THE ULTIMOBRANCHIAL ORIGIN OF CALCITONIN

A STUDY BASED ON

ULTIMOBRANCHIAL GLAND EXTRACTS

by

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ABSTRACT

Acid extracts of the ultimobranchial glands of domestic fowl *Gallus domestica* and the dogfish *Squalus suckleyi* had a very potent hypocalcemic effect when injected into young rats, while extracts of the corresponding thyroid glands were devoid of such activity. The log. dose response paralleled that obtained with mammalian calcitonin preparations. The data indicate that the ultimobranchial gland, rather than the thyroid, is the source of calcitonin in these species.
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Introduction

Godwin postulated in 1937 that the "interfollicular" cell (parafollicular or "C" cell) of the dog thyroid originated from ultimobranchial tissue (34). The significance of this postulate was not appreciated until thirty years later when Pearse (61) and Bussolati et al. (7) reported that the parafollicular or "C" cell in the thyroid was the source of calcitonin. As a test of the Godwin hypothesis and in an attempt to establish the ultimate origin of calcitonin, it seemed logical that we should study ultimobranchial gland extracts for the presence of calcitonin-like activity. Accordingly, we concentrated our investigation on non-mammalian vertebrates in which the ultimobranchial gland remains a separate structure. We chose the domestic fowl and the dogfish for our study. Being a homeothermic animal and possessing a bony skeleton, the chicken is physiologically close to the mammal. The dogfish on the other hand, has a cartilagenous skeleton and we felt that if calcitonin was shown to be present it would provide an opportunity for studying the action of this hormone in an animal which lacks a bony skeleton.
Since our initial reports on the ultimobranchial origin of calcitonin (24, 25), the entire lower vertebrate spectrum has been examined for the presence of this hormone. There was initially some doubt as to whether the hypocalcemic factor of ultimobranchial gland extracts was indeed calcitonin. However, the structure of calcitonin from a lower vertebrate, the salmon, has now been determined (59) and is basically the same as that of mammalian calcitonins.
Part I

a) History of Calcitonin

Until the discovery of calcitonin in 1961 by Copp et al. (18), it was generally accepted that the precise control of the calcium level in body fluids was mainly dependent upon a feedback mechanism involving the parathyroid glands (51). Hypocalcemia was thought to cause release of parathormone from the parathyroids which in turn raised the calcium level. Hypercalcemia was believed to suppress parathormone release. The first indication of endocrine control of hypercalcemia was noted in this laboratory in 1957 in a study of the effect of EDTA infusions in the dog in which the thyroid and parathyroids were removed at the end of the experiment. Instead of the expected fall in plasma calcium level, the calcium level rose above normal when infusion was stopped (Fig. 1). This was so contradictory to the generally accepted theory of parathyroid physiology at the time that the result was thought to be a technical error.

The following year it was again noted that the removal of the thyroid and parathyroids impaired the control
of hypercalcemia. On this occasion (Fig. 2), ten dogs were given 8-hour infusions of parathyroid extract (1 U/kg/hr) during which time the plasma calcium rose to 11 mg % and remained at that level for several hours after the infusion was discontinued. However, when thyroparathyroidectomy was performed at the end of the infusion, the plasma calcium immediately rose to 13-14 mg %. This and the earlier observations were not reported because they contradicted the accepted theory of calcium homeostasis at the time.

In 1961 Copp et al. observed a rapid fall in plasma calcium when the isolated thyroparathyroid complex was perfused with high-calcium blood. According to the McLean feedback hypothesis (51), this fall was due to the suppression of parathormone release resulting from the hypercalcemic state. If this were indeed correct, removal of the source of parathormone should logically cause the same drop in calcium level. However, it was found that removal of the thyroparathyroid complex at the end of the perfusion experiment actually caused the calcium level to rise where it remained for more than ten hours (Fig. 3). It was further noted that the high-calcium perfusate from the original dog caused a drop in plasma calcium when injected into a second dog. These observations strongly suggested the presence of an active calcium-lowering factor which was produced by either
the thyroid or parathyroid or both in response to hypercalcemia. With this evidence at hand, Copp et al. (18) in 1961 postulated a new calcium-regulating hormone which they named calcitonin. Their work was confirmed by Kumar et al. (49) in 1963.

Initially it was thought that calcitonin originated from the parathyroids (19, 22). It soon became clear that a hypocalcemic factor was produced by mammalian thyroids (9, 28, 39). The name "thyrocalcitonin" was introduced to suggest its possible identity to calcitonin and to indicate its thyroid origin.

In 1967, Bussolati et al. (7) found calcitonin in parafollicular or "C" cells of the hog and dog thyroids using an immunoflorescent technique. This same procedure showed that calcitonin was absent from the follicular cells. Godwin had postulated the ultimobranchial origin of the "interfollicular" (parafollicular) cells of the dog thyroid in 1937 (34). It was supported by Pearse and Carvalheira (62) when they reported cytochemical evidence for an ultimobranchial origin of "C" cells in rodent thyroid in 1967. In the same year Copp, Cockcroft and Kueh reported calcitonin-like activity in acid extracts of the ultimobranchial glands of domestic fowl (24, 25) and dogfish (25). Later
in the year, Tauber (79) also reported independently that the ultimobranchial glands of the domestic fowl contained calcitonin.* This discovery has opened up great opportunities for research on the physiology of this hormone and its possible role for therapy in man. The historic development of the calcitonin concept is traced in Figure 4.

*Tauber's paper (79) was submitted to the Proc. Nat. Acad. Sci. in August 1967. The paper by Copp et al. (24) was submitted to the Can. J. Physiol. and Pharmacol. in June 1967.
b) Chemical Structure of the Known Calcitonins

The structures of hog (65), human (55) and salmon (59) calcitonins have now been elucidated. They are all straight chain polypeptides with 32 amino acid residues and a molecular weight of approximately 3500. Each chain has a disulfide bridge at the N-terminus and an amide group at the C-terminus. Table I compares the amino acid composition and biological activity of salmon, human and hog calcitonins. Figure 5 shows the structure of these hormones and it is seen that the basic structure appears to have persisted during evolutionary development. However, salmon calcitonin has much more potent hypocalcemic effect in young rats and its action is also more prolonged.
c) Therapeutic Importance of Calcitonin

The level of calcium in the extracellular fluid is regulated with such precision that the diurnal variation in plasma calcium in healthy male subjects is found to be less than ± 3% (10). An optimum level of ionic calcium is vital to many important body processes including the activity of many enzyme systems, neuromuscular excitability and muscular contractions. Its maintenance depends upon the relative rates of calcium deposition in and out of bone, renal excretion of calcium and absorption through the gastrointestinal wall. Both parathormone and calcitonin are involved in these processes.

The hypocalcemic effect of calcitonin is believed to be mediated primarily by its inhibition of bone resorption. This mechanism of action has been demonstrated in studies both in vitro (31) and in vivo (37). Foster et al. (30) reported that calcitonin caused increased trabecular bone formation at the metaphyses of the caudal vertebrae in growing rats. Brown et al. (6) however, observed that the hormone had no major role in the growth of the skeleton in chicken under normal conditions but that it did apparently aid in controlling hypercalcemia.
In man, a deficiency of calcitonin had been suggested as possible factor in the condition of osteoporosis and hypercalciuria (2). However, in clinical trials using i.v. calcium infusions, O'Brien and McIntosh (58) found the response of idiopathic osteoporotic patients inconclusive of a state of calcitonin deficiency.

Foster et al. (29) reported the use of porcine calcitonin in the acute treatment of hypercalcemia in patients with malignant disease, viz., terminal carcinoma of the breast. They found that calcitonin had a greater hypocalcemic effect in these patients than in the normal controls. Mazzuoli et al. (53) documented a patient with a long history of tetany and diffuse non-toxic goitre in whom they suspected an excess production of calcitonin. Thyroidectomy followed by thyroxine replacement reversed the condition of the patient.

The proven hypocalcemic effect of porcine (29) and salmon (66) calcitonins in man and the fact that the hormone has been identified in the human thyroid (1) suggest that it probably plays an important role in calcium metabolism in man. However, further studies on the physiology of calcitonin will have to be made before its importance in the therapy of pertinent human diseases can be evaluated.
Part II

a) Embryology of the Ultimobranchial Gland

The ultimobranchial gland is present in all vertebrates except the cyclostomes (86). In elasmobranchs it is also referred to as the suprapericardial body, a name introduced by van Bemmelen (82) to indicate the relationship of this structure to the pericardium in these animals. Other names such as postbranchial and telobranchial body, accessory and lateral thyroid are also encountered in the literature. "Ultimobranchial" was the name suggested by Greil (35) and is preferred over the others as it suggests the embryological origin of the gland.

The ultimobranchial gland arises from the most caudal outgrowth of the pharyngeal epithelium, the terminal pouch (86). The origin of the ultimobranchial varies in different genera. In teleost it originates from the dorsal portion of the fifth gill-pouch, in amphibians and birds from the sixth, in rodents from the third and in most other mammals from the fourth branchial pouch. In elasmobranchs only the left gland develops fully and persists in the adult. The right counterpart undergoes atrophy in early embryological development (8).
Among amphibians, the ultimobranchial is single in adult urodèles but is paired in anura. It is variable in reptiles. Birds are reported to have paired ultimobranchials (41, 42, 86) except in the sparrow (42). In mammals the gland arises as a paired structure but fails to remain as discrete entities. Godwin (34) had hypothesized the ultimobranchial origin of the "interfollicular" cells in dog thyroid. There is good embryological evidence (3,4,34,45) that mammalian ultimobranchial tissue becomes incorporated in the thyroid. Cytochemical evidence for an ultimobranchial origin of rodent "C" cells (parafollicular cells of the thyroid) has been reported by Pearse et al. (62).
b) Histology of the Ultimobranchial Gland

The chicken ultimobranchial gland consists largely of cords of cells in a loose network of connective tissue and blood capillaries. That of the dogfish is largely follicular (Figs. 7, 8, 9).

In the chick (13, 14) two distinct cell types have been identified and studied in the ultimobranchial gland. They are the light and the dark cells. At the level of the electron microscope, the dark cells have irregular outlines and closely packed organelles which impart to the cytoplasm a rather dense appearance. The light cell which is the predominant cell is morphologically similar to the para-follicular or "C" cell of the mammalian thyroid. It contains a pale cytoplasm with electron dense secretory granules, a rough surfaced endoplasmic reticulum, a Golgi apparatus and mitochondria. It has, therefore, the appearance of a secretory unit. Chan et al. (13, 14) also noted that degranulation occurred in the light cells when young fowls were maintained on a high-calcium diet. It was observed, too, that the endoplasmic reticulum was well developed and that the Golgi was hypertrophyied, indicating that these cells were in active synthesis (Figs. 10, 11).
c) **Functional Status of the Ultimobranchial Gland Prior to 1967**

From the late 19th century, embryologists and histologists have shown a keen interest in the ultimobranchial gland. However, practically nothing was known about the function of the ultimobranchial gland despite the exhaustive studies on the embryology and morphology of this structure. Verdun (84) who carried out a comprehensive study of the branchial derivatives of vertebrates reported in his doctoral dissertation in 1898 that he did not think the "lateral thyroid" produced colloid. He therefore cautioned the use of the name lateral thyroid, so as not to "prejudge its nature and significance." He also regarded the ultimobranchial gland as a special gland for birds only.

Uhlenhuth and McGowan also failed to demonstrate a thyroid-like function for the ultimobranchial gland in their study on the salamander (80). Kingsbury (43, 44, 45) reported in his work on the mammalian embryo that "a study of its (ultimobranchial) morphology gave no suggestion of a specific function either past or present" and concluded that it was "unnecessary to ascribe any specific function to that curious pharyngeal derivative."
In 1933, Watzka published an extensive study on the morphology of the ultimobranchial glands of representatives of all vertebrate classes but concluded that "in man and animals it can hardly possess significant physiological value." He suggested that in non-mammals it has a "probable but unknown endocrine function" (86).

Robertson et al. (69) reported in 1964 that the ultimobranchial body in *Rana pipiens* is an active secretory gland which "responds to fluctuations in environmental conditions (including starvation)," as illustrated by its altered structure and varied types of secretion when stressed. They identified the secretion histochemically as a heterogeneous substance consisting of three components, alpha (acid mucopolysaccharide with a carbohydrate-protein complex), beta (mucroprotein) and gamma (sudanophilic) moieties. They noted that under normal environmental conditions, the alpha and beta components predominated whereas environmental stresses resulted in a predominance of the alpha component.

Rasquin and Rosenbloom came close to the current understanding of the function of the ultimobranchial gland. In their study of the Mexican cave fish, they felt that the ultimobranchial gland played some role in calcium metabolism but misinterpreted it as paralleling that of the parathyroid (67).
Thus prior to 1967 the ultimobranchial gland was considered a structure of little functional significance. It was important mainly to the histologists and embryologists who painstakingly studied its development and morphology but were unaware of its function.
Part III

Materials

Chicken glands (ultimobranchial, thyroid and parathyroid) were obtained from reject birds provided by Panco Poultry Limited, North Surrey, B.C. Fresh dogfish glands were obtained from Squalus suckleyi, a small Pacific coast shark. Live 3-week old chicks were supplied by the Poultry Science Division of the Department of Agriculture, University of British Columbia.

Methods

The glands were identified and removed from the chickens within two hours after slaughter. Their relative positions in the avian thorax are shown in Figure 12. These glands were immediately frozen solid in dry ice (CO\(_2\)). The dogfish glands were similarly treated. The dogfish ultimobranchial is found only on the left side, in the triangle formed by the coracobranchial muscle, the ceratobranchial and basibranchial cartilage. A ventral and transverse view of the gland and the associated structures are shown in Figures 13 and 14 respectively. Each chicken ultimobranchial gland weighed approximately 6-7 mg while that of the dogfish weighed in the range of 12 - 20 mg.
Owing to the high fat content in the chicken ultimobranchials, it was necessary to first extract them (3X) with ten volumes acetone before final extraction with 0.1 NHCl, using the method of Hirsch et al. (39). Since the dogfish glands were relatively low in fat content, they were directly extracted with the acid. The acid extracts were allowed to stand at room temperature for one hour before being spun down in a clinical centrifuge at approximately 3200 rpm to precipitate out cellular debris. The supernatant was stored at -20 degrees centigrade in the frozen state up to two weeks. No loss of activity was observed in extracts stored in the frozen state for several months. Thyroid and parathyroid glands from Gallus domestica and thyroid glands from Squalus suckleyi were treated in the same way.

Initially, 3-week old chickens were used to assay the activity of the chicken-gland extracts. Intravenous injections of the extracts were given followed by blood sampling at 0, +1/2 and +1 hour. As we were unable to demonstrate any hypocalcemic effect of the gland extracts in young chicks, we decided to use 7-week old rats, adopting the procedure of Copp and Kuczerpa (23). The frozen extracts were allowed to thaw completely before they were injected into the rats. The pH was adjusted to 4 by addition of 0.1 N NaOH. Concentrations or doses of the extracts were varied using 0.9% NaCl as vehicle. These doses were expressed in terms of fresh
weight of glands used to prepare the extracts. At least five animals were used for each test dose and five controls were included in each series of experiments.

Rats which had been fasted overnight were given i.p. injections of extracts of different amounts of ultimobranchial tissue extracts in a constant volume of 0.5 cc and at pH 4. Control animals received i.p. injections of vehicle only, also at pH 4. Blood samples (0.5 cc) were obtained from the tail at 0, +1, +3 and +6 hours. The response to the extracts was assayed by measuring the fall in the plasma calcium levels at these times using the EDTA titration method of Copp (21). The intensity and duration of the hypocalcemic effect was evaluated by the method of Copp and Kuczerpa (23) and is expressed as the area between the plasma calcium curve after injecting the extract and the plasma calcium level of the control.
Results

I. Response of 7-Week Old Rats to Chicken Ultrimobranchial Extracts

Using the bioassay method of Copp and Kuczerpa (23) we obtained pronounced drops in plasma calcium levels in 7-week old rats of the Long Evans strain as early as 1/2 hour after injection of the extracts. We used doses ranging from 0.1-5.0 mg of gland tissue. It was found that the larger the dose given the greater the fall in calcium level and the longer it took for the level to return to normal (Table II and Figure 15). It is apparent from the results presented in Table II that extracts of chicken thyroid and parathyroid had no significant hypocalcemic activity whereas ultimobranchial extracts were a rich source of the hypocalcemic factor.

The relationship between response and the logarithm of the dose (Fig. 16) is a straight line, the slope of which is parallel to that of the standard (beef-thyroid calcitonin), suggesting similar biological activity. From the curves obtained, the calcitonin activity was estimated to be 30-120 MRC mU/mg wet weight which is 10-30 X the activity of calcitonin in hog thyroids.
II. Response of 7-Week Old Rats to Dogfish Ultimobranchial Extracts

The response to dogfish ultimobranchial extracts is given in Table III and Figure 17. Again, the thyroid extracts showed no hypocalcemic activity whereas the ultimobranchial extracts were strongly hypocalcemic when assayed in 7-week old rats.

The log-dose response curve (Fig. 16) is also parallel to that of the beef-calcitonin standard. The activity was estimated at about 25-35 MRC μU/mg wet weight, which is approximately 10X more active than hog thyroid.

III. Response of 3-Week Old Chicks to Chicken Ultimobranchial Extracts

Our initial attempts to demonstrate hypocalcemic activity of extracts of checken ultimobranchial glands using 3-week old chicks failed (Table IV). The extracts given to each chick were obtained from two ultimobranchial glands having an activity of approximately 900 MRC μU. Urist (81) also was unable to obtain any hypocalcemic effect when porcine calcitonin was injected into chickens.
We did not parathyroidectomize the chicks before the experiment. Kraintz and Intscher (47) later showed that partially parathyroidectomized chickens responded to avian ultimobranchial gland extracts at dose levels of 300 MRC mU/kg weight with a marked fall in plasma calcium within an hour following injection.
Discussion

It is clear from our study that the ultimobranchial gland of *Gallus domesticus* and *Squalus suckleyi* is an important source of calcitonin. It is also clear that calcitonin is localized in the ultimobranchial gland of these two lower vertebrates as no calcitonin-like activity could be demonstrated in the extracts of thyroid and parathyroid. Since the mammalian thyroid "C" cells (parafollicular cells) are believed to have arisen embryologically from ultimobranchial tissue (3,4,34,45) and since cytochemical evidence supports this belief (62), mammalian calcitonin is in fact an ultimobranchial hormone. Therefore, it is more appropriate when speaking of the mammalian hormone to use the original name calcitonin, rather than thyrocalcitonin since the hormone in addition has also been found in parathyroid and thymus of man (32).

We were unable to demonstrate hypocalcemic activity when chicken ultimobranchial extracts were injected into intact 3-week old chicks. Since Kraintz and Intscher (47) obtained marked drops in plasma calcium levels in partially parathyroidectomized cockerels in response to avian ultimobranchial gland extracts, it is possible that in the intact
chicken the rapid action of the parathyroids masks the hypocalcemic effect of any exogenously introduced calcitonin. Walker et al. (85) showed that the removal of the ultimobranchial gland in the young turkey impaired the response of the animal to calcium infusion. Brown et al. (6) demonstrated that ultimobranchiectomized chicken showed an abnormal response to the hypercalcemic effect of parathyroid extracts when compared with sham-operated controls. These observations strongly suggest that calcitonin is essential in controlling hypercalcemia in the chicken.

The response of the ultimobranchial gland to calcium stresses merits special consideration. Urist (81) observed that the ultimobranchial glands of laying hens were hyperplastic. This could be attributed to the response of the glands to the mobilization of large amounts of calcium during the egg-laying cycle. Robertson reported increased mitotic activity and depth of glandular epithelium in ultimobranchial glands of frogs which were given injections of vitamin D₂ (72). These observations indicate a relationship in which prolonged hypercalcemia results in hyperplasia of the glands whereby exhausted cells are replaced and the absolute number of secretory cells are increased. In addition, Gittes et al. (33) reported a reduction (35±5 %) in
calcitonin contents in the thyroids of rats subjected to prolonged systemic hypercalcemia induced by methods including gavage feeding of calcium lactate and a very high calcium diet. They also reported increased calcitonin contents of thyroid up to 12X the normal in hypocalcemic parathyroidectomized rats. These observations find a possible explanation in the electron micrographic studies of Chan, Cipera and Belanger (14). The ultimobranchial cell from the chick placed on a high-calcium diet showed fewer secretory granules than that of the chick on the low-calcium regimen. The former, however, was noted to have prominent Golgi apparatus and a very well developed rough-surfaced endoplasmic reticulum suggesting that it is actively synthesizing hormone (73). These observations suggest that the ultimobranchial gland responds to hypercalcemia by a constant discharge of secretory granules (i.e. calcitonin). Synthesis apparently continues in the absence of the hypercalcemic stimulus resulting in an accumulation of granules in the normal cell.

Our study indicates that calcitonin in Gallus domesticus and Squalus suckleyi is essentially an ultimobranchial hormone. The observations of Walker et al. (85) and Brown et al. (6) suggest that the ultimobranchial gland is an important endocrinological participant in calcium homeostasis in the chicken.
Conclusion

Acid extracts of ultimobranchial glands of Gallus domesticus and Squalus suckleyi contain potent hypocalcemic activity when assayed in 7-week old rats. As we were unable to demonstrate any significant hypocalcemic activity in extracts of thyroid and parathyroid glands from these animals, we conclude that in these animals, calcitonin is localized in the ultimobranchial gland.
Fig. 1. Effect of thyroparathyroidectomy after a prolonged period of hypocalcemia produced by intravenous infusion of EDTA (From Copp, D.H., unpublished data).
Fig. 2. Effect of thyroparathyroidectomy following continuous infusion of parathyroid extract (1 U/kg/hr) (After Copp, D.H., Oral Surgery, Oral Medicine, Oral Pathology, 16:972, 1963).
Fig. 3. Effect of successive perfusions of the thyroid and parathyroid glands of a dog with blood high and low in calcium. Thyro-parathyroidectomy was carried out at the time indicated in the figure (After Copp et al., 1961).
Fig. 4. The historic development of the calcitonin concept.
Fig. 5. Chemical structure of salmon, human and hog calcitonins.
Fig. 6. Branchial derivatives of major classes of vertebrates. All except the cyclostome have at least one ultimobranchial gland as represented by the last outpouching of the pharynx.
Fig. 7. Epon-embedded section of the ultimobranchial gland of 4-week old chick showing the distribution of light (L) and dark (D) cells and capillaries. Toluidine blue stain X590 (After Chan, Cipera, and Belanger [14]).
Fig. 8. Showing the characteristics of the light (L) and dark (D) cells of the 4-week old chick ultimobranchial gland. Uranyl and lead X8500 (After Chan, Cipera, and Belanger [14]).
Fig. 9. Light micrograph of an epon-embedded ultimobranchial gland from the elasmobranch *Squalus suckleyi* showing the follicular organization. Toluidine blue stain. Prepared by W.A. Webber (Department of Anatomy, U.B.C.).
Fig. 10. Parts of two light cells to show the distribution of secretory granules (sg). Uranyl and lead X9000 (After Chan, Cipera, and Belanger [14]).

Fig. 11. Portion of a light cell to show the well developed, rough surfaced endoplasmic reticulum (Rer), the Golgi (G), mitochondria (M) and free ribosomes (r). Uranyl and lead X1700 (After Chan, Cipera, and Belanger [14]).
Fig. 12. The relative positions of the endocrine glands in the avian thorax.
Fig. 13. Ventral view of a wax reconstruction of the caudal half of the pharynx of a shark embryo 95 mm. long x 12. L.s.b-R.s.b., left and right suprapericardial bodies (ultimobranchial glands); Th., thymus; IV to VI, fourth to sixth gill-pouches (From Camp [8]).
Fig. 14. Transverse section through the pharynx of a 95 mm. shark embryo at the level of the ultimobranchial gland. X24. L.sb., left suprapericardial body (ultimobranchial gland); M.cb., coracobranchial muscle; C.c., ceratobranchial cartilage; Cb.c., basibranchial cartilage (From Camp [8]).
Fig. 15. Dose-response curves--The hypocalcemic activity of chicken ultimobranchial gland extracts in 7-week-old rats.
Fig. 16. Showing the log-dose response curves of chicken and dog fish ultimobranchial gland extracts as parallel to hog calcitonin and the beef standard. The abscissa represents the wet weight of the glands extracted.
Dose Response to Dogfish Ultimobranch Extracts

(7-week old Rats)

Fig. 17. Hypocalcemic effect of dogfish ultimobranchial extract in 7-week-old rats at the specified doses in mg wet weight.
Table I. Amino Acid Composition of Salmon, Human and Hog Calcitonin.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Salmon (59)</th>
<th>Human (55)</th>
<th>Hog (65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Arginine</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Asparagine</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Cysteine</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Histidine</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Leucine</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lysine</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Glycine</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Methionine</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Proline</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Serine</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Threonine</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Valine</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>32</strong></td>
<td><strong>32</strong></td>
<td><strong>32</strong></td>
</tr>
</tbody>
</table>

| Molecular Weight | 3427 | 3419 | 3604 |
| Biological Activity | 5000 | 102  | 200  |

(MRC U/mg) as reported by the authors.
Table II. Plasma Calcium Changes in 7-Week Old Rats Following Injections of Extracts of Chicken Glands.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Rats</th>
<th>Dose (mg, wet wt.)</th>
<th>Calcium Levels (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0-hour</td>
</tr>
<tr>
<td>*Vehicle</td>
<td>25</td>
<td>--</td>
<td>9.56±0.11</td>
</tr>
<tr>
<td>**Parathyroid</td>
<td>6</td>
<td>5</td>
<td>9.41±0.19</td>
</tr>
<tr>
<td>***Thyroid</td>
<td>4</td>
<td>500</td>
<td>9.20±0.25</td>
</tr>
<tr>
<td>****Ultimobranchial</td>
<td>7</td>
<td>0.1</td>
<td>9.49±0.13</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.25</td>
<td>10.02±0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.5</td>
<td>9.52±0.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.0</td>
<td>9.41±0.22</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.5</td>
<td>9.27±0.24</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.0</td>
<td>9.21±0.18</td>
</tr>
</tbody>
</table>

Significance of Calcium Changes in one hour Following Injections of Gland Extracts

* Insignificant p >> 0.1  ** Insignificant p >> 0.1  *** Insignificant p >> 0.1

**** At 0.1 mg, significant p = 0.001; at 0.25 mg, significant p << 0.001;
at 0.5 mg, significant p << 0.001; at 1.0 mg, significant p << 0.001;
at 2.5 mg significant p << 0.001; at 5.0 mg, significant p < 0.001.
### Table III. Plasma Calcium Changes in 7-Week Old Rats Injected with Extracts of Dogfish Glands.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Rats</th>
<th>Dose (mg wet wt.)</th>
<th>Calcium Levels (mg %)</th>
<th>0-hour</th>
<th>1-hour</th>
<th>3-hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Thyroid</td>
<td>6</td>
<td>200</td>
<td>9.98±0.13</td>
<td>10.18±0.11</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td><strong>Ultimobranchial</strong></td>
<td>3</td>
<td>2</td>
<td>10.84±0.26</td>
<td>9.24±0.56</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>10.30±0.22</td>
<td>7.59±0.26</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7.5</td>
<td>10.38±0.21</td>
<td>7.48±0.22</td>
<td>7.96±0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>15</td>
<td>10.49±0.25</td>
<td>7.69±0.11</td>
<td>7.33±0.43</td>
<td></td>
</tr>
</tbody>
</table>

Significance of Calcium Changes in One Hour Following Injections of Gland Extracts

* Insignificant p > 0.1

** At 2 mg, significant p = 0.05

At 4 mg, significant p << 0.001

At 7.5 mg, significant p << 0.001

At 15 mg, significant p << 0.001
Table IV. Lack of Response in Intact 3-Week Old Chicks to Chicken Ultimobranchial Gland Extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Chicks</th>
<th>Calcium Levels in mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-hour</td>
<td>1-hour</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3</td>
<td>10.65</td>
</tr>
<tr>
<td>Thyroid</td>
<td>6</td>
<td>10.21</td>
</tr>
<tr>
<td>Parathyroid</td>
<td>5</td>
<td>9.76</td>
</tr>
<tr>
<td>Ultimobranchial*</td>
<td>4</td>
<td>10.45±.24</td>
</tr>
</tbody>
</table>

* t = 0.78, degrees of freedom = 6, p >> 0.1, insignificant.


84. Verdun, P. Derives branchiaux chez le vertebres superieurs, These, Toulouse, 1898.

