

COMPARISON OF BIRDS SHOWING SYMPTOMS OF CAGE LAYER FATIGUE WITH BIRDS EVIDENCING NO SYMPTOMS OF THE DISEASE, TRIAL 1

Factors[1]	Ultimobranchialectomized 6" x 8" cage[2]	Sham operated 6" x 8" cage[2]
Number of birds		
Normal	4	4
Cage fatigue	3	3
Bone density index		
Normal	1.45±0.08	1.59±0.07
Cage fatigue	1.18±0.04*	1.38±0.04
Number of eggs per bird		
Normal	25±1.5[3]	23±3.0[3]
Cage fatigue	21±1.6	24±1.7
Blood Calcium (mg./100 ml.)		
Range	18.8-24.8	14.5-20.5
Normal	20.2±0.8	16.1±1.8
Cage fatigue	22.6±1.3	18.7±0.9
Percent hematocrit		
Normal	32.9±0.8	32.8±1.2
Cage fatigue	34.3±0.8	32.8±1.5
Ultimobranchial glands weight (mg.)		
Normal	--	8.4±0.3
Cage fatigue	--	9.1±0.4
Adrenal weight (mg.)		
Normal	194.8±29.3	168.9±8.0
Cage fatigue	205.4±26.1	187.6±20.0

[1]Measurement taken at 40 weeks of age.

[2]Significant differences between means for each factor is indicated by asterisk ($P \leq 0.05$) as determined by Student's "t" Test.

[3]For 28 days.

TABLE VI

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND CAGE SIZE ON SPECIFIC GRAVITY SCORE OF EGGS, TRIAL 1

Treatments	Specific gravity score[1,2]				
	Laying periods[3]				Av. for entire laying period
	2	3	4	5	
Ultimobranchialectomized					
6" x 8" cages (7)[4]	5.9 ± 0.5^{b}	4.0 ± 0.3^{a}	3.6 ± 0.3^{a}	3.4 ± 0.4^{a}	4.2 ± 0.2^{ab}
10" x 16" cages (8)	6.3 ± 0.4^{b}	5.4 ± 0.3^{a}	4.9 ± 0.3^{b}	3.9 ± 0.5^{a}	5.1 ± 0.1^{b}
Sham operated					
6" x 8" cages (7)	4.8 ± 0.4^{a}	3.9 ± 0.5^{a}	3.4 ± 0.5^{a}	3.1 ± 0.5^{a}	3.9 ± 0.4^{a}
10" x 16" cages (8)	5.9 ± 0.3^{b}	4.0 ± 0.6^{a}	4.3 ± 0.5^{b}	4.0 ± 0.6^{a}	4.7 ± 0.3^{ab}

[1] Mean ± S. E.

[2] Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[3] Twenty-eight days each.

[4] Number of birds per group.

in 6 x 8 inch cages. These results indicate that removing the ultimobranchial glands resulted in eggs of greater shell density and that confining pullets to the 6 x 8 inch cages resulted in eggs of lower shell density.

Among the treatment groups, the specific gravity scores of eggs of the UBX pullets kept in 10 x 16 inch cages were not significantly different from those of the UBX pullets kept in 6 x 8 inch cages at the end of the second, third and fifth 28 day laying periods. At the end of the fourth 28 day collection period, the UBX pullets in 10 x 16 inch cages had significantly higher specific gravity scores than those kept in 6 x 8 inch cages. A significant difference in the average specific gravity score of eggs was found between sham operated pullets kept in 6 x 8 inch and 10 x 16 inch cages during the second 28 day period only. These data indicate that there is a resultant decline in specific gravity score as the cage size decreases.

Egg production. The effect of ultimobranchialectomy and cage size on the number of eggs produced during the four 28 day laying periods and percent of egg production is shown in Table VII.

Although none of the differences in average number of eggs produced were significantly different, there was a tendency for the pullets kept in 6 x 8 inch cages to lay fewer eggs than those kept in 10 x 16 inch cages.

Egg and body weights. The effect of ultimobranchialectomy and housing conditions on average egg weight and body weight is shown in Table VIII.

TABLE VII

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND CAGE SIZE ON EGG PRODUCTION, TRIAL 1

Treatments	Egg production[1,2]				
	Laying periods[3]				Av. for entire laying period
	2	3	4	5	
	number of eggs				percent
Ultimobranchialectomized					
6" x 8" cages (7)[4]	19.9±1.4	21.6±1.2	21.6±1.2	23.4±1.3	77.3±5.0
10" x 16" cages (6)	21.0±2.8	25.7±1.1	25.9±1.1	25.1±1.0	87.2±3.4
Sham operated					
6" x 8" cages (7)	19.4±2.2	22.3±2.6	22.9±1.4	23.4±1.4	78.6±4.4
10" x 16" cages (8)	24.6±1.1	23.4±1.3	24.4±1.0	25.6±1.0	87.5±1.8

[1]Mean ± S. E.

[2]Means in each column did not differ significantly ($P > 0.05$) when tested by analysis of variance.

[3]Twenty-eight days each.

[4]Number of birds per group.

TABLE VIII

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON EGG AND BODY WEIGHTS, TRIAL 1

Treatments	Egg weight[1,2]	Body weight[1,2,3]
	grams	grams
Ultimobranchialectomized		
6" x 8" cages (7)[4]	59.9±0.5[c]	1694±52[a]
10" x 16" cages (8)	56.0±0.6[a]	1790±65[a]
Floor pens (12)	--	1698±41[a]
Sham operated		
6" x 8" cages (7)	58.8±1.3[bc]	1621±32[a]
10" x 16" cages (8)	57.7±1.0[ab]	1869±80[a]
Floor pens (14)	--	1731±33[a]

[1]Mean ± S. E.

[2]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[3]At 40 weeks of age.

[4]Number of birds per group.

Over the experimental period of 112 days, there were no significant differences in egg weights due to the removal of the ultimobranchial glands. The egg weights of the UBX pullets maintained in 6 x 8 inch cages were significantly greater than those of UBX pullets maintained in 10 x 16 inch laying cages.

Body weights were not significantly influenced by ultimobranchialectomy or by housing condition. However, there was a trend for those pullets maintained in small cages to weight less than those maintained in large cages.

Bone breaking strength. The effect of ultimobranchialectomy and housing conditions on bone breaking strength of the left and right tibiae is presented in Table IX.

No significant differences were found in the breaking strength of the left tibia regardless of treatment. However, there was a tendency for the breaking strenth to increase as housing space increased. There were no significant differences found in the breaking strength of the right tibiae among the six treatments with the exception of the sham operated groups. A significant difference in breaking strength was found between the sham operated pullets kept in 6 x 8 inch cages and in floor pens. As was for the left tibiae, there was also a tendency for the breaking strength of the right tibiae to increase as the housing space increased.

A significant correlation ($P \leq 0.05$) was found between bone density and bone breaking strength. The correlation coefficient as calculated by the least squares method was 0.782.

Gland weights. Ultimobranchialectomy and housing condition

TABLE IX

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON BONE BREAKING STRENGTH OF LEFT AND RIGHT TIBIAE OF LAYING PULLETS, TRIAL 1

Treatments	Breaking strength[1,2] (lbs.) Left tibia	Right tibia
Ultimobranchialectomized		
6" x 8" cages (7)[3]	77.7±6.2[a]	77.6±6.3[ab]
10" x 16" cages (8)	80.9±6.3[a]	79.1±4.6[ab]
Floor pens (12)	81.0±5.4[a]	84.3±5.3[ab]
Sham operated		
6" x 8" cages (7)	72.1±7.0[a]	71.0±5.9[a]
10" x 16" cages (8)	83.5±5.5[a]	81.0±6.4[ab]
Floor pens (14)	97.5±7.1[a]	93.2±6.3[b]

[1]Means ± S. E.

[2]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[3]Number of birds per group.

effects on parathyroid, thyroid, adrenal and ultimobranchial weights are presented in Table X.

Although the parathyroid weights of the treatment groups did not differ significantly in this trial, those of the pullets maintained in 6 x 8 inch cages were larger than those of pullets in other groups.

The thyroid glands of the UBX pullets maintained in 6 x 8 inch cages were significantly larger than those of the other treatment groups.

The adrenal weights of the UBX pullets tended to be larger than those of the sham operated birds, also housing space influenced adrenal weights. The UBX pullets from the 6 x 8 inch cages had adrenal glands significantly larger than those of all groups except similarly operated birds in the 10 x 16 inch cages. Restriction of housing space tended to result in enlarged ultimobranchial glands. Glands from sham operated pullets maintained in 6 x 8 inch cages were significantly greater in weight than those of the sham operated pullets maintained in the floor pens.

Calcitonin content. The effect of housing conditions upon the hypocalcemic potency of the ultimobranchial glands of laying pullets is presented in Table XI.

Ultimobranchial extract taken from the pullets kept in floor pens produced significant hypocalcemia ($P \leq 0.05$) in assay rats at 0.5 mg. and 1.0 mg. dose levels when compared to that from birds maintained in 6 x 8 inch cages at similar dose levels.

No significant difference in hypocalcemic response was found between extract from glands secured from pullets kept in

TABLE X

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON PARATHYROID, THYROID, ADRENAL, AND ULTIMOBRANCHIAL WEIGHTS OF LAYING PULLETS, TRIAL 1

Treatments	Average weights (mg.)[1,2,3]			
	Parathyroid	Thyroid	Adrenal	Ultimobranchial
Ultimobranchial-ectomized				
6" x 8" cages (7)[4]	21.4±1.8[a]	272.5±42.7[b]	199.3±18.6[c]	---
10" x 16" cages (8)	21.0±1.7[a]	175.5±11.0[a]	190.4±8.5[bc]	---
Floor pens (12)	20.4±0.8[a]	172.0±13.9[a]	165.9±8.4[a]	---
Sham operated				
6" x 8" cages (7)	21.1±3.3[a]	196.7±13.9[a]	176.9±9.7[ab]	8.7±0.3[a]
10" x 16" cages (8)	15.6±5.3[a]	167.3±23.3[a]	175.6±9.6[ab]	8.1±0.5[ab]
Floor pens (14)	17.8±3.3[a]	196.6±29.1[a]	160.4±7.3[a]	7.3±0.4[b]

[1]Mean ± S. E.

[2]Combined weight of left and right glands.

[3]Means with different superscripts within a column differ significantly ($P \leq 0.05$)

[4]Number of birds per group.

TABLE XI

THE EFFECT OF HOUSING UPON THE HYPOCALCEMIC POTENCY OF ULTIMOBRANCHIAL GLANDS OF LAYING PULLETS, TRIAL 1

Preparation and dose level	Number of rats[2]	Plasma Ca (mg./100 ml.)[1]		
		Initial	One hour post injection	Change[3]
Saline (0.5 ml.)	6	9.14±0.51	9.20±0.41	+0.06±0.20[a]
Ultimobranchial glands (6" x 8" cages)				
0.25 mg.	9	9.18±0.15	8.85±0.21	-0.33±0.11[b]
0.50 mg.	9	9.36±0.17	8.75±0.21	-0.61±0.17[b]
1.00 mg.	9	9.51±0.18	8.33±0.21	-1.18±0.26[c]
Ultimobranchial glands (floor pens)				
0.25 mg.	9	9.08±0.17	8.51±0.15	-0.57±0.14[b]
0.50 mg.	9	9.19±0.15	8.01±0.14	-1.18±0.25[c]
1.00 mg.	9	9.85±0.41	7.21±0.21	-2.64±0.26[d]

[1]Mean ± S. E.

[2]The extract was injected at three dose levels with three rats per dose level.

[3]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

small cages and those from pullets kept in large cages at the 0.25 mg. dose level.

The data on hypocalcemic response following the injection of 0.5 mg. and 1.0 mg. dose levels indicate that confining laying pullets to 6 x 8 inch cages resulted in a measurable depletion of the calcitonin content of the ultimobranchial glands.

The log-dose response one hour following the injection of ultimobranchial extracts is presented in Figure 1. Data indicate a linear relationship between the dose levels and the mean change in plasma calcium.

Serum calcium and hematocrit. Data regarding the effect of ultimobranchialectomy and housing conditions on serum calcium and hematocrit readings are presented in Table XII.

No significant differences were found with regard to serum calcium levels and hematocrit percentages between the UBX pullets maintained in small or large cages and floor pens and those of the sham operated groups maintained under similar housing conditions. The UBX pullets tended to have greater but not significantly higher serum calcium levels and hematocrit percentages.

Histological studies. Sections of parathyroid, thyroid, adrenal, and ultimobranchial glands were used for the histological studies.

No histological changes in the thyroid, parathyroid or adrenal glands were found that could be associated with ultimobranchialectomy or housing conditions as studied under the compound microscope.

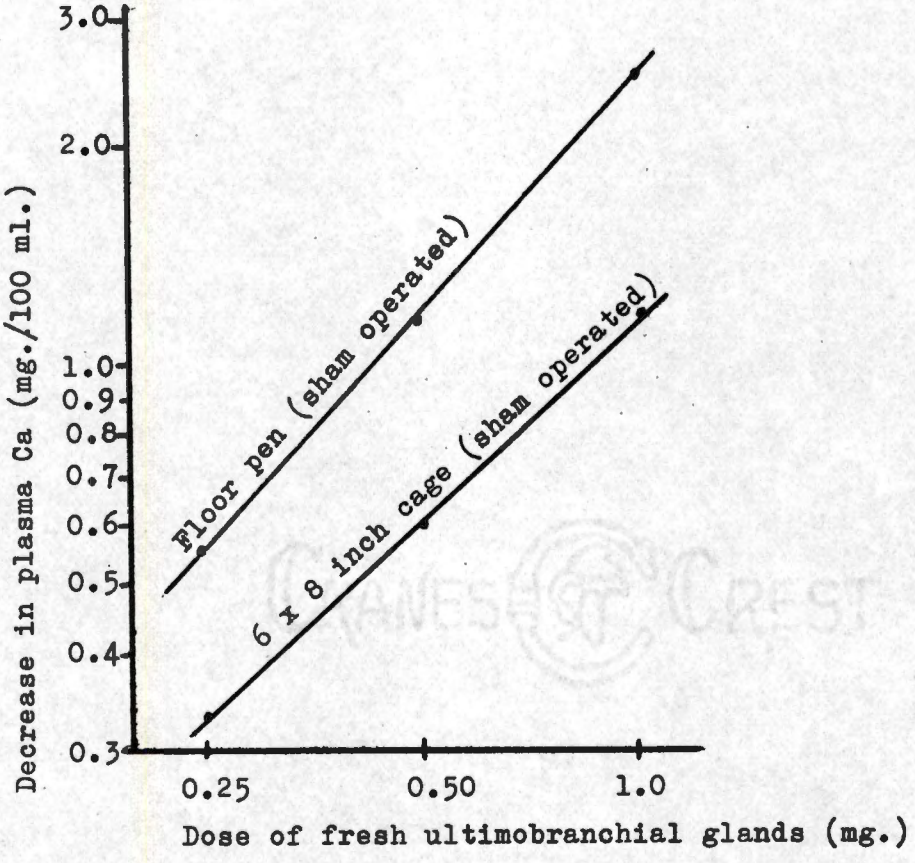

Figure 1. Log-dose response one hour following the injection of acid extracts of ultimobranchial glands of sham operated pullets kept in 6 x 8 inch cages and in floor pens, Trial 1. Each point represents the mean of 9 rats at each dose level.

TABLE XII

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON SERUM CALCIUM AND HEMATOCRIT OF LAYING PULLETS, TRIAL 1

Treatments[1]	Serum calcium[2,3]	Hematocrit[2,3]
	mg./100 ml.	percent
Ultimobranchialectomized		
6" x 8" cages	21.6±1.0	33.5±0.5
10" x 16" cages	23.2±1.3	34.0±0.4
Floor pens	20.6±3.3	33.5±0.5
Sham operated		
6" x 8" cages	17.2±1.0	32.3±0.9
10" x 16" cages	20.2±0.3	33.1±0.2
Floor pens	19.5±2.1	31.7±0.5

[1]Blood samples were taken from five birds at 8:00 a.m. for serum calcium and hematocrit determination.

[2]Mean ± S. E.

[3]Means in each column did not differ significantly ($P > 0.05$) when tested by analysis of variance.

Sections taken from sham operated pullets maintained in floor pens showed hyperplasia of the ultimobranchial cells.

Sections taken from the sham operated pullets maintained in 6 x 8 inch cages exhibited hypertrophy of several ultimobranchial cells.

Sections of the ultimobranchial glands from pullets kept in small or large cages or on the floor showed the existence of accessory parathyroid embedded in them. Similar observations were reported by Chan et al. (1969), and Hurst and Newcomer (1969). The latter investigators found that the accessory parathyroid tissue is functional in chicks.

Photographs illustrating these observations are shown in Figures 2, 3, and 4.

Serum calcium and phosphorus response to parathormone. The response of laying pullets to intramuscular injection of bovine parathyroid extract is shown in Table XIII.

Although the differences were non-significant, the ultimobranchialectomized pullets had distinctly higher serum calcium and lower serum phosphate levels than the sham operated pullets, at three hours following the injection of parathormone.

At six and nine hours post injection the serum calcium and phosphorus levels of UBX pullets were significantly higher than for the sham operated pullets.

Nine hours after parathormone injections, the mean serum calcium levels of the sham operated pullets fell to a hypocalcemic level which differed significantly from that of the UBX pullets. The mean serum phosphorus level of the sham operated group was

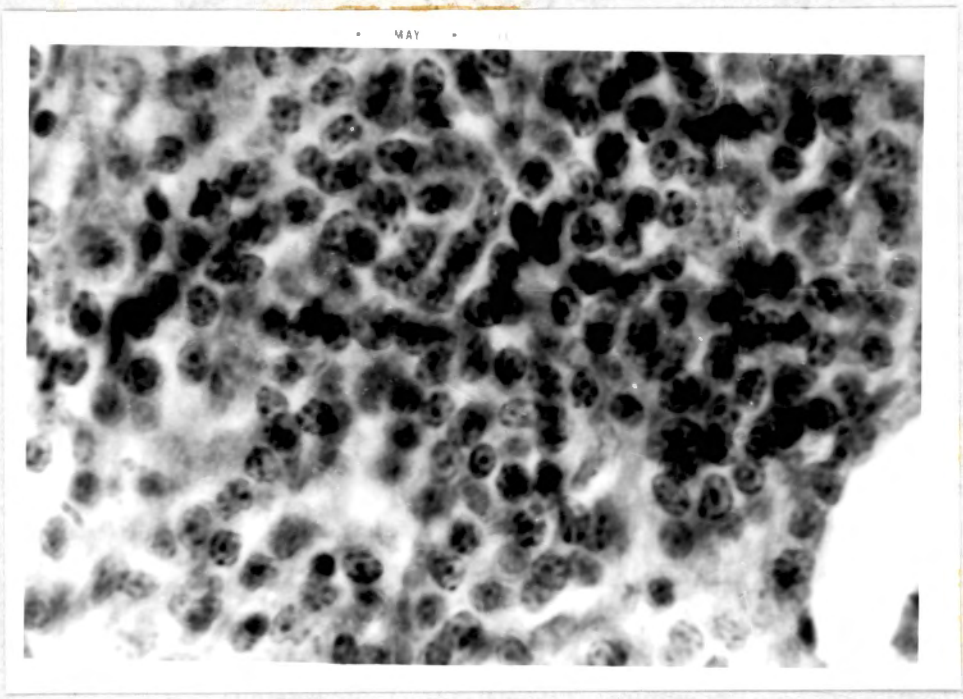

Figure 2. Ultimobranchial gland from floor pen pullet showing hyperplasia of the cells. X1070.

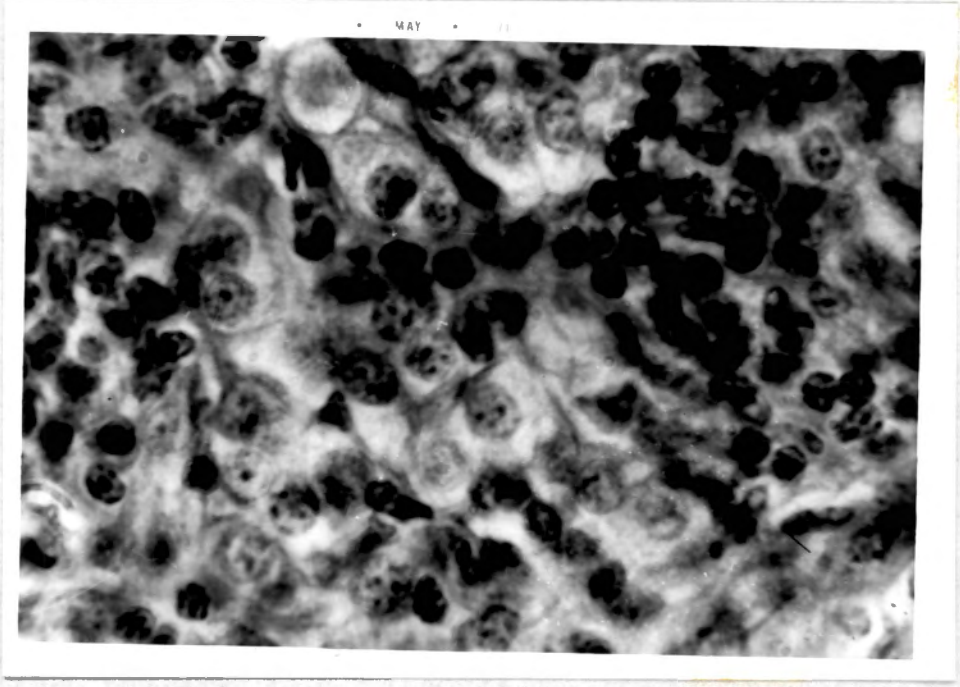

Figure 3. Ultimobranchial gland from small cage pullet showing hypertrophic cells. X1070.

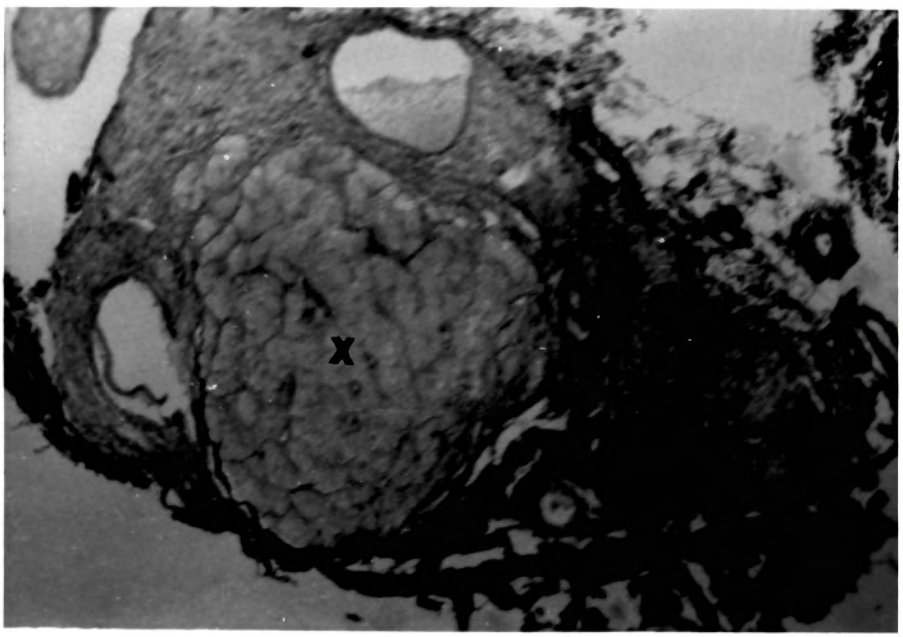

Figure 4. Ultimobranchial gland showing the accessory parathyroid tissue (X) imbedded in its parenchyma. X85.

TABLE XIII

CHANGE IN SERUM CALCIUM AND PHOSPHORUS OF ULTIMOBRANCHIALECTOMIZED AND SHAM OPERATED PULLETS IN RESPONSE TO BOVINE PARATHYROID EXTRACT, TRIAL 1

Serum components	Treatments[2]	Serum levels (mg./100 ml.)[1]			
		Initital	Hours post injection		
			3	6	9
			change from initial		
Calcium	UBX	14.20±1.70	+6.70±2.20	+4.74±2.10 *	+2.12±1.10 *
	sham operated	17.98±3.30	+4.30±1.40	-2.89±1.40	-3.31±0.05
Phosphorus	UBX	8.70±0.58	-2.10±2.00	-4.00±2.10 *	-5.36±1.20 *
	sham operated	6.22±0.36	-0.56±0.62	-0.50±1.30	+1.67±0.50

[1]Mean ± S. E.

[2]Three pullets per treatment.

*Indicates significant difference between values for UBX and sham operated pullets within each column for each of the two minerals ($P \leq 0.05$).

significantly higher than that of the UBX group.

Trial 2

Bone density. The effects of ultimobranchialectomy and housing conditions are shown in Table XIV.

None of the comparisons between the means of the various treatments was significantly different.

By the end of the second period, the floor pen pullets tended to show higher bone density indices than pullets maintained in cages. However, an exception was found in the bone density of the sham operated floor pullets at the end of the fourth and fifth laying periods which tended to be lower than those of the pullets kept in the 10 x 16 inch cages.

The data on the effect of ultimobranchialectomy are reported in Table XV. When the bone density indices of the three housing conditions were combined, no significant differences were found between the ultimobranchialectomized and the sham operated pullets at the end of the five laying periods, however, mean values of the sham operated birds tended to be higher.

In Table XVI, the pooled data regarding the effect of housing conditions on UBX and sham operated pullets is presented.

No significant differences in bone density indices were found between the treatment means. However, bone density indices tended to increase as house space per bird increased.

In this trial, no symptoms of cage layer fatigue were observed in laying pullets maintained in small and large cages in floor pens.

TABLE XIV

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON BONE DENSITY OF LAYING PULLETS, TRIAL 2

Treatments	Pre-laying period[6]	Bone density index[1,2,3,4] Laying periods[5] 1	2	3	4	5
Ultimobranchialectomized						
6" x 8" cages (6)[7]	1.51±0.06	1.83±0.05	1.73±0.07	1.56±0.05	1.42±0.04	1.52±0.07
10" x 16" cages (6)	1.43±0.07	1.55±0.09	1.69±0.15	1.49±0.05	1.48±0.06	1.44±0.04
Floor pens (11)	1.41±0.08	1.79±0.10	1.78±0.06	1.66±0.06	1.71±0.07	1.62±0.04
Sham operated						
6" x 8" cages (6)	1.42±0.08	1.80±0.10	1.65±0.07	1.64±0.06	1.60±0.08	1.60±0.08
10" x 16" cages (6)	1.44±0.05	1.85±0.07	1.69±0.15	1.69±0.19	1.73±0.14	1.76±0.23
Floor pen (10)	1.37±0.04	1.59±0.09	1.70±0.07	1.71±0.07	1.67±0.06	1.63±0.07

[1] X-ray equivalent grams of alloy per cubic centimeter of bone.

[2] Mean ± S. E.

[3] Left tibia.

[4] Means in each column did not differ significantly ($P > 0.05$) when tested by analysis of variance.

[5] At 24 weeks of age and at 28-day intervals thereafter.

[6] At 20 weeks of age.

[7] Number of birds per group.

TABLE XV

THE EFFECT OF ULTIMOBRANCHIALECTOMY ON BONE DENSITY OF LAYING PULLETS, TRIAL 2

Treatments[6]	Bone density index[1,2,3,4] Laying periods[5] 1	2	3	4	5
Ultimobranchial-ectomized (23)[7]	1.74±0.06	1.69±0.04	1.59±0.04	1.58±0.03	1.55±0.05
Sham operated (22)	1.72±0.05	1.69±0.05	1.68±0.06	1.67±0.05	1.66±0.07

[1]X-ray equivalent grams of alloy per cubic centimeter of bone.

[2]Left tibia.

[3]Mean ± S. E.

[4]Means in each column did not differ significantly ($P > 0.05$) when tested by analysis of variance.

[5]At 28-day intervals.

[6]Pullets were kept in 6" x 8", 10" x 16" cages and in floor pens.

[7]Number of birds per group.

TABLE XVI

THE EFFECT OF HOUSING CONDITIONS ON BONE DENSITY OF LAYING PULLETS, TRIAL 2

Housing conditions[6]	Bone density index[1,2,3,4] Laying periods[5]				
	1	2	3	4	5
6" x 8" cages (12)[7]	1.82±0.05	1.69±0.05	1.60±0.05	1.51±0.05	1.56±0.05
10" x 16" cages (12)	1.70±0.07	1.59±0.08	1.59±0.09	1.60±0.08	1.60±0.11
Floor pens (21)	1.69±0.07	1.74±0.04	1.77±0.05	1.69±0.04	1.63±0.05

[1]X-ray equivalent grams of alloy per cubic centimeter of bone.

[2]Left tibia.

[3]Means ± S. E.

[4]Means in each column did not differ significantly ($P > 0.05$) when tested by analysis of variance.

[5]At 28-day intervals.

[6]Ultimobranchialectomized and sham operated pullets combined.

[7]Number of birds per group.

Specific gravity score. The effect of ultimobranchialectomy and cage size on egg specific gravity score is presented in Table XVII.

The analysis of variance and the F values show that the specific gravity scores of eggs produced by UBX pullets maintained in 6 x 8 inch and 10 x 16 inch cages did not differ significantly from those of the sham operated pullets maintained under identical housing conditions.

Among the treatment groups, eggs laid by pullets maintained in small laying cages exhibited significantly lower specific gravity scores than eggs from pullets maintained in large cages at the end of the first 28 day period.

At the end of the second, third and fifth laying periods, there was a tendency for eggs of birds maintained in small cages to have lower specific gravity scores than the eggs of birds maintained in large cages.

Egg production. The data on the effect of ultimobranchialectomy and cage size on the number of eggs laid per pullet during the four 28 day laying periods are given in Table XVIII.

None of the differences in average number of eggs produced per hen in each 28 day laying period was significant ($P \leq 0.05$). However, production was at a slightly higher rate for birds confined to 6 x 8 inch cages as compared with the production of birds confined to 10 x 16 inch cages during the five 28 day laying periods.

Eggs and body weights. The effect of ultimobranchialectomy

TABLE XVII

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND CAGE SIZE ON SPECIFIC GRAVITY SCORES OF EGGS, TRIAL 2

Treatments	Specific gravity score[1,2]					
	Laying periods[3]					Av. for entire laying period
	1	2	3	4	5	
Ultimobranchialectomized						
6" x 8" cages (6)[4]	5.2±8.4[a]	4.2±2.1[a]	3.3±0.9[a]	4.2±1.6[a]	3.3±1.8[a]	4.0±1.0[a]
10" x 16" cages (6)	6.7±0.7[b]	5.7±0.5[a]	3.7±0.5[a]	3.8±1.1[a]	4.8±0.7[a]	4.9±0.5[a]
Sham operated						
6" x 8" cages (6)	5.2±0.4[a]	4.0±0.2[a]	3.7±0.9[a]	3.2±1.2[a]	3.3±0.7[a]	3.9±0.4[a]
10" x 16" cages (6)	6.2±0.8[b]	5.6±1.4[a]	4.0±1.4[a]	2.6±0.8[a]	4.8±0.8[a]	4.6±0.7[a]

[1] Mean ± S. E.

[2] Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[3] Twenty-eight days each.

[4] Number of birds per group.

TABLE XVIII

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND CAGE SIZE ON EGG PRODUCTION, TRIAL 2

Treatments	Egg production[1,2]				
	Laying periods[3]				Av. for entire laying period
	2	3	4	5	
	number of eggs				percent
Ultimobranchialectomized					
6" x 8" cages (6)[4]	19.2±3.0	26.7±0.8	24.0±1.4	21.5±2.6	81.6±4.1
10" x 16" cages (6)	23.5±1.8	26.2±0.8	24.2±1.4	21.8±2.3	85.4±2.8
Sham operated					
6" x 8" cages (6)	15.5±1.8	22.3±1.9	20.3±2.5	22.7±1.7	72.2±4.7
10" x 16" cages (6)	21.0±2.0	23.4±1.9	22.8±2.0	23.2±1.6	80.7±6.5

[1]Mean ± S. E.

[2]Means in each column did not differ significantly ($P > 0.05$) when tested by analysis of variance.

[3]Twenty-eight days each.

[4]Number of birds per group.

and housing conditions on mean egg and body weight is shown in Table XIX.

In this trial, there were no significant differences found in average weight of eggs laid by UBX pullets and that of eggs laid by sham operated pullets kept in the two cage sizes. However, the average egg weight of the UBX pullets in 6 x 8 inch cages was greater than that of UBX laying pullets in 10 x 16 inch cages.

Body weight was not significantly influenced by any of the treatments.

Bone breaking strength. The effect of ultimobranchialectomy and housing conditions on bone breaking strength for trial 2 is given in Table XX.

The breaking strength of the tibiae of the UBX pullets was influenced to a greater degree by the housing treatments than was that of the sham operated pullets. Tibiae of the UBX pullets kept in floor pens had breaking strengths significantly greater than those kept in cages. Whereas, differences between housing groups of the sham operated birds were not significant.

Significant correlations ($P \leq 0.05$) were found between bone density of the left tibia and breaking strength. The correlation coefficient was 0.823.

Gland weights. The data on parathyroid, thyroid, adrenal and ultimobranchial glands are presented in Table XXI.

There were no significant differences found among the six treatment groups in parathyroid, and thyroid weights. In both the UBX and the sham operated groups, pullets in small cages exhibited

TABLE XIX

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON EGG AND BODY WEIGHTS, TRIAL 2

Treatments	Egg weight[1,2]	Body weight[1,2,3]
	grams	grams
Ultimobranchialectomized		
6" x 8" cages (6)[4]	54.9±1.7	1751±66
10" x 16" cages (6)	53.1±1.8	1758±53
Floor pens (11)	--	1698±42
Sham operated		
6" x 8" cages (6)	53.2±1.9	1710±40
10" x 16" cages (6)	53.1±3.9	1883±38
Floor pens (10)	--	1780±54

[1]Mean ± S. E.

[2]Means in each column did not differ significantly ($P > 0.05$) when tested by analysis of variance.

[3]At 40 weeks of age.

[4]Number of birds per group.

TABLE XX

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON BONE BREAKING STRENGTH OF LEFT AND RIGHT TIBIAE OF LAYING PULLETS, TRIAL 2

Treatments	Breaking strength[1,2] (lbs.) Left tibia	Right tibia
Ultimobranchialectomized		
6" x 8" cages (6)[3]	70.5 ± 4.9^{ab}	74.9 ± 3.7^{ab}
10" x 16" cages (6)	65.8 ± 5.3^{a}	68.3 ± 2.5^{a}
Floor pens (11)	89.4 ± 3.7^{c}	87.9 ± 3.8^{bc}
Sham operated		
6" x 8" cages (6)	79.2 ± 8.7^{abc}	79.1 ± 6.2^{abc}
10" x 16" cages (6)	87.5 ± 6.1^{c}	88.4 ± 4.2^{c}
Floor pens (10)	84.9 ± 5.5^{bc}	82.9 ± 5.8^{bc}

[1] Mean ± S. E.

[2] Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[3] Number of birds per group.

TABLE XXI

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON PARATHYROID, THYROID, ADRENAL, AND ULTIMOBRANCHIAL WEIGHTS OF LAYING PULLETS, TRIAL 2

Treatments	Average weights (mg.)[1,2,3]			
	Parathyroid	Thyroid	Adrenal	Ultimobranchial
Ultimobranchial-ectomized				
6" x 8" cages (6)[4]	19.8±1.0[a]	215.6±19.9[a]	196.5±20.6[b]	---
10" x 16" cages (6)	20.2±2.2[a]	224.5±33.9[a]	165.0±18.4[a]	---
Floor pens (11)	16.8±1.3[a]	157.2±10.2[a]	152.7±8.1[a]	---
Sham operated				
6" x 8" cages (6)	15.2±2.6[a]	283.9±68.4[a]	200.7±13.7[b]	8.9±0.4[a]
10" x 16" cages (6)	18.0±2.5[a]	174.5±18.5[a]	164.5±6.5[ab]	7.6±0.3[b]
Floor pens (10)	16.9±1.5[a]	222.1±25.1[a]	154.8±8.5[a]	6.9±0.2[b]

[1] Mean ± S. E.

[2] Combined weight of left and right glands.

[3] Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[4] Number of birds per group.

significantly heavier adrenal weights than those in the floor pens.

The ultimobranchial glands of pullets in the small laying cages were significantly heavier than those of pullets maintained in large cages and in the floor pens. The glands of the latter two groups were similar in weight.

Calcitonin content. The effect of housing on the calcitonin content of ultimobranchial glands is presented in Table XXII.

As in trial 1, the ultimobranchial glands of the birds in the 6 x 8 inch cages evidenced a lesser calcitonin content than those from birds kept in floor pens. Differences between the two treatment groups were statistically different at the 1.0 mg. dose level.

The log-dose response in Figure 5 shows a linear relationship between doses of fresh ultimobranchial gland and plasma calcium changes. As the dose level increased, the fall in plasma calcium doubled. The response, however, is dose dependent.

Serum calcium and hematocrits. Serum calcium and hematocrit values for the UBX and sham operated pullets are shown in Table XXIII.

The UBX pullets tended to have higher calcium levels and hematocrit percentages than did the sham operated pullets. Although differences were not statistically significant, both the serum calcium and hematocrit values of the UBX pullets were higher when the means of the same housing groups are compared. Also, values of the floor pen groups were higher than those of the cage groups.

TABLE XXII

THE EFFECTS OF HOUSING UPON THE HYPOCALCEMIC POTENCY OF ULTIMOBRANCHIAL GLANDS OF LAYING PULLETS, TRIAL 2

Preparation and dose levels	Number of rats[2]	Plasma Ca (mg./100 ml.)[1]		
		Initial	One hour post injection	Change[3]
Saline (0.5 ml.)	6	9.36±0.12	9.38±0.13	+0.02±0.11[a]
Ultimobranchial glands (6" x 8" cages)				
0.25 mg.	9	9.36±0.32	9.00±0.26	-0.36±0.31[b]
0.50 mg.	9	9.92±0.28	9.20±0.23	-0.72±0.28[bc]
1.00 mg.	9	9.61±0.28	8.36±0.28	-1.25±0.28[d]
Ultimobranchial glands (floor pens)				
0.25 mg.	9	9.05±0.20	8.53±0.28	-0.52±0.32[b]
0.50 mg.	9	8.21±0.40	7.14±0.37	-1.07±0.28[cd]
1.00 mg.	9	9.06±0.37	6.97±0.42	-2.09±0.37[e]

[1]Mean ± S. E.

[2]The extract was injected at three dose levels with three rats per dose level.

[3]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

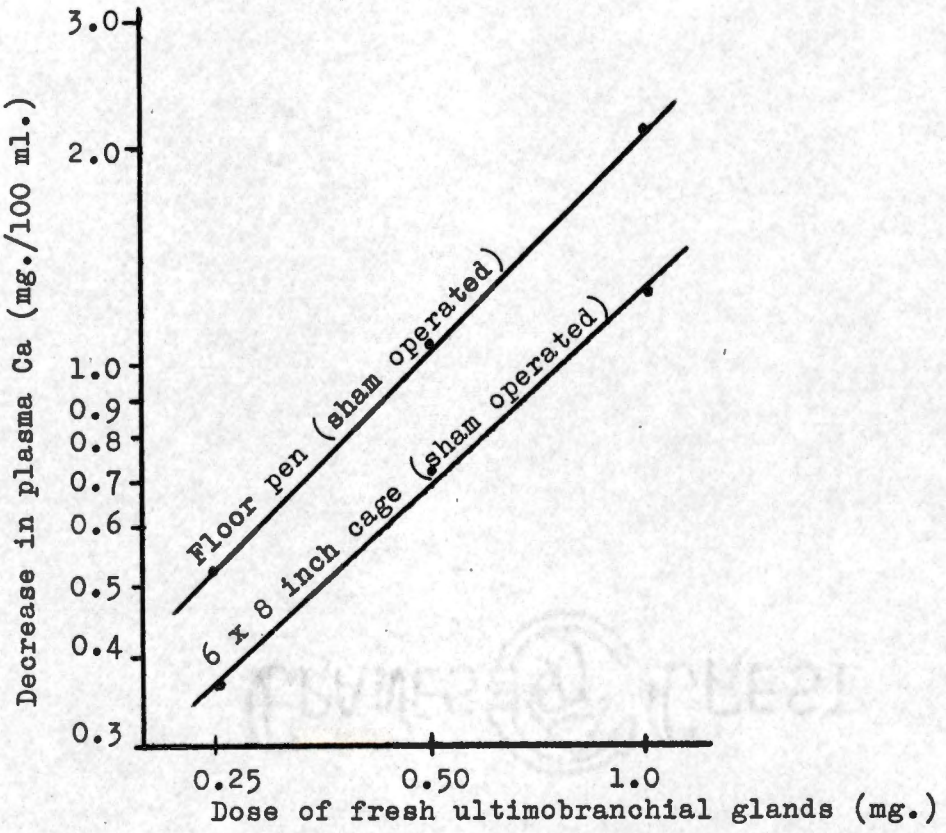

Figure 5. Log-dose response one hour following the injection of acid extracts of ultimobranchial glands of sham operated pullets kept in 6 x 8 inch cages and in floor pens, Trial 2. Each point represents mean of 9 rats at each dose level.

TABLE XXIII

THE EFFECT OF ULTIMOBRANCHIALECTOMY AND HOUSING CONDITIONS ON SERUM CALCIUM AND HEMATOCRIT OF LAYING PULLETS, TRIAL 2

Treatments[1]	Serum calcium[2,3]	Hematocrit[2,3]
	mg./100 ml.	percent
Ultimobranchialectomized		
6" x 8" cages	22.4±2.1	32.6±0.6
10" x 16" cages	20.9±0.9	33.7±0.5
Floor pens	27.5±3.7	33.6±0.9
Sham operated		
6" x 8" cages	20.3±5.1	31.8±1.1
10" x 16" cages	18.6±1.9	32.8±0.1
Floor pens	22.7±2.3	33.5±0.3

[1]Blood samples were taken from four birds at 8:00 a.m. for serum calcium and hematocrit determination.

[2]Mean ± S.E.

[3]Means in each column did not differ significantly ($P > 0.05$) when tested by analysis of variance.

Histological studies. Use of the light microscope disclosed no histological changes in the parathyroid, thyroid and the adrenal glands of all experimental groups of pullets in trial 2.

In this trial, ultimobranchial cells of the sham operated pullets maintained in floor pens showed hyperplasia. On the other hand, sections of glands taken from small cage laying pullets showed several hypertrophic cells. These histological changes are shown in figures 6 and 7.

Serum calcium and phosphorus response to parathormone. The response of UBX and sham operated pullets to intramuscular injections of bovine parathormone is given in Table XXIV.

Although non-significant, the increase in serum calcium in response to bovine parathormone three hours post injection was greater for the ultimobranchialectomized than for the sham operated pullets. At six and nine hours post injection, the serum calcium remained at a significantly higher level in the UBX pullets as compared with the sham operated pullets.

At three hours post injection, the serum phosphorus level was significantly higher in the UBX pullets as compared with the sham operated pullets. Although the differences were non-significant at six hours following parathormone injection, the serum phosphorus level in the UBX birds remained higher as compared with that of the sham operated. However, at nine hours post injection, the UBX exhibited a significantly lower level of serum phosphorus than the sham operated pullets.

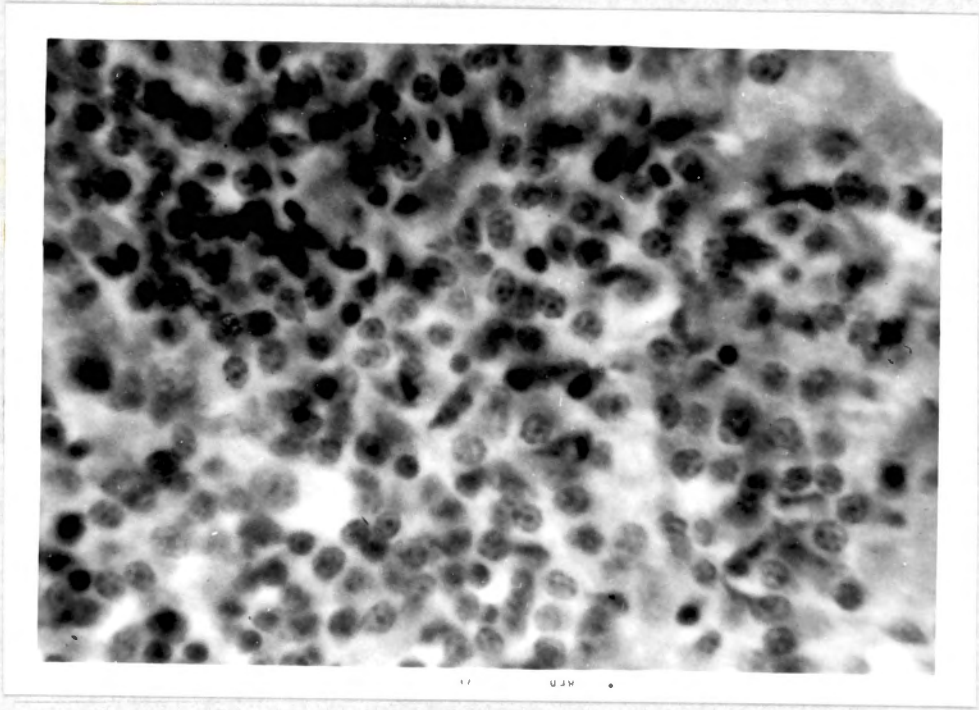

Figure 6. Ultimobranchial gland from floor pen pullet showing hyperplasia of the cells. X1070.

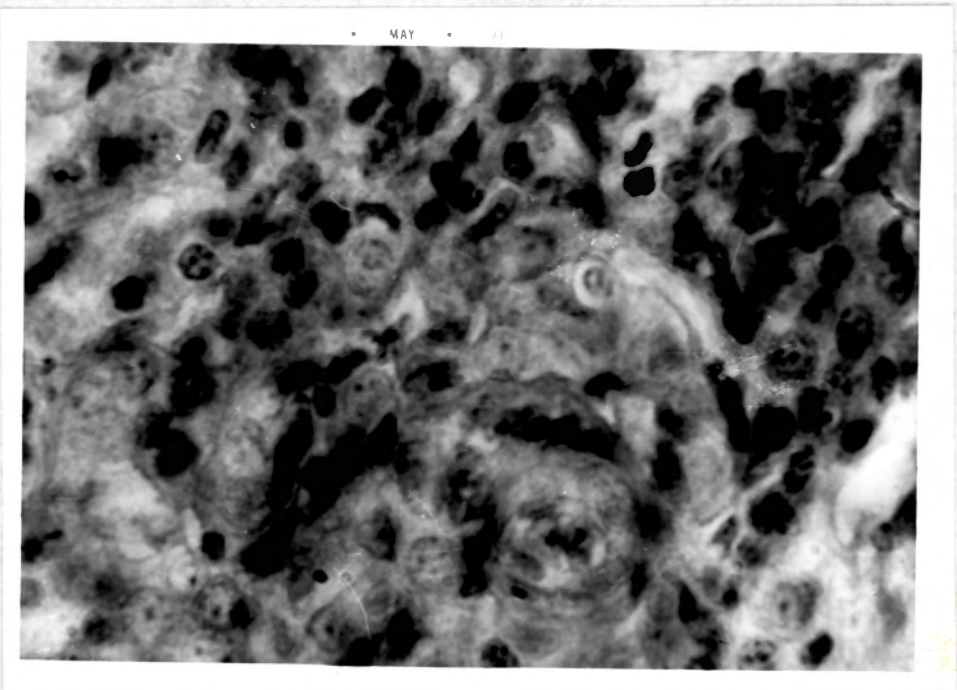

Figure 7. Ultimobranchial gland from small cage pullet showing hypertrophic cells. X1070.

TABLE XXIV

CHANGES IN SERUM CALCIUM AND PHOSPHORUS OF ULTIMOBRANCHIALECTOMIZED AND SHAM OPERATED PULLETS IN RESPONSE TO BOVINE PARATHYROID EXTRACT, TRIAL 2

Serum components	Treatments[2]	Serum levels (mg./100 ml.)[1]			
		Initial	Hours post injection		
			3	6	9
			change from initial		
Calcium	UBX	19.33±0.45	+3.64±1.02	-0.93±0.26 *	-0.93±0.26 *
	sham operated	15.12±0.10	+2.16±1.02	-2.95±0.23	-3.98±0.27
Phosphorus	UBX	5.67±0.46	+0.60±0.23 *	-0.47±0.31	-1.37±0.18 *
	sham operated	7.53±0.50	-0.26±0.47	-1.28±0.30	+1.92±0.52

[1] Mean ± S. E.

[2] Three pullets per treatment.

[3] Indicates significant difference between values for UBX and sham operated pullets within each column for each of the two minerals ($P \leq 0.05$).

VI. DISCUSSION

Ultimobranchialectomy combined with the stress of confinement resulted in a significant increase in the weights of the thyroid and adrenal glands. In addition, the operation also resulted in non-significant increases in specific gravity scores and weights of eggs, parathyroid weights, serum calcium and hematocrit values. The opration also resulted in a significant decrease in bone density and bone breaking strength. The response of UBX birds to bovine parathyroid extract as measured by serum calcium was found to be greater than that of the sham operated controls.

There was no significant increase found in the incidence of cage layer fatigue as a result of removing the ultimobranchial glands. This would indicate that calcitonin apparently does not play a major role in this disorder under the conditions of low stress. However, the effects of ultimobranchialectomy were greater in the birds confined to the smaller cage sizes, 6 x 8 inches, as compared to those in large cages and on the floor. Thus, the homeostatic mechanism provided by calcitonin may become very important when other factors such as stress of confinement result in a marginal situation in respect to calcium sufficiency.

Confinement of pullets to laying cages resulted in a significant decrease in bone density and in bone breaking strength. Calcitonin content of the ultimobranchial glands as determined by bioassays was also significantly reduced by confinement.

That confinement affects the function of the ultimobranchial glands is also supported by the finding that the size

of these glands increased and showed definite histological changes suggestive of secretory depletion.

The precentage of pullets evidencing symptoms of cage layer fatigue was higher in the caged birds as compared to pullets maintained on the floor. In addition, caging resulted in a non-significant decrease in egg production and specific gravity scores of eggs. Non-significant increases in the weights of the parathyroid, thyroid and adrenal glands of the caged birds were also found.

Thus in comparing the effects of removal of the ultimobranchial glands of pullets and confinement to cages, it appears that in respect to a number of parameters the effects are similar. The results of these studies would indicate that both ultimobranchialectomy and close confinement of pullets results in a reduction in the efficiency of calcium metabolism particularly as it is reflected in bone structure. The results of these studies also indicate that the effects of close confinenemt and ultimobranchialectomy are additive in respect to several of these parameters.

Considering the data obtained in these experiments along with that reported in the literature on the influence of calcitonin in calcium metabolism it is concluded that close confinement of pullets increases the incidence of cage layer fatigue and eventually results in a reduction in the function of ultimobranchial glands as evidenced by lowered calcitonin content and change in size and histology of these glands.

It is postulated that the stress of confinement results in

an increase in corticoids production by the adrenal glands which in turn increases calcium mobilization from the bone and augments the plasma calcium. When the plasma calcium rises, calcitonin from the ultimobranchial glands is released. The release of calcitonin is a physiological effort to inhibit further calcium mobilization resulting from the action of corticoids and thus, to indirectly prevent the elevation of plasma calcium. However, if the stress of confinement persists, the ultimobranchial glands can not produce calcitonin sufficient to inhibit calcium resorption.

As a result, the ultimobranchial glands increase in weight, calcitonin content declines and the ultimobranchial cells increase in size and show signs of secretory exhaustion. In the meantime, the adrenal glands enlarge due to the increased production of corticoids under hormonal influence of the anterior pituitary gland.

The possibility exists that the adrenal corticoids act directly upon the ultimobranchial glands and influence their histology and functioning.

If the ultimobranchial gland is removed the inhibitory effect of calcitonin on the elevation of plasma calcium, as induced by the adrenal corticoids acting on bone resorption is eliminated, thus calcium loss occurs at a more rapid rate resulting in lower bone density or osteoporosis. That the reduction in bone density brought about by close confinenemt is due to stress acting through increased adrenal corticoids secretion and not due to inactivity per se, is evidenced by the work of Urist and Deutsch (1960) in which they showed that injection of ACTH resulted in osteoporosis of hens. Rowland and Harms (1971) found no significant difference

in bone characteristics between 8 week-old broilers grown in floor pens with litter and those grown on wire floors. This is evidence that the decrease in bone density brought about by close confinement is not due to the inability of the birds confined in cages to recycle nutrients.

VII. SUMMARY

The effect of ultimobranchialectomy and close confinement on calcium metabolism in the laying hen was studied in two trials involving a total of 113 Single Comb White Leghorn pullets.

Ultimobranchialectomy and sham operations were performed on pullets at one week of age. They were reared to maturity in floor pens and housed through the laying period in 6 x 8 and 10 x 16 inch laying cages and in floor pens.

In trial 1, significant differences ($P \leq 0.05$) in bone density were evident at the end of the fourth and fifth 28 day laying periods between the UBX pullets kept in large and small laying cages and the sham operated pullets kept in the same cage sizes. In trial 2, however, no significant differences in bone density indices were found between the UBX and sham operated birds maintained in the three housing conditions at any time during the five 28 day periods.

In both trials, when the housing effect was eliminated, ultimobranchialectomy per se resulted in no significant reductions in bone density.

In trial 1, ultimobranchialectomy combined with the stress of close confinement resulted in non-significant reductions in bone breaking strength. Whereas, in trial 2, a significant reduction was evidenced as a result of the operation combined with the stress of confinement.

With regard to thyroid and adrenal weights, in trial 1 significant differences were found between UBX pullets kept in

small cages and sham operated pullets kept in similar size cages. In trial 2, however, none of the differences between treatments was significant.

In both trials no significant differences in specific gravity scores of eggs, percent egg production, parathyroid weights, serum calcium and hematocrit values were found between the operated and sham operated pullets kept in the three housing conditions.

There were no significant differences found between the UBX and the sham operated birds for mean body weights.

In trial 1, confinement to cages resulted in significant reductions in bone density and bone breaking strength. In trial 2, no significant differences in bone density were found between cage and floor housed pullets. In the latter trial significant differences in bone breaking strength of the left and right tibiae were found between cage and floor pullets.

In both trials, confinement of laying pullets to small cages resulted in significant changes in the ultimobranchial glands as follows: (1) increased weights, (2) hypertrophy of cells, and (3) a decrease in calcitonin content.

Only in trial 1 were symptoms of cage layer fatigue observed in both the operated and the sham operated pullets confined to small cages. No symptoms of cage layer fatigue were observed in pullets confined to large cages or in the floor pens.

In conclusion, the results of these studies indicate that the ultimobranchial gland does play a physiological role in the laying hen, particularly in relation to calcium metabolism and bone structure. Removal of the ultimobranchial gland results in a reduction in the efficiency of calcium utilization.

REFERENCES

REFERENCES

Baud, C. A., J. Desiebenthal, B. Langer, M. R. Tupling and R. C. Mach, 1969. The effect of prolonged administration of thyrocalcitonin in human senile osteoporosis. Calcitonin 1969: Proc. Sec. Ingr. Symposium. William Heinemann Medical Books, LTD, London, 541-545.

Bell, D. J., and W. G. Siller, 1962. Cage layer in Brown Leghorn Res. Vet. Sci. 3:219-230.

Bell, D. J., W. G. Siller and J. C. Campbell, 1959. Observations on cage layer fatigue in hens. J. of Biochem. 72:32p.

Brown, D. M., D. Y. E. Pery, P. B. Dent and R. A. Good, 1969. Effect of chronic calcitonin deficiency on the skeleton of the chicken. Proc. Soc. Exptl. Biol. Med. 130:1001-1004.

Chan, A. S., J. D. Cipera and L. F. Belanger, 1969. The ultimobranchial gland of the chick and its response to a high calcium diet. Rev. Can. Biol. 28:19-31.

Copp, D. H., E. C. Cameron, B. A. Cheney, A. G. F. Davidson, and K. G. Henze, 1962. Evidence for calcitonin--a new hormone from the parathyroid that lowers blood calcium. Endocrinology 70:638-649.

Copp, D. H., C. E. Brooks, B. S. Low, F. Newsome, R. K. O'Dor, C. O. Parker, V. Walker and E. G. Watts, 1969. Calcitonin and ultimobranchial function in lower vertebrates. Calcitonin 1969: Proc. Sec. Intr. Symposium. William Heinemann Medical Books, LTD, London 281-292.

Copp, D. H., D. W. Cockcroft and Yankoon Kueh, 1967. Ultimobranchial origin of calcitonin. Hypocalcemic effect of extracts from chicken glands. Can. J. Physiol. and Pharmacol. 45:1095-1009.

Copp, D. H., D. W. Cockcroft and Yankoon Kueh, 1967. Calcitonin from ultimobranchial glands of dogfish and chicken. Science 158:924-925.

Copp, D. H., and C. O. Parkes, 1968. Extraction of calcitonin of calcium from ultimobranchial tissue. In parathyroid hormone and thyrocalcitonin (calcitonin). Excerpta Medica Foundation. Amsterdam. 47-82.

Copp, D. H., 1969. Endocrine control of calcium homeostasis. J. Endocrinology 43:137-161.

Copp, D. H., W. A. Webber, B. Low, Y. Kueh and J. Diely, 1968. Effect of dietary calcium on ultimobranchial morphology in chickens. Proc. Sec. Can. Fed. Biol. Soc., II, 34. (Abstract).

Copp, D. H., 1967. Hormonal control of hyercalcemia.. Historic development of the calcitonin concept. Am. J. Med. 23: 648-655.

Couch, J. R., 1955. Cage layer fatigue: A new problem for poultrymen. Poultry Dig. 14:385-388.

Dudley, J., 1942. Ultimobranchial of the fowl. Am. J. Anat. 71:65-97.

Duncan, B. D., 1955. Multiple range and multiple F test. Biometric 11:1-42.

Fiske, C. H., and Y. Subbarow, 1925. The colormetric determination of phosphorus. J. Biol. Chem. 66:375-400.

Foster, G. V., F. H. Doyle, G. F. Joplin, F. R. Singer, T. R. Fraser and I. MacIntyre, 1968. Clinical application of calcitonin. In parathyroid hormone and thyrocalcitonin (calcitonin). Excerpta Medica Foundation. Amsterdam. 100-107.

Francis, D. W., 1957. Strain differences in the incidence of cage layer fatigue. Poultry Sci. 36:181-183.

Gardiner, T., 1965. Cage layer fatigue. Agr. N. Ireland. 39:81.

Gaillard, P. J., 1963. Observation on the effect of thyroid and parathyroid secretions on explanted mouse radius rudiments. Develp. Biol. 7:103-116.

Gittes, R. F., S. U. Toverud and C. W. Cooper, 1968. Effect of hypercalcemia and hypocalcemia on the thyrocalcitonin content of rat thyroid glands. Endocrinology 82:83-90.

Grumbles, L. C., 1959. Cage layer fatigue (cage paralysis). Avian Diseases 3:122-125.

Hirsch, P. F., G. F. Gauthier and P. L. Munson, 1963. Thyroid hypocalcemic principle and recurrent laryngeal nerve injury factors affecting the response to parathyroidectomy in rats. Endocrinolgy 73:244-252.

Hirsch, P. F., E. F. Boskel and P. L. Munson, 1964. Thyrocalcitonin: hypocalcemic hypophosphatemic principle of the thyroid gland. Science 146:412-413.

Hodges, R. D., 1969. Effect of vagal stimulation upon blood calcium in the fowl. Calcitonin 1969: Proc. Sec. Intr. Symposium. 67. (Abstract).

Hurst, J. G. and W. S. Newcomer, 1969. Functional accessory parathyroid tissue in ultimobranchial bodies of chickens. Proc. Soc. Exp. Biol. Med. 132:555-557.

King, D. F., 1965. Effects of cage size on cage layer fatigue. Poultry Sci. 44:898-900.

Kingsbury, B. F., 1935a. On the fate of the ultimobranchial body within the human thyroid. Anat. Record 61:155-173.

Kingsbury, B. F., 1935b. Ultimobranchial and thyroid glands in the fetal calf. Am. J. Anat. 56:445-479.

MacIntyre, I., 1967. Calcitonin: A general review. Calc. Tiss. Res. I:173-182.

Norris, L. C., and F. H. Kratzer, 1968. Effect of supplying adrenal hormone in the diet on calcium metabolism in the chicken. Proc. Cornell Nutr. Conf. 5-11.

Robertson, D. R., 1968. The ultimobranchial body in Rana pipiens. IV. Hypercalcemia and glandular hypertrophy. Z. Zellforsch: 85:441-452.

Rowland, L. O., and R. H. Harms, 1971. Bone characteristics of broilers grown on wire and on litter. Proc. 68th Annual Convention Assoc. South. Agr. Workers. (Abstract).

Simpson, C. F., P. W. Waldrop, C. B. Ammerman and R. H. Harms, 1963. Relationship of dietary calcium and phosphorus levels to the cage layer fatigue syndrome. Avian Diseases 8:92-100.

Snedecor, C. W., 1956. Statistical Methods. Fifth Edition. The Iowa State College Press, Ames, Iowa.

Stoeckel, M. E. and Aime Porte, 1969. Etude Ultrastructurale des crops ultimobranchiux du poulet. I. Aspect normal et developpement embryonnaire. Z. Zellforsch. 94:495-512.

Urist, M. R., 1959. The effect of calcium deprivation upon the blood, adrenal cortex, ovary and skeleton in domestic fowl. Recent Prog. Hormone Research 15:455-481.

Urist, M. R., 1967. Avian parathyroid physiology: Including a special comment on calcitonin. Am. Zoologist 7:883-851.

Urist, M. R., and Nancy Marie Deutsch, G. Pomerants and F. C. McLean, 1960. Inter-relation between actions of parathyroid hormone and estrogens on bone and blood in avian species. Am. J. Physiol. 199:851-855.

Urist, M. R., and Nancy Marie Deutsch, 1959. Osteoporosis in the laying hen. Endocrinology 66:377-391.

Urist, M. R., 1960. Cage layer osteoporosis. Endocrinology 67:870-871.

Urist, M. R., and Nancy Marie Deutsch, 1960. Influence of ACTH upon avian species and osteoporosis. Proc. Soc. Exptl. Biol. Med. 104:35-38.

Van Dyke, J. H., 1943. The behavior of ultimobranchial tissue in the postnatal thyroid of sheep. Anat. Record 85:432. (Abstract).

Young, D. Y., and C. C. Capen, 1969. Thyrocalcitonin: Response to experimental hypercalcemia induced by vitamin D in cows. Calcitonin 1969: Pro. Sec. Intr. Symposium. William Heinemann Medical Books, LTD, London, 140-151.

Ziegler, R., G. Delling and E. F. Pfeiffer, 1969. The secretion of calcitonin by the perfused ultimobranchial gland of the hen. Calcitonin 1969. Proc. Sec. Intr. Symposium. William Heinemann Medical Books. LTD, London, 301-310.

VITA

Abdul Karim Gaidan Al-Khazraji was born in Ali-Garbi, Iraq, on April 1, 1935. He was graduated from Primary Teachers' Training College, Baghdad, Iraq, in 1956. From 1956 until 1958 he was a teacher in arts and visual aids in Baghdad. In June 1958, he was awarded a scholarship by the Iraqi-Government to study poultry in the United States. From 1958 until 1962 he attended The University of Tennessee, Knoxville; he received a Bachelor of Science degree in Agriculture, majoring in poultry, in the latter year. From 1962 until 1966 he was a Second-Lieutenant in the Iraqi Army. In November 1966, he was awarded a scholarship by the Iraqi-Government to study toward a Ph.D. degree in poultry. In January, 1967, he entered The University of Tennessee Graduate School. In August 1968, he received his Master of Science degree. In June 1971, he is to be granted a Doctor of Philosophy in Animal Science. The major work was in the area of animal physiology with emphasis on poultry. He is a member of Alpha Zeta, Gamma Sigma Delta, and Sigma Xi.