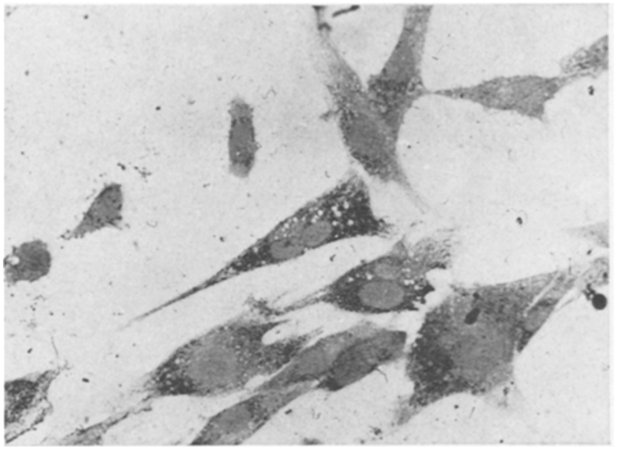

Fig. 1. Fibroblast-like cell cultures derived from α-1-4-glucosidase deficient muscle.

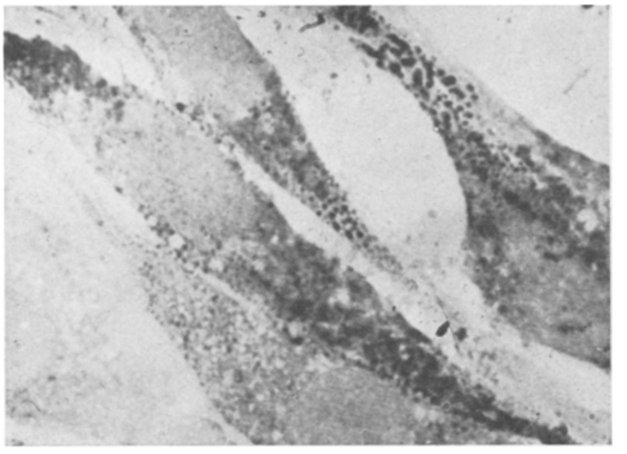

Fig. 2. High magnification: the cytoplasm is filled with PAS positive diastase digestible granules.

to muscle cultures, could be related to the different features of glycogen metabolism of these cells.

It is possible, because the glycogen synthesis occurs in the cell cultures[9], that it comes about slowly in skin cultures and very rapidly in muscle cultures. In the former, but not in the latter, glycogenolysis could take place completely by an alternate pathway to the α-1-4-glucosidase degradation. However, our observation could also mean that the α-1-4-glucosidase defect is not the only cause of glycogen deposition. This would be in agreement with the presumption that there is another defect apart from the α-1-4-glucosidase one. On the whole, in vitro culture results also show the difficulty of simplifying the pathogenesis of glycogenosis type II to a lysosomal enzymatic defect.

These results demonstrate that the disease's features can be reproduced in vitro. Further studies of tissue cultures might produce a better understanding of glycogenosis type II.

Riassunto. È stata eseguita la coltura in vitro di frammenti di muscolo e di cute di un soggetto affetto da glicogenosi di tipo II°. È stato osservato un accumulo di materiale PAS positivo, digeribile con la diastasi, nelle cellule derivate dal muscolo ma non in quelle derivate dalla cute.

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Partial Nervous Control of the Avian Ultimobranchial Body

There is now strong evidence that the mammalian thyroid parafollicular, or C, cells are the cells of origin of the hypocalcaemic hormone calcitonin[1,2]. Embryological[3] and cytochemical[4] studies have shown that the C cells are derived from the ultimobranchial bodies.

In birds there are no thyroid C cells but the ultimobranchial bodies persist as distinct structures into adult life. Recently COPP et al.[5] have shown that they contain high levels of calcitonin activity.

Unlike the mammalian thyroid C cells the ultimobranchial body of the fowl is well innervated. The vagus nerve lies adjacent to its dorso-lateral border and the recurrent nerve runs close to its medial boundary. Nerve fibres supplying the ultimobranchial come mainly from the vagus and to a lesser extent from the recurrent nerve and the sympathetic system[6]. NONIDEZ[7] described a single branch, often initially in association with the accessory depressor nerve, which leaves the vagal ganglion nodosum to supply the ultimobranchial body. The situation is, however, more complex than this. An interanastomosing group of several fine nerve bundles leaves the ganglion nodosum to supply both the carotid body and the ultimobranchial body, and the 2 bodies may also be interconnected by nerves. Similarly, there may be more than one nerve bundle passing from the recurrent nerve into the ultimobranchial[6,8].

Within the ultimobranchial body the nerves branch and pass out into the tissues but, before doing so, some of the fasciculi, particularly from the vagus, may become associated with small groups of ganglion cells located superficially within the gland[6,9].

The structure and innervation of the ultimobranchial gland of the laying hen has been studied with light microscope, silver impregnation techniques and by

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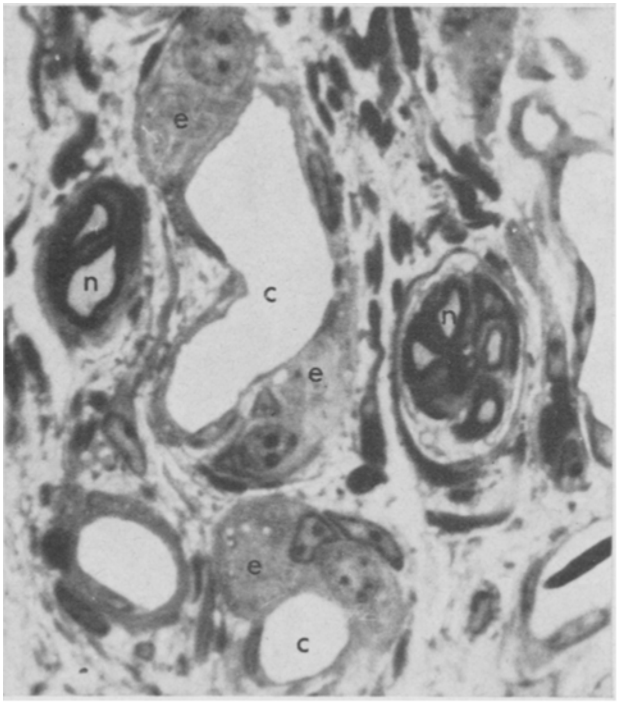

Fig. 1. Light micrograph of ultimobranchial epithelioid cells. c, capillary; e, epithelioid cell; n, myelinated nerve fibre. × 2000.

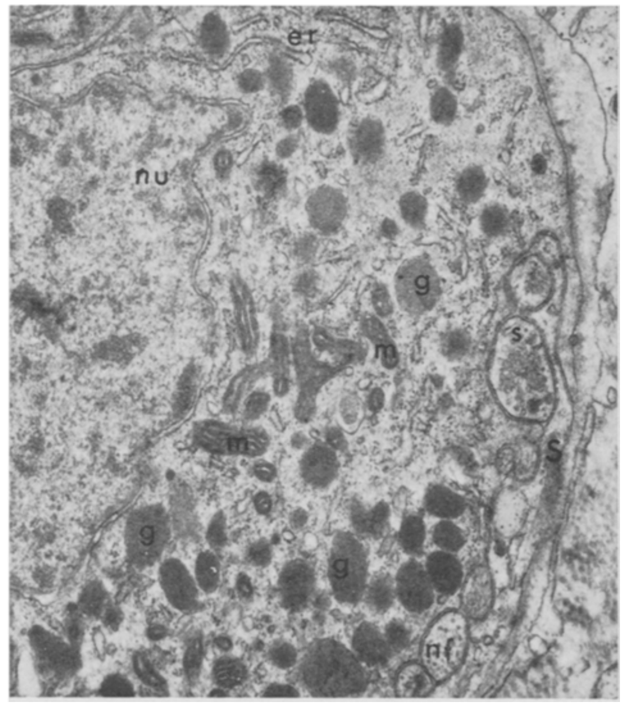

Fig. 2. Electron micrograph of part of an ultimobranchial epithelioid cell. er, endoplasmic reticulum; g, dense cytoplasmic granule; m, mitochondrion; nf, unmyelinated nerve fibre; nu, nucleus; s, synaptic terminal containing many agranular synaptic vesicles and one small dense-cored vesicle; S, Schwann cell process. × 18,000.

electron microscopy and, in particular, close attention has been paid to the left gland. In this gland the ganglion cells are usually found just within its anterior end and nerve bundles pass posteriorly from them into the main mass of glandular tissue. The ultimobranchial epithelioid cells are scattered throughout the gland in strands and groups[10] (Figure 1), and after silver impregnation can be seen to contain numerous argentophilic granules. Certain groups of these cells, superficially indistinguishable from the remainder, appear to be innervated. These groups occur anywhere within the gland but are most frequently seen in an anterior position close to the ganglion cells. Nerve fibres leave the main nerve tracts within the gland and eventually come into close relationship with these groups of cells. Very fine, apparently unmyelinated, fibres can be seen passing around and between the cells. No direct light microscope evidence of nerve terminations on the cells has yet been found, although the unmyelinated fibres are clearly intimately related with the cells. These innervated cells comprise not more than 10% of the epithelioid cell population.

The fine structure of fowl ultimobranchial epithelioid cells is very similar to that of the mammalian thyroid C cells. The cells are ovoid to elongate in shape (Figure 1) and are characterized by the presence of numerous dense membrane-bound cytoplasmic granules (Figure 2). The nucleus tends to be located towards one end of the cell and usually contains 1 or 2 nucleoli. Rod-like mitochondria have longitudinally arranged cristae. The rough endoplasmic reticulum consists of solitary, or occasionally stacked, elongated profiles. Free ribosomes or polysomes are found scattered throughout the matrix. The Golgi complex consists of 4 or 5 smooth-membraned cisternae and a varying number of associated smooth-membraned vesicles. The characteristic dense granules are found throughout the cytoplasm and reach a maximum size of about 1.0 μ. Other organelles seen in the cells are lysosomal dense bodies, multivesicular bodies and tonofilaments.

The fine structure of the innervated epithelioid cells from the anterior end of the gland is very similar to that of the epithelioid cells just described, but they differ in that unmyelinated nerve fibres enter into intimate contact with the cells and end synaptically on them (Figure 2). Further, much of the epithelioid cell surface is ensheathed by Schwann cell processes, especially where nerve fibres are found in close association with it. The synaptic terminals contain mainly non-granulated synaptic vesicles and occasionally small dense-cored vesicles (Figure 2).

This anatomical evidence may be interpreted in 2 ways. Firstly, that the innervated epithelioid cells are aberrant paraganglionic cells, resembling those of the nearby carotid body[9,11,12]. Or, secondly, that they are a special form of the ultimobranchial epithelioid cells, possibly concerned in calcitonin secretion, and in part under central nervous control via the vagus.

To test the latter hypothesis, young, anaesthetized cockerels with 1 wing vein cannulated had their left vagi exposed. A total of eight 1 ml blood samples were taken at 15 or 20 min intervals. Between the second and third samples the vagus was stimulated electrically. Control birds were not stimulated. It can be seen from Figure 3 that stimulation was followed by a fall in total plasma

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calcium which was significantly greater ($P < 0.01$, t-test) than in the control samples at 7 min after stimulation, and also at 20 min after stimulation ($P < 0.05$). Recovery to non-significant levels occurred within 35 min. A second significant fall ($P < 0.05$) occurred between points 6 and 7. This fall in plasma calcium in response to vagal stimulation was prevented by atropinization and could also be initiated by injections of the parasympathomimetic drug Carbachol. Section of the left vagus gave no response, indicating that any nervous control is stimulatory and not inhibitory as has been found in the frog ultimobranchial[13].

Although it has been demonstrated anatomically that there is a direct connection between the vagus and the innervated, densely-granulated, epithelioid cells within the ultimobranchial body, there is as yet no direct evidence that the drop in plasma calcium following vagal stimulation is caused by release of calcitonin from the innervated cells. Such evidence is at present being sought.

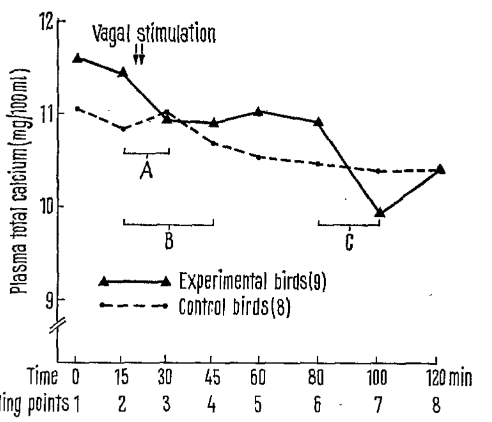

Fig. 3. Effects of vagal stimulation (arrows) upon plasma total calcium in young, anaesthetized cockerels compared with unstimulated, control animals. (A) The difference between points 2 and 3 in the experimental birds was significantly different ($P < 0.01$, t-test) to that in the controls. (B) and (C) The differences between points 2 and 4 and 6 and 7 in the experimental birds was significantly different ($P < 0.05$) to that in the controls.

The comparatively small and transient, though significant, decrease in plasma calcium is of interest when considered in the light of other results. The failure by several workers[14–16] to demonstrate a fall in plasma calcium in intact chickens of various ages after the injection of either allogeneic or xenogeneic calcitonin has been ascribed to the probable ability of the fowl parathyroids to respond rapidly to a slight hypocalcaemia[14,16]. Such a hypothesis would help to account for the transience of the response found here.

A neural mechanism of this nature, partially controlling calcitonin secretion, could conceivably have considerable significance in birds where there is a particularly active calcium metabolism associated with egg-shell formation.

Résumé. Le corps ultimobranchial de la poule a une forte innervation, provenant en particulier du nerf vague. On a constaté que des fibres nerveuses se terminent sur quelques groupes de cellules C. La stimulation du vague produit une chute significative du taux de calcium du plasma, ce qui démontre les propriétés d'un effet provoqué par voie parasympathique. On propose l'hypothèse que l'hypocalcémie peut être causée par une décharge de calcitonine venant des cellules C innervées.

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Narrowings of the Synaptic Cleft in Myoneural Junctions

In the course of electron microscopic studies[1] on myoneural junctions in several species, narrowings of the synaptic cleft were observed in motor nerve terminals of the striated intraocular muscle fibres of the chicken (Figure 1) and in extraocular muscle fibres of the rat (Figure 2). In these narrowings, the 5-layered synaptic membrane complex of the myoneural junction is reduced to 3 layers; the plasma membranes of the nerve terminal and the muscle fibre, which are usually separated by a 500 Å gap containing a basement membrane layer, approach as close as 160 Å without interposition of basement membrane material (Figure 3).

According to previous investigations[2], the sphincter pupillae muscle of the chicken, in which narrowings of the synaptic cleft were found several times, consists only of muscle fibres with one motor endplate. These endplates do not have junctional folds. The nerve terminals contain numerous synaptic vesicles both near the normal 5-layered synaptic membrane complex and near the 3-layered narrowings of the synaptic cleft (Figure 1).

The motor ending shown in Figure 2 was found in a multiply innervated muscle fibre[3] of the orbital region in a lateral rectus bulbi muscle of the rat. The motor

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[3] On the basis of a detailed study of muscle fibre types and their innervation in rat extraocular muscles (R. Mayr, in preparation) we are now able to discern multiply innervated fibres in these muscles using fine structural criteria.