

The phylogenesis and ontogenesis of the human pharyngeal region focused on the thymus, parathyroid, and thyroid glands

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Abstract

The pharyngeal (branchial) region represents a classic example where the relationship between ontogenesis and phylogenesis has been demonstrated. It is a region where the development of gills during ontogenesis of all chordates has been recapitulated. In the process of evolution the pharyngeal region has undergone marked changes. While it functioned to ensure blood oxygenation and regulation of a constant internal environment in aquatic animals, it had to adapt to new and more complex functions in terrestrial vertebrates. The lungs have taken on the main role of blood oxygenation and the salivary glands now regulate ionic balance. The immune organs in mammals such as the thymus and the palatine tonsil, endocrine organs such as the parathyroid glands and the parafollicular cells of the thyroid gland, which produces calcitonin (originally as independent ultimobranchial bodies), as well as a part of the ear developed from the pharyngeal region. This article briefly summarizes the current knowledge regarding the phylogenesis and development of the human thymus, parathyroids, and the thyroid gland with a focus on the influence of neural crest cells during development.

PHYLOGENESIS OF PHARYNGEAL REGION

A conserved feature in all vertebrate embryos is the presence of a series of bulges on the lateral surface of the head, i.e. the pharyngeal arches (Veitch *et al.* 1999; Graham *et al.* 2005). The pharyngeal (branchial) region is a classic example where the relationship between ontogenesis and phylogenesis has been repeatedly demonstrated. The branchial region was in direct contact with the external environment and therefore it had to react to environmental changes during the transition of vertebrates from an aquatic environment to a terrestrial environment.

With changes in the external environment, the function of this region also progressively changed, from branchial to the gastropulmonary system, supplemented with a system of branchial derivatives. As the respiratory and osmoregulatory functions of the branchial region were lost, the branchial arches, clefts and pouches were gradually reduced. Contact with oxygen was useful not only for tissue oxygenation but also for protection of the organism (cytotoxic function of leukocytes and macrophages). Secondary, more complicated structures arose from the gills which performed homeostatic and protective functions in the organism. Thus the pharyngeal pouches gradually started to change into immune and endocrine organs and their epithelium became the source for the thymus, which develops early in association with all pharyngeal pouches. The thymus and other new structures in branchial region appeared in connection with development of the thyroid gland. During evolution the number of thymus primordias became reduced and from the same primordia the parathyroid glands and ultimobranchial bodies developed (Slípka, 1986).

Phylogenetic development of vertebrates went through the stage of simple aquatic animals (having the common name *Protochordata*), which filtered water through the an enlarged pharynx with numerous slits (pharyngeal basket). Later in evolution, the jaw developed with the associated function of food intake. The filtering "basket" of the primitive pharynx was transformed into a series of mobile arches (gills) in aquatic animals. An external, well vascularized, pharyngeal surface took over respiratory functions. The inner endodermal epithelium of the gills also had to adapt to this new function, i.e. regulation of the internal environment. In addition to the osmoregulatory function, the endodermal epithelium also has homeostatic and excretory functions. Primitive excretory organs in the subphylum *Cephalochordata* – segmentally arranged nephridias – opened into the branchial slits. Formations resembling gills appeared early in the development of all vertebrates. However, further development of the pharyngeal region in fish species is distinct from terrestrial species. During the process of vertebrate evolution, the lungs developed and adapted to undertake respiratory

functions while the salivary glands developed to handle ionic regulation. Endocrine structures, specifically the parathyroid glands and parafollicular cells of the thyroid gland (originating from ultimobranchial body) formed from the endoderm of the pharyngeal pouches; as did the central immune organ, the thymus. From the second pouch, and from the same epithelium, the youngest organ, from an evolutionary perspective, arose which had immune functions, i.e. the palatine tonsil. The tonsil's epithelial part (surface and deep crypts) originates in the reticulation of epithelium which we can compare with the stroma of thymus. The first pouch, which is an exception, persists and from which the tympanic cavity and pharyngotympanic tube develop. The visceral skeleton and the head and neck muscles are evolutionary modifications of the mesenchyme of the pharyngeal arches. The pharyngeal region has had different functions during vertebrate evolution:

- intimate contact between the organism and the external environment
- primary nutrition and filtration functions, regulation of the internal aqueous and ionic environment
- maintenance of physiological balance
- exchange of respiratory gases between blood and the external aqueous environment through well vascularized gills – respiratory function
- lymphoepithelial function
- exocrine and endocrine function
- immune function

STRUCTURE OF PHARYNGEAL REGION DURING EARLY ONTOGENESIS OF VERTEBRATES

The branchial region consists of mesenchymal branchial arches that are separated from each other externally by ectodermally lined branchial clefts and internally by endodermally lined pharyngeal pouches. During ontogenesis, specific adult structures are derived from each branchial arch and its related cleft and pouch (Benson *et al.* 1992). The question regarding the development of branchial (pharyngeal) organs in vertebrates belongs to the most complicated organogenetic problems. A number of studies have tried to answer the question regarding the origin of the individual formations of the oral, pharyngeal and laryngeal cavity from the various parts of the original visceral apparatus. However, the results of these studies are markedly different. The fate of the oral part of the visceral apparatus causes no major problems. The first three visceral arches, i.e. maxillo-mandibular, hyoid arch, and the first branchial arch retain their original structure during development. However, the study of the development of the more distal parts of the visceral apparatus is quite challenging. Not even the basic question, regarding the number of arches an

visceral apparatus in *Amniota*, has been definitively resolved. Numbering of arches was bedeviled by the finding that in some animals the fourth pharyngeal slit protrudes into a shallow pouch, terminating in a small caruncula which could be considered as an indication of a fifth visceral arch. Termination of this vessel also corresponds with the termination of the branchial arch, which is only a phylogenetic mark, but without ontogenetic significance. The real existence of the fifth arch in birds was demonstrated by finding the appropriate associated vessel, i.e. the fifth aortal arch and by finding innervations from the *ramus postrematicus nervi vagi* (Slípka, 1956).

During the early ontogenesis of vertebrates, not only do the locations of the branchial arches change, but so do the ectodermic clefts and pharyngeal pouches. The first three visceral arches maintain their original structure during ontogenesis. The skeleton, muscular, nervous, and vascular equipment of these arches can be observed in all vertebrates, humans included. The more caudally located arches, slits, and pouches have changed their location, structure and relationship to one another. Slípka (1979) studied evolutionary changes in the branchial region during development of amphibians, as animals transitioned from an aqueous to a terrestrial environment. He found proliferation of the epithelium in all slits except the fourth (in subphylum *Anura*, primordia of the thymus, in the second as well as in the first slit, have been described) even though the actual thymus is based in the third slit which was confirmed by the course of cranial nerves IX and X as they passed near the thymus. The thymus remains located near the otic capsule and is closely adjoined to the lymphatic sinus. The epithelium lining the pharyngeal slits and covering the pharyngeal lamellas forms plates, from tall cells with cilia, in the epibranchial parts. In folds (sinuses) of the pharyngeal lamellas, particularly in the fourth slit, the low epithelium changes to clusters of giant cells containing pale cytoplasm and which are responsible for salts excretion.

In *Amniota* (e.g. class *Reptilia*) the participation of the branchial slits during thymus development is reduced to the dorsal parts of two slits. In mammals, the epithelium of the ventral part of the 3rd and 4th pouch participate in the development of the thymus; in humans only the bottom of the 3rd pouch and primordia of parathyroid gland cells move dorsally. The thymus, thyroid gland, and parathyroid glands descend with the heart, during which the thymus loses its tubular shape and changes into a solid trabecula. The question, "why the thymus was transferred from the dorsal to the ventral part in mammals" still remains poorly elucidated.

The basic question of how many arches the branchial region, in *Amniota*, actually consisted of was not resolved for a long time. The presence of the fifth, rudimentary arch in birds was clarified by finding the appropriate vessel (the fifth aortic arch) and its innervations. The loss of this vessel also meant the loss of

the arch and the fifth pharyngeal pouch becomes associated with the development of the ultimobranchial body. Slípka (1956) studied the early ontogenesis of the pharyngeal region in six bird species. He proposed that the diverticulum of the fourth pharyngeal pouch was a rudimentary fifth pouch. Their common branchiopharyngeal orifice is quite wide, wider than the orifice of the other pouches. The fifth pouch is lined with the same endodermal epithelium as the others and the comment that unlike other pouches its course is parallel with *pharynx* (the others are perpendicular to the *pharynx*) is insufficient. At first, the course of the fifth pouch is parallel with the fourth and only after a shift of the *aditus laryngis*, in the oral direction, does it become perpendicular to the fourth pouch. Afterwards, the fifth pouch together with the fourth closes the independent, but quite rudimentary, 5th visceral arch with its own vessel supply from the fifth aortic arch during ontogenesis. The loss of this vessel is also associated with the disappearance of the arch which is actually only a phylogenetic residual, without any ontogenetic significance (Slípka, 1956).

During evolution, immune organs were first located in close contact with the rostral part of the digestive tube exposed to exogenous antigens. Under their influence in invertebrates (e.g. phylum *Annelida*) and lower vertebrates (class *Cyclostomata*) an organ of protection *typhlosolis* (spiral fold which enlarge the gut surface) was formed. It can be considered as a homologous organ of the present spleen and hematopoietically similar to bone marrow. With the evolution of the gastrointestinal organs and excretory nephridia, in representatives of the class *Cyclostomata* and the subphylum *Cephalochordata*, a connection with the circulatory system was formed. Nephridia were originally excretory organs for osmotic regulation, later they served for elimination of antigens. During the following stages of the evolution of the excretory system, pro- and mesonephros were also hemopoietic organs. In *Gnathostomata*, the hemopoietic function was transferred to the mesentery where the liver developed. Hepatolienal hematopoiesis became the main source of all blood elements; multipotent stem cells also differentiated into lymphocytes. Excretory antigen elimination was shifted in a cranio-caudal direction. Nephridia of *Amphioxus* still opened into the branchial slits, but in all real vertebrates the pronephros stayed situated behind the last pharyngeal arch, caudal to the pharyngeal region. The pharyngeal slits now became free for other functions. They gradually begin to take part in the immune processes and their epithelium became the basis for the central immune organ, the thymus. In the class *Cyclostomata*, there is no real thymus; primordia of this organ might depend on the appearance of the thyroid gland.

The thymus first appears in sharks, in the dorsal ectodermal part of all slits. In the shark, *Notorynchus cepedianus* (syn. *Heptanchus*), a connection between the thymus and the branchial slits can be seen. Due to

this epithelial duct, the organ gains an excretory character which resulted probably from modifications of the "branchionephros" of *Amphioxus*. The rest of this duct is also observed in the ontogenesis of the human thymus (Slípka, 1986).

In representatives of the class *Osteichthyes*, the reduction of the thymus primordia begins in the proximal branchial slits, while in the terminal slits the lymphopoietic tissue develops together with the pronephros. In representatives of the class *Amphibia*, the hematopoietic mesenchyme has shifted into a more protected place, i.e. to the bone marrow. After reduction of human thymic primordia, development of the parathyroid glands begins, hormones of which also represent growth stimulators of lymphatic tissue, especially in the thymus (Shields, 1976). In *Amniota*, i.e. vertebrates, which left the aqueous environment, the branchial region develops only during early ontogenesis. In the gut, the system of lymphatic follicles develops with fragmentation of lymphatic pouches, and the lymphatic nodes are formed. In *Sauropsida*, a separate primary lymphatic organ, the *bursa Fabricii*, developed in the cloaca. The thymic primordia gradually reduce to the 3rd pouch in humans and regulation of ions shifts to *gll. parathyroideae* which originates from the 3rd and 4th pouch; even though the thymus does not develop in the second pouch in humans. Slípka and Pospíšilová (1995) found a proliferation of epithelium in the early ontogenetic stage that is homologous with thymic primordia in the 2nd pouch in *Sauropsida*, and in the 3rd pouch in humans. In the second month of human gestation thymic primordia in the second pouch are lost and the *sinus tonsillar* is infiltrated with lymphocytes during the 3rd month. Ontogenetically the palatine tonsil develops as the last derivate of the branchial region after other organs have already started their histogenesis. It corresponds to the late appearance of tonsils in phylogenesis: the palatine tonsil resembles the thymus in its origin in the branchial region (especially by reticulation of the covering epithelium). In regard to the lymphoreticular structure, it resembles the palatine tonsil, lymphatic nodes, and, by its involution, it resembles the thymus as well. *Tonsilla palatina* is a typical mammalian organ and phylogenetically is the youngest organ of the immune system; it developed from the same region as the thymus in lower vertebrates. It actually integrates both structures, i.e. the thymus and lymph nodes (Slípka, 1986).

The study of developmental changes in the pharyngeal region and the pharyngeal organs is extremely difficult. The reason is the complexity of the formations, considerable spatial changes and insufficient methodological possibilities during the study of derivatives of the pharyngeal arches. Not only have the location of the pharyngeal arches changed but so have the ectodermal clefts and pharyngeal pouches (Graham, 2003).

The changes in the structure of the vertebrate pharyngeal region from a phylogenetic point of view can be summarized as follows:

- number of branchial arches have been reduced from 7 to 5
- the thymus from an evolutionary point of view is the oldest organ of the immune system and originally developed in each branchial slit
- during evolution the thymus appears together with the *glandula thyroidea*
- the number of thymic primordia has been reduced
- the thymus has changed from an excretory organ to the central organ of the immune system
- from the pharyngeal endoderm other endocrine glands have been formed (*glandulae parathyroideae*, *glandula thyroidea*, and the parafollicular cells of the thyroid gland),
- phylogenetically, the youngest organ of the immune system is the *tonsilla palatina*.

THYMUS EVOLUTION

Phylogenetically, the development of thymus proceeded simultaneously with the development of the thyroid gland. The thymus played a key role in the evolution of animals during development of an adaptive immune system; therefore it is an important element which separates higher vertebrates from other animals (Bowden *et al.* 2005). The development of the thymus and T-cells is a highly conserved process in vertebrate evolution. Mammals, birds, reptiles and fish have many common molecular signaling pathways regulating the development of adaptive immunity. Recently, the most frequently used animal model in the study of the development of the thymus and genetic mutations affecting immunobiology of T-cells is the aquaria fish *Danio rerio* (Langenau & Zon, 2005).

Studies by Pospíšilová and Slípka (1994) of thymic primordias in the evolution of vertebrates shows that thymic primordia were shifted within the pharyngeal region. It can be generalized that in phylogenetically lower vertebrates, several pharyngeal pouches/slits participated in thymic primordia; the number of which was reduced during evolution, so in vertebrates it is limited to the 3rd and 4th pouch, and in human to only the 3rd pouch. According to the studies of the aforementioned authors, thymic primordia have also been found in the 2nd and 4th pouch in early human development, but do not develop further. This observation has supported the existence of an evolutionarily encoded "thymus potency" of all pharyngeal pouches and clefts. Thymic development starts with proliferation of the endodermal epithelium and progress in a caudal direction. Against these proliferations, cleft ectoderm associated with the *sinus cervicalis*, becomes deeper. In the 3rd cleft, epibranchial placodes in the form of finger ductules develop, which are located parallel to the pouch and forms the basis of the ectoderm-endodermal complex.

This close connection with the 3rd pouch and the 3rd cleft is necessary for thymic development. The loss of the ectoderm/endodermal interaction in the 2nd cleft/pouch and development of an operculum from the 2nd arch can lead to lost development of a complete thymus, and the same is true for the 4th cleft. The studies of the aforementioned authors dealing with ontogenetic processes of the branchial region in almost 100 human embryos, from the 2nd lunar month of development, confirmed that the ability to form thymic primordia is equal in all pouches, except the first, and that this potency is encoded during evolution.

Slípka and Pospíšilová (1995) found quite advanced development of "thymus secundus" in human embryos. The potency of the 2nd pouch is suppressed by the 2nd cleft in order to keep this region free, so that it can later act as the basis for the palatine tonsil. Even though the endoderm in mammals plays a leading role in thymic differentiation, the organ can not develop if the epithelial basis is not connected with mesenchymal tissue and vice versa. Early determination of thymic endoderm is able to induce the ectomesenchyma to participate in the histogenesis of the thymus. The mesenchyme originates from the neural crest of rhombencephalon. Neural crest cells migrate to the pharyngeal arches and form the ectomesenchyme (Bockman & Kirby, 1984). In vertebrate embryogenesis the neural crest has a close relation to neuroplacodes. Unlike the neural crest, neuroplacodes have, more or less, limited potency. Neural crest cells, as well as neural placodes, lose their epithelial character and gain considerable migratory ability. It is known that a series of neuroplacodes at the embryonic head of vertebrates represent the primordia of sensory structures. The neuroplacodes not only form the ganglia of the visceral cephalic nerves but they also contribute to cephalic morphogenesis in vertebrates (Webb & Noden, 1993). During evolution it is possible that the neural crest and neural placodes originate from the epidermal nerve plexus of lower chordates and is associated with the ciliated epithelium and diffuse sensory organs at the body surface and around the gut (Northcutt & Gans, 1983).

Thymus evolution can be summarized as follows:

- sharks, class *Chondrichthyes* – it is the first time real thymus to appear in all branchial slits (they have 7 thymuses, in some species even have ducts). It is also the first appearance of the *glandula thyroidea* and ultimobranchial corpus
- class *Osteichthyes* – thymus is reduced into the more distal slits, however, thymic primordias also appear in the first two slits
- class *Amphibia* – thymus reduction continues (already limited to the third slit, thymic primordias present in the first and second pharyngeal slits), appearance of *glandulae parathyroidae*,

- class *Reptilia* – thymus appears in the second and third pharyngeal pouch
- class *Aves* – thymus is localized in the third and fourth pharyngeal pouch, thymic primordias can be also found in the second pouch
- class *Mammalia* – thymic transport from the dorsal to ventral wall of the third and fourth (in humans only the third) pharyngeal pouch, first appearance of the youngest immune organ, the *tonsilla palatina*

NEURAL CREST CELLS AND PHARYNGEAL DERIVATES

The neural crest is a paired structure, and appears only in early embryonic developmental stages and exists for only a short time. The neural crest forms the border between the neural plate (the future central nervous system) and the non-neural ectoderm (future skin). At the beginning of development, the neural crest is present as a part of ectoderm and have an epitheloid character. Then, the neural crest cells undergo a complex process of epithelial-mesenchymal transition (Kang & Svoboda, 2005). Neural crest cells migrate and differentiate into a variety of cell lineages such as melanocytes, neurons, glial cells, myofibroblasts, chondrocytes, and osteoblasts (Anderson, 1997; Bronner-Fraser, 1995; Ito & Sieber-Blum, 1993; Sakai & Wakamatsu, 2005).

Neural crest cells are also necessary for the normal differentiation of the pharyngeal arches (Graham, 2003). These ectomesenchymal cells surround the growing mass of epithelial cells emerging from the wall of the pharynx and future branchiogenic organs, such as the thymus, parathyroid and thyroid glands. Cranial neural crest cells has been shown to play a critical role during the development of the thymus, parathyroid glands, and thyroid gland, initially by providing the mesenchymal cells that populate the pharyngeal region (Bockman, 1997; Manley, 2000; Xu *et al.* 2002; Yamazaki *et al.* 2005). Bockman & Kirby (1984) confirmed the theory, that the elimination of the cranial parts of the neural crest in chickens (between the 1st and the 5th somits) considerably reduces the size of the thymus or even causes its absence. The parathyroid glands and the thyroid gland were similarly reduced. In some cases, the parathyroid glands were absent (unilaterally or bilaterally) and developmental malformations of the heart also appeared. Hence, the development of thymus depends on a direct connection between the mesenchymal derivatives of the neural crest and the epithelium of the future pharynx. Kuratani & Bockman (1990) used E/C8 monoclonal antibodies for the localization of derivatives from the neural crest in chicken embryos. They detected that the quantity of reactive products had a positive correlation with the size of thymic primordia. The authors concluded that mesenchymal derivatives of the neural crest participated in the early development

of thymic primordia, and that they played an important role in its development and thus also in the development of the immune system.

We can conclude that mesenchymal cells which originate from the neural crest induce and participate in the normal development of several organs (Pospíšilová *et al.* 2008):

1. thymus – neural crest cells condition the attractiveness of the epithelial thymic primordia for precursor T-cells, which migrate from hemopoietic organs and which are necessary for normal histogenesis and function of the thymus. Neural crest cells form the condensing mesenchymal capsule and connective tissue,
2. parathyroid gland – the neural crest cells condition the normal development, structure and function of the parathyroid glands, and contribute to the connective tissue of the parathyroids,
3. thyroid gland – the neural crest cells constitute the connective tissue of the thyroid gland and form the parafollicular cells (calcitonin producing cells) of the thyroid gland,
4. face and neck – the neural crest cells partake in the development of bones, cartilages, connective tissues and some muscles of the head, face, and neck,
5. heart – the neural crest cells partake in the septation of the outflow tract of the heart and separation of the great vessels and heart's ventricles.

An example of a disturbed formation of the pharyngeal region and failure of neural crest migration is DiGeorge syndrome. DiGeorge syndrome is the most frequent microdeletion syndrome in humans. It's characterized by cardiovascular, thymic and parathyroid, and craniofacial anomalies, as a result of malformation of the third and fourth pharyngeal pouches (Bockman & Kirby, 1984; Thomas *et al.* 1987; Wurdak *et al.* 2006; Markert *et al.* 2007).

ONTOGENESIS OF HUMAN THYMUS

During human ontogenesis, the thymus passes through marked morphological changes, including rapid prenatal development and postnatal progressive physiological involution.

Ontogenesis can be divided into 3 stages:

1st stage: *thymus epithelialis* – The thymus arises, together with primordia of parathyroid glands, from the 3rd (also from the 4th) pharyngeal pouches during the 5th week following fertilization. Both the ectodermo-endodermal epithelial complex and mesenchyme from the neural crest give rise to the primordia of these organs. Epithelium (of the pouches) forms the reticuloepithe-

lial network of the future thymus, *thymus epithelialis*. The epithelial primordia of thymus grow medially and caudally (*descensus thymi*) with the primordium of the heart (*descensus cordis*). The paired primordia of the thymus then fuse and migrate to the interior surface of the pericardium. However, lobes of the thymus remain separated from each other by a thin layer of connective tissue for the remainder of life. The innervation, blood supply, and lymphatic vessels of each lobe are separate (Kendall, 1991; Slípka *et al.* 1998).

The most widely accepted model of thymus organogenesis suggests that both the third pharyngeal cleft ectoderm and the third pharyngeal pouch endoderm contribute physically to the thymus during organogenesis, such that the epithelial component of the cortex is generated from ectodermally derived cells, while cells of endodermal origin are generated from medullary epithelium (Blackburn & Manley 2004). Only Gordon *et al.* (2004) dispute this dual origin for the epithelial cells. In a steady they transplanted isolated mouse pharyngeal endoderm, without any ectodermal cells, to a secondary location on the mouse, which resulted in a normally developed thymus, and which differentiated into a cortex and medulla. This fact is in contrast with the opinion of Cordier & Haumont (1980) who studied the thymic development in nude mice (mice without a thymus gland). The ectoderm of the 3rd pharyngeal cleft in immunodeficient mice gets involved in the early intrauterine developmental stage and their thymus never becomes a lymphatic organ.

2nd stage: *thymus lymphaticus* – Precursors of T-lymphocytes quickly migrate from hematopoietic tissues (from the fetal liver and postnatally from the bone marrow) and colonize the epithelial thymus. It is assumed that the epithelial cells produce chemokines (selective attractive substances) which mediate the migration of precursors through the walls of blood vessels. The human thymus is also a generalized hematopoietic tissue between the 7th to 9th weeks of ontogenesis. Various elements of hematopoiesis have been identified intrathymically: B- lymphocytes, plasma cells, erythropoietic and granulocytopenic cells, antigen presenting dendritic cells, and mast cells (Bodey *et al.* 1998; Crivellato *et al.* 2005). Hence, the *thymus epithelialis* is gradually transformed into the definitive lymphoepithelial organ – *thymus lymphaticus*. It acquires its characteristic structure during the 6th month of intrauterine development.

3rd stage: *thymus adiposus* – The conception of postnatal age-related thymus involution is known as organ atrophy and leads to a loss of the thymopoiesis. The highest immunological activity of the thymus is 6 months after birth, when the thymus contains the greatest overall numbers of thymocytes (Weerkamp *et al.* 2005). After the 1st year of life, this organ starts to undergo changes, which has become considerable by

puberty and adulthood. By this time a marked involution has occurred and adipose tissue has accumulated to the detriment of the lymphatic tissue. All the connective septa have enlarged, the thymic cortex has reduced in size and the numbers of large Hassall's bodies have increased. These age-related changes in structure of the thymus are called physiological involution (Bodey *et al.* 1997; Staal *et al.* 2001; Varga *et al.* 2008). The thymus loses its cellularity (by 3–5% a year) until middle age, after which the process slows down to 1% a year (Bodey *et al.* 1997).

ECTOPIC THYMUS

Genetic research has confirmed the theory that the migration process of thymic primordia is controlled by neural crest cells, which are present on the surface of these primordia. Patients with a deletion in the HOX gene group (expressed by neural crest cells) have a normal sized thymus, but it is located ectopically (generally superior to its normal location, often called "thymus cervicalis" (Manley & Blackburn, 2003). An ectopic thymus is a rare congenital anomaly. The thymus can be located anywhere along its pathway, from the mandibular angle to the upper mediastinum, which constitutes its normal migration route. An ectopic thymus usually does not cause severe symptoms. Pathological-anatomical studies have shown that 20% of the population has parts of the thymus or other lymphatic tissue in various locations within the neck; even though this tissue cannot be considered as an ectopic thymus (Gilmour, 1941). This tissue is usually found randomly during operations, and only becomes important after thymectomy in *myasthenia gravis*. An ectopic thymus can undergo hyperplastic changes during the first decade of life, as well as after an infectious disease or after vaccination. In such cases it can resemble lymphadenopathy (Scott *et al.* 2002).

In spite of the rare and usually asymptomatic occurrence of an ectopic thymus, some severe cases, with acute symptomatology, of this inborn diagnosis have been described in the literature. Shah *et al.* (2001) reported the case of a 6-week old boy with an ectopic thymus who was hospitalized for acute cough and cyanosis. Bistrizter *et al.* (1985) described a case of a 5-month old boy with dyspnea and dysphagia. Prasad *et al.* (2006) published a case-report about edema in the submandibular region and loud snoring, and Pai *et al.* (2005) reported on a case of acute respiratory insufficiency; both cases involved 3-month old boys with an ectopic thymus. Hence, the symptoms of ectopic thymus can be varied and severe.

ONTOGENESIS OF PARATHYROID GLANDS

The identity of the parathyroid glands in man was established long ago, although certain morphological and functional aspects have yet to be clarified. Currently, it is one of the most intensively studied endocrine glands, both in respect to its organogenesis and its function (García-García *et al.* 1985; Garcia-García *et al.* 1987; Mérida-Velasco, 1991). The parathyroid glands have been classified as a part of the Amine Precursor Uptake & Decarboxylation (APUD) system (Pearse, 1977) and also as members of the paraneuron system (Fujita, 1980).

In humans, parathyroid primordia can be first observed in embryos (6 mm in length); after the third and fourth pharyngeal pouches are already present (García-García *et al.* 1985). Traditional morphogenetic dogma has suggested that the inferior parathyroid glands are derived from the third pharyngeal pouches, while the superior parathyroid glands develop from the fourth pharyngeal pouches. The inferior parathyroid glands (parathyroid III) detach from the pharyngeal wall and migrate inferiorly and medially, coming to rest, during the seventh week, on the dorsal side of the caudal parts of the thyroid lobes. The superior parathyroid glands (parathyroid IV) detach from the pharynx wall, as well. They migrate inferiorly and medially, coming to rest, during the seventh week, in a position slightly superior to the inferior parathyroid glands (Hilfer & Brown 1984; Larsen, 2001). According to Le Lievre & Le Douarin (1975), the connective tissue of the parathyroid in the chicken embryo is of neural crest origin. Observations by García-García *et al.* (1985) have led us to reject the concept of an endodermal origin for human parathyroid glands. Based on an embryological analysis of 11 human embryos, the parathyroid glands have been shown to develop from sectors of the epicardiac ectodermal placode.

ONTOGENESIS OF THYROID GLAND AND ULTIMOBRANCHIAL BODY

The thyroid gland is formed by the fusion of 2 structures having separate embryonic origins: the **thyroid diverticulum**, derived from the endoderm of the floor of the pharynx; and the **ultimobranchial bodies**, formed from invaginations of the fifth pharyngeal pouches. During embryonic development, the thyroid gland migrates through the thyroglossal duct from the pharyngeal endoderm to the anterior cervical region. This duct is normally ultimately obliterated and disappears (Soscia *et al.* 2004; Manzano *et al.* 2005). Human thyroid prenatal development is divisible into three stages (Sugiyama, 1969):

1st stage: primordial development (5–7 weeks) – form-modeling and descent as *ductus thyreoglossus*

- 2nd stage:** preparatory development, subdivisible:
 8–9 weeks – increased formation of cell cords
 10–11 weeks – increased formation of immature follicles
 11–12 weeks – production of immature colloid
3rd stage: final development (13–40 weeks) – occurrence of mature follicles.

The development of the ultimobranchial body has been studied extensively by many authors including Fontaine (1979), Merida-Velasco *et al.* (1988), and Mérida-Velasco *et al.* (1989). Ultimobranchial body development can be divided into 4 stages (Sugiyama, 1969):

- 1st stage:** branchial pouch stage (5–7 weeks)
2nd stage: separation stage (7–8 weeks)
3rd stage: incorporation stage (8–9 weeks)
4th stage: dissolution stage (from 9th week)

The literature has shown the ultimobranchial body to have no consistent origin among different species. All authors agree that during prenatal development, the fifth endodermic pharyngeal pouches, prior to their incorporation into the thyroid gland, are colonized by precursors of the parafollicular cells. Some authors have demonstrated that these colonizing cells are derived from the neural crest (Pearse & Polak, 1971; Fontaine, 1979). Mérida-Velasco *et al.* (1989) suggest that in human embryos, the ultimobranchial body originates solely from the fifth pharyngeal pouch, which is colonized by ectodermic cells derived from the posterior margin of the fourth branchial groove.

Extrathyroid scattered, calcitonin-producing, cells have also been detected in several locations in the pharynx. On the other hand, calcitonin production has been demonstrated in other pharyngeal derivatives, such as the thymus and the parathyroid glands (Kameda, 1971).

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