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Development of Olfactory and Related Structures in Staged Human Embryos*

J. Bossy

Laboratoire d'Anatomie, Faculté de Médecine de Montpellier-Nîmes, Avenue Kennedy, 30000 Nîmes and Carnegie Laboratories of Embryology, University of California, Davis, California 95616, USA

Summary. The staged sequence of development of the olfactory and related structures has been established from the serially sectioned human embryos of the Carnegie collection, from stage 11 to stage 23.

The nasal epiblastic thickening appears at stage 11 and the nasal field is well outlined at stage 12. At stage 15, a continuous cellulovascular strand is observed between the nasal groove and the olfactory field. The vomeronasal groove appears at stage 16 (O'Rahilly 1967). During stage 17, the olfactory nerve is organized into two plexuses, lateral and medial, the latter mingled with the terminal-vomeronasal complex. The olfactory bulb begins to appear at stage 18. Stage 19 is characterized by the individualization of the olfactory bulb and nuclei. In addition, the distinction between olfactory structures and terminal and vomeronasal ones begins to be clear. The structure of the olfactory bulb is evident at stage 21. At stage 23, the olfactory strands are well individualized, and olfactory and terminal-vomeronasal fibers are easily distinguishable.

The terminal ganglion is rather terminal-vomeronasal with an autonomic terminal contingent and a sensory one attached to the vomeronasal system.

Key words: Human embryo – Developmental stages – Olfactory structures – Terminal nerve – Vomeronasal complex.

Introduction

Since the beginning of the 20th century, a number of important studies have appeared on the olfactory structures either in human embryos and fetuses (Hines 1922; Humphrey 1940; Lazorthes 1944; van Campenhout 1948; Larsell 1950; Humphrey 1963, 1966, 1967, 1968) or in comparative material (van Campenhout 1936). Very few works, however, have been devoted to staged human embryos (O'Rahilly 1965, 1967).

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The importance of embryonic staging (O'Rahilly 1973) in order to allow justifiable comparisons to be made between various specimens is now universally admitted. Only one parameter (e.g., size, organ, menstrual data) is not sufficient to establish a stage. Investigations need to be undertaken to determine the staged development of each system and organ.

The present work was undertaken to establish the staged sequence of development of the olfactory and related structures during the embryonic period proper, and especially the relationships between the nasal field (on the epiblastic side) and the olfactory field (on the neural side).

Material and Methods

The olfactory structures were studied in all the serially sectioned human embryos of the Carnegie Collection from stage 11 to stage 23. Data in the literature that refer to embryos of this collection can now be considered in terms of staging and hence can be compared more precisely, because the embryos have subsequently been staged. The ages used throughout this article are in postovulatory days (O'Rahilly 1973).

Results

Sequence of Events

Stage 11 (ca. 2.5–4.5 mm; 13–20 Pairs of Somites; ca. 24 Days)

Among the 16 usable specimens of this stage, 10 show a small epiblastic thickening on each side of the rostral neuropore. This thickening is distinguished by the shape and orientation of its cells, which, from an arrangement parallel to the skin surface, become perpendicular with an ovoid nucleus, the long axis of which is perpendicular to the skin. Moreover, a second cellular layer begins to appear in some areas. This primordium is a little less advanced than that for the lens, which is dorsocaudal (Fig. 1). O'Rahilly (1965, 1967) noted the appearance of the nasal fields at stage 12.

Stage 12 (ca. 3–5 mm; 21–29 Pairs of Somites; ca. 26 Days)

Twenty-one specimens of this stage were selected for study. All show a nasal field, and, in 14 the nasal disc is well defined. Between the rostral neuropore, or its scar, and the lens cup, an epiblastic thickening appears with 2 or 3 cell layers. Its surface follows the general curve of the cephalic end, and a basement membrane may begin to appear. There are no epiblastic buddings similar to those described by van Campenhout (1948) for the trigeminal neural crest at this same stage. On the cerebral part, a thickening of the neural epithelium may appear facing the nasal field, with a cellular condensation beneath it and some strands oriented towards the nasal field. Moreover, in comparison with the other parts of the brain, mitotic figures are more numerous in this primordial olfactory field.

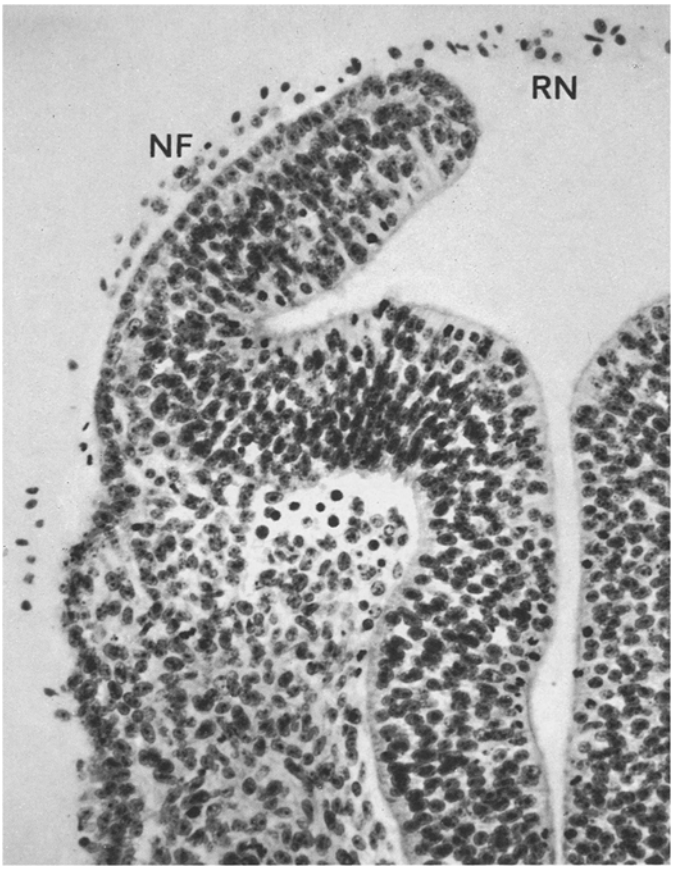

Fig. 1. Stage 11. Epiblastic thickening, lateral to the rostral neuropore and corresponding to the first Anlage of the nasal field. (Embryo No. 7611:1-3-8). *NF* nasal field; *RN* rostral neuropore

Stage 13 (ca. 4–6 mm; 30 or More Pairs of Somites; ca. 28 Days)

Nineteen specimens were considered suitable for the study of the olfactory structures. This stage is characterized by the appearance of an important vascular layer between the nasal and olfactory fields. Fairly frequently the shape of the nasal field is still convex. It is a pluristratified epithelium and shows unquestionable cellular buds imbricated with vessels, and some cellular passages though the basement membrane are seen. From the central side, the thickening is not always evident, but a remarkable mitotic activity appears in the olfactory field. The cellular buds issuing on the nasal side, already described by van Campenhout (1948) and O'Rahilly (1965, 1967), seems to be a characteristic of this stage. O'Rahilly (1967) noted that the nasal disc is better defined by stage 13.

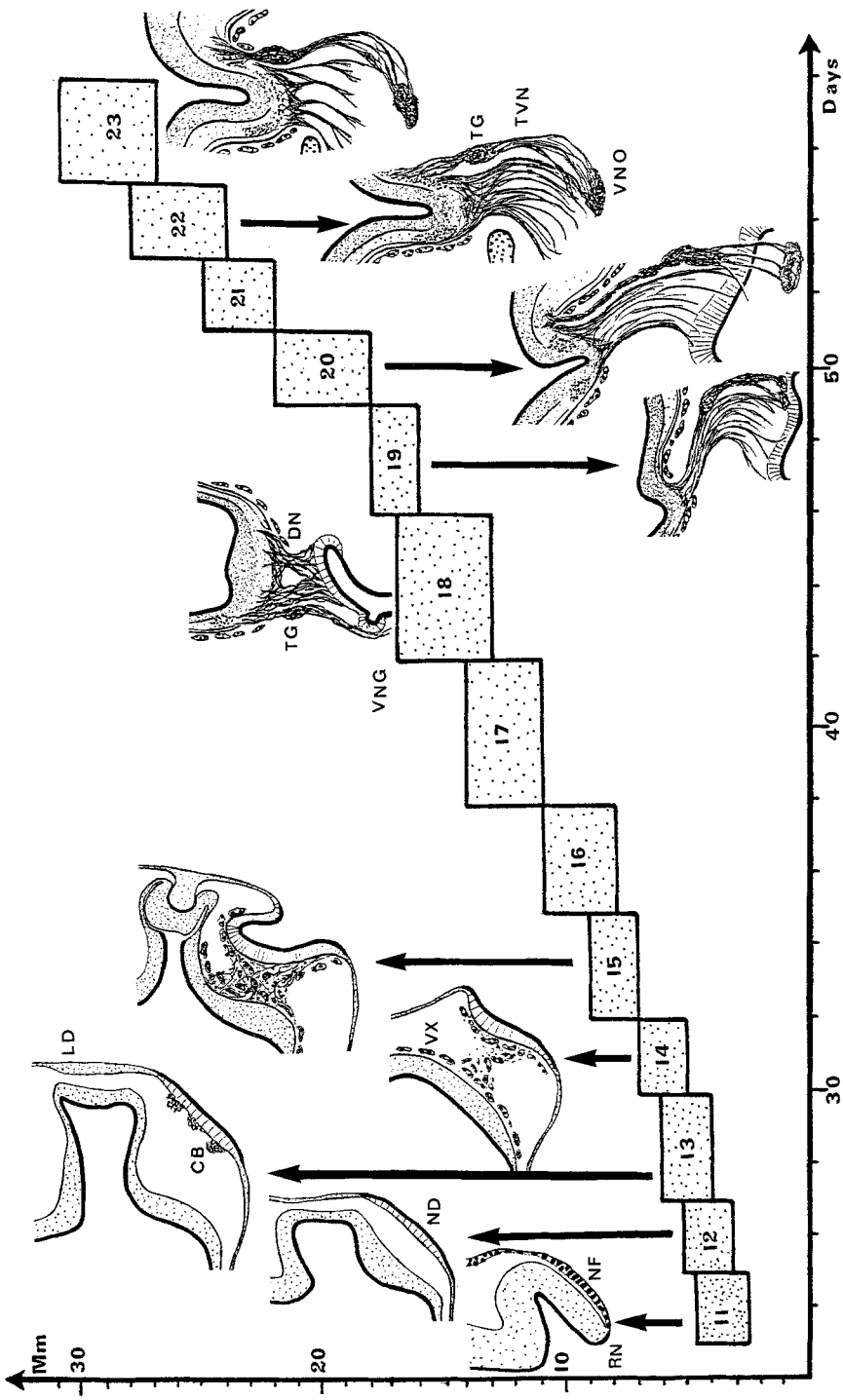

Fig. 2. Semischematic representation of the main features related to the stage development. Stages 11 to 15 and 18: transverse sections; Stages 19, 20, 22, and 23: sagittal sections. *CB* Cellular buds; *LD* Lens disc; *ND* Nasal disc; *NF* Nasal field; *ON* Olfactory nerve; *RN* Rostral neuropore; *TG* Terminal ganglion; *TVN* Terminal-vomeronasal nerve; *VNG* Vomeronasal groove; *VNO* Vomeronasal organ; *VX* Vessels

Stage 14 (ca. 5–7 mm; ca. 32 Days)

Among the 33 usable specimens, 15 are excellent and 3 of them were studied already by Van Campenhout (1948) for epiblastic buddings. This stage is characterized by the spreading out of the vascular layer, which constitutes a strand between the nasal placode and the olfactory field. The epiblastic buddings are usually clearly comparable with those of the facial nerve (Van Campenhout 1948), but are not evident in all specimens. The placode seems to get ready for the next stage: cells are more numerous and are arranged close to the basement membrane; they become a spindle-shaped; and fairly frequently cellular passages through the basement membrane are seen. From the central side, some images evoke cellular passages in a placodal direction. The placode, most commonly flat, may be concave in shape.

Stage 15 (ca. 7–9 mm; ca. 33 Days)

Twenty-seven specimens were taken into account. An evident and continuous cellulovascular strand exists between the nasal and olfactory fields. The concavity of the nasal field is constant, even if it is not clear-cut.
The cells of the nasal field are spindle-shaped and some fibres begin to appear in the cellular buddings, which are always not so well marked as in the other epiblastic thickenings, the facial for instance (Van Campenhout, 1948). The basement membrane is well developed. On the central side, the olfactory field shows numerous mitotic figures, and it becomes thicker, with sparse cellular passages. This olfactory field, however, is better outlined between the lamina terminalis and the primordial lateral ridge, which appears at this time (Humphrey 1968).

Stage 16 (ca. 8–11 mm; ca. 37 Days)

Among the 38 good specimens of this stage, 13 were sectioned coronally and were found to be the most suitable for this study. The nasal field always shows a pronounced groove, on the medial wall of which there usually appears the vomero-nasal groove (O'Rahilly 1967).

The Anlage of the olfactory nerve, which extends between the nasal groove and the olfactory field, is seen to be oriented rostrally in the sagittal sections, and laterally in the coronal and transverse ones. It always includes vascular structures, and sometimes it begins to show a fibrillar appearance.

One characteristic of this stage is the delineation of the olfactory field by sulci, its thickening and the advent of a mantle layer. Hines (1922) had already noted the existence of the ventricular sulcus medial to the primordium hippocampi, but she did not consider the olfactory field. This nasal field is well outlined peripherally by a hiatus in the vascular stratum and the marginal layer. The cellular passages are always debatable and surely inconstant throughout the marginal layer of the olfactory field.

Stage 17 (ca. 11–14 mm; ca. 41 Days)

The fibrillar aspect of the olfactory nerve is obvious. Cells accompany the olfactory fibres, which probably will give the ensheathing neuroglia and come from the nasal sac. This was confirmed later (29 mm C.-R,) by Fagnart (1950). The olfactory nerve begins to be slightly vertical in a transverse plane, but its rostrocaudal direction is always pronounced. The terminal-vomeronasal crest (O'Rahilly 1965, 1967) gives rise to a fibrocellular strand that proceeds towards the brain.

This olfactory nerve is already divided into two plexus-like laminae, medial and lateral, the latter being the smaller. The medial plexus receives the terminal-vomeronasal strand, the cells of which constitute a fairly individualized ganglionic mass. The medial olfactory and terminal-vomeronasal elements are directed rather to the caudal part of the Anlage of the olfactory bulb.

The Anlage of the olfactory bulb is better limited, sometimes by two sulci. This area, where the fibrocellular strand ends, was described by Hines (1922) as the primordium of the corpus striatum (embryo No. 940, 17-1-3). In this olfactory field, the mantle layer may have a columnar shape, mitotic figures are still numerous, and some passages through the basement membrane are observed. A hiatus in the vascular stratum and in the marginal layer always exists at the level of the olfactory field. Fibers pass through the basement membrane of the olfactory field.

The septal nuclei begin to be individualized medial to the Anlage of the olfactory bulb, but no connection with the terminal-vomeronasal system is noted.

Stage 18 (ca. 13–17 mm; ca. 44 Days)

Thirty-five specimens were examined. The olfactory nerve and bulb are in the same sagittal plane, but nevertheless their direction always remains rostrocaudal. Frequently the olfactory nerve is flattened against the brain and only a vascular layer lies between them.

The olfactory field is well outlined by two sulci in transverse sections. Its ependymal wall is either flat or convex. However, in sagittal sections, an olfactory ventricle is seen to be taking form. The terminal-vomeronasal complex is always present and presents a well-shaped terminal ganglion, although it lies in a peripheral position. The Anlage of the olfactory bulb shows a slight thickening of the ependymal layer, and the mantle layer is a little widened, with cellular and fibrillar strands arranged in columns. The marginal zone stops at the attachment area of the olfactory nerve, which is located principally on the summit and on the medial side of the olfactory field. The terminal and vomeronasal fibres, usually darker than the olfactory fibres (Pearson 1941), are the most medial.

Stage 19 (ca. 16–18 mm; ca. 48 Days)

Twenty-two specimens of this stage were studied. The origins of the olfactory nerve and the terminal-vomeronasal complex are well differentiated. The two

structures are well separated from each other by the intensity of their staining and even of their silver impregnation. The terminal ganglion is clearly delimited.

The development of the base of the skull and the face places the olfactory bulb and the roof of the nasal cavity on the same vertical plane, so that the olfactory nerve appears almost vertical in sagittal sections. Nevertheless, it describes a rostrally concave curve due to a mesenchymal condensation of the base of the skull, which has appeared at stage 18 (O'Rahilly 1967).

In sagittal sections, the olfactory bulb is usually well outlined with a ventricular recess. In transverse sections, however, the olfactory field appears as a thickening limited by two obvious angles: medial to the lateral corpus striatum and rostral to the medial corpus striatum. Hines (1922) had already described the olfactory bulb and tubercle in one embryo of this series (No. 8965). In one embryo at this age, Humphrey (1940) noted the appearance of the anterior olfactory nucleus, pars dorsalis, the presence of which is almost constant at this stage in our series.

The olfactory nerve is located in the medial dihedron between the brain and the primordium hippocampi. It reaches the olfactory bulb in its dorsal part, and, at the level of its extremity, the fibres are arranged in small threads that pass through the vascular stratum and the marginal layer.

In one excellent silver-impregnated specimen, the course and distribution of the fibres of the terminal and vomeronasal nerves could be traced. The fibres of the terminal nerve have their peripheral origin from the whole length of the nasal roof, and hence are mixed with olfactory fibres at this level; they then branch off to enter the brain behind and medial to the olfactory bulb in the septal area, behind the olfactory nucleus. It is not obvious that cells accompanying these fibres are of ganglionic type; Pearson (1941) has suggested that they are destined to form a glial sheath. The vomeronasal fibres have a very peculiar peripheral origin; they constitute a well-individualized nerve trunk, which seems to end in a ganglion applied against the side of and behind the olfactory nerve; beyond this ganglion the vomeronasal fibres are mixed with posterior olfactory ones, and the nerve loses its individuality.

Stage 20 (ca. 18–22 mm; ca. 51 Days)

The olfactory nerve is almost vertical in the 18 specimens employed for this study; it ends in the posteromedial, superior, and apical areas of the olfactory bulb. The ending of the fibres of the terminal nerve is always isolated, and the vomeronasal fibres are still intermingled with the posterior and medial olfactory fibres beyond the ganglionic mass. The olfactory ventricle becomes definite with the formation of a constriction.

In the ventral part of the olfactory bulb, it is possible to see between the septal area and the bulb, a thick area, which is rectilinear on the ventricular side and which corresponds to the primordium hippocampi.

Stage 21 (ca. 22–24 mm; ca. 52 Days)

The olfactory nerve is arranged in small threads in the mesenchymal area of the future cribiform plate, to penetrate the olfactory bulb, which began at

stage 19. It still shows a marked rostral concavity at the level of the future ethmoid bone.

The terminal nerve is well individualized, especially in its intracranial course; the right and left nerves approach one another, and almost touch in the median plane, so suggest that the appearance of a decussation is suggested in specimens with an asymmetrical crista galli.

The ganglionic mass is located at the limit of the base of the skull, and seems essentially connected to the vomeronasal nerve. All the fibres of the terminal nerve are directed towards the medial septal area, where a nucleus appears.

The olfactory bulb is directed medially, rostrally, and vertically. The shape of the olfactory bulb is well marked by a central constriction of the olfactory ventricle. The arrangement of the olfactory bulb becomes definite with a superficial fibrillar layer, plexiform and in part limited by a marginal velum, a cellular layer (mitral cells), a poorly cellular layer, and an ependymal one. The dorsal and ventral parts of the anterior olfactory nucleus are recognizable; this corresponds to the age and size of the embryos used by Humphrey (1963).

Stage 22 (ca. 23–28 mm; ca. 54 Days)

The study of 18 good specimens has shown that the olfactory bulb grows longer, increases in size, particularly in its medial wall, and takes a bulbar shape. Its structure becomes definite: the plexiform cap is clear-cut; the next cellular layer includes many fibres; then a clear and poorly cellular stratum and the ependymal layer. Nuclei are sharply outlined and increase in size: dorsal and ventral parts of the anterior olfactory nucleus, and the medial nucleus, which seems to belong to the septum rather than to the olfactory bulb (Humphrey 1963).

The olfactory nerve ends as a mass at the level of the cap, and by isolated threads on the dorsal aspect (possibly the vomeronasal nerve). The cap seems to be olfactory, and the medial and posterior threads appear to belong to the terminal-vomeronasal complex.

In a limited area, behind the crista galli, and in the median plane, the fibres that touch belong to the terminal nerve. The crossing suggested in some sections is due to asymmetry of the median skeleton (crista galli and nasal septum).

Stage 23 (ca. 27–31 mm; ca. 57 Days)

Twenty-two specimens of this stage have permitted a study of the state of the olfactory structures at the end of the embryonic period proper. The olfactory bulb, the olfactory nerve, and the nasal cavity are in an almost vertical axis.

The organization of the olfactory bulb is such that it is possible to trace its boundaries with neighbouring structures. The terminal ganglion decreases in size. The olfactory filaments divide into medial and lateral. Graphic recon-

structions show that a crossing of the fibres of the terminal nerve can be eliminated; at this level, however, this nerve sends some recurrent superodorsal fibres, precisely paramedian in position, and probably to the falx cerebri.
The vomeronasal fibres are gathered into two posterior filaments, which arrive at the vomeronasal ganglion, on the dorsalmedial aspect of the olfactory bulb, and end in the medial part of this last in a precise and perhaps specific area.

The fibres of the terminal nerve, especially the posterior, extend more caudally in the mucosa of the nasal septum.

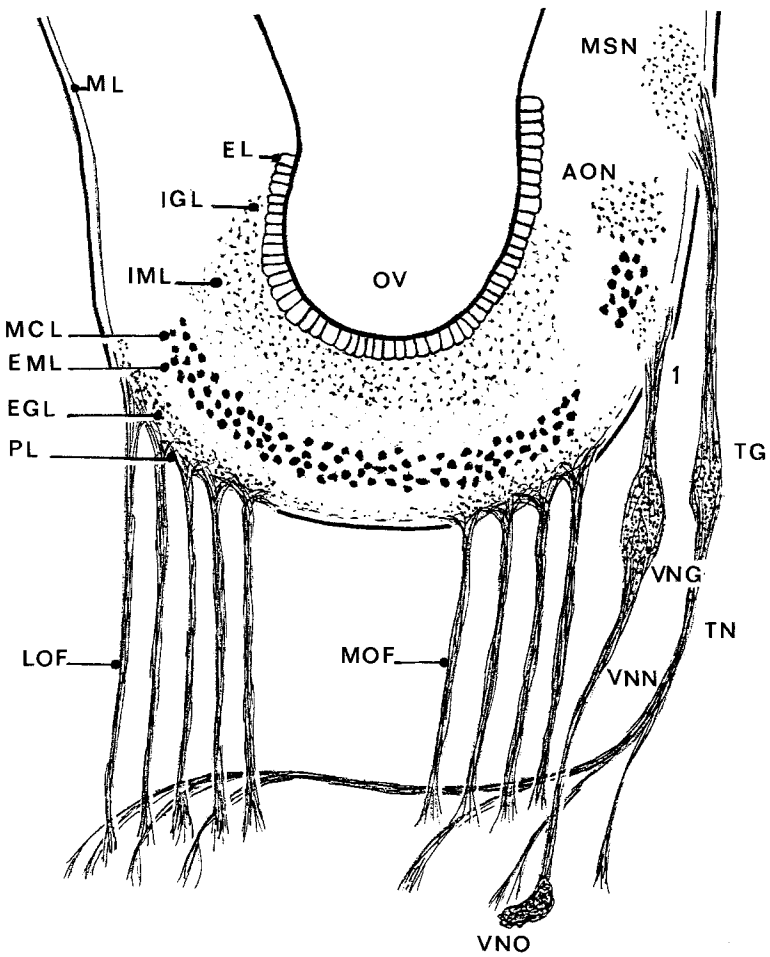

Fig. 3. Schematic representation of the nervous olfactory structures at the stage 23. *AON* Anterior olfactory nucleus; *EL* Ependymal layer; *EGL* External granular layer; *EML* External molecular layer; *IGL* Internal granular layer; *IML* Internal molecular layer; *LOF* Lateral olfactory fila; *MCL* Mitral cell layer; *ML* Marginal layer; *MOF* Medial olfactory fila; *MSN* Medial septal nucleus; *OV* Olfactory ventricle; *PL* Plexiform layer; *TG* Terminal ganglion; *TN* Terminal nerve; *VNG* Vomeronasal ganglion; *VNN* Vomeronasal nerve; *VNO* Vomeronasal organ; *1* accessory olfactory formation; according to Humphrey (1940) the external granular layer of this formation is deep and does not mix with the vomeronasal fibres, in contrast to the intermingling of olfactory fibres and external granular layer of the olfactory bulb

Discussion and Conclusions

(a) Olfactory Structures

The nasal epiblastic thickening appears at stage 11. At stage 12, modifications in the cerebral olfactory field begin. At stage 13, cellular buddings imbricated with vessels are found; during the next stage, some cellular passages through the basement membrane may be observed. At stage 15, a continuous cellulovascular strand is observed between the nasal groove and the olfactory field. At stage 16, a fibrillar appearance begins. Masy (1955) emphasized cellulofibrillar strands to the telencephalon in a human embryo of 9 mm C.-R. Humphrey (1940) thought that the primitive olfactory bulb is distinguishable only by the entering nerve fibres, that is, two or three stages later. During stage 17, the olfactory nerve is organized into two plexuses, the medial of which mingles with the terminal-vomeronasal complex. The beginning of the olfactory bulb appears at stage 18. Stage 19 is characterized by the individualization of the olfactory bulb and nuclei; olfactory fibres are distinguishable from the terminal-vomeronasal fibres (Pearson 1941). The olfactory bulb takes form at stage 20, and, at stage 21, its structure is evident and distinct from the hemispheric wall, contrary to Pearson's opinion (1941). At stage 23, the olfactory strands are well individualized; olfactory and terminal-vomeronasal fibres are easily distinguishable.

According to Verwoerd and van Oostrom (1979), who described the olfactory placodes in the mouse, the neural plate and placodes "do not originate as thickenings of previously thin ectoderm, but are remnants of the original area of thick ectoderm. Thickening of the ectoderm of the neural plate and the placodes takes place at a later stage." This does not agree with observations in human embryos.

The difference between the appearance of the nasal field (stage 11) and the first central differentiation (stage 12) seems to eliminate direct induction of differentiation of the nasal placode by the telencephalon according to one of three forms of *primum movens* suggested by van Campenhout (1967).

According to Detwiler (1936), reciprocal relationships exist between olfactory nerve and brain. It is possible that increasing mitotic activity at stage 13, followed by cellular passages at stage 14, would attract a cellular strand at stage 15. At stage 16, fibres enter the olfactory field and induce growth, which causes significant mitotic activity, with the appearance of olfactory nuclei, and then an increase in olfactory volume, leading to the olfactory bulb during the next stages.

(b) Terminal-Vomeronasal Complex

The time when it is possible to distinguish between the terminal-vomeronasal complex and olfactory fibres is rather late. The vomeronasal groove appears at stage 16 (O'Rahilly 1967). During the next few stages, the terminal ganglion passes from a peripheral to an intracranial location. At the end of the embryonic period proper, it begins to regress.

The distinction between olfactory structures and terminal and vomeronasal ones begins to be clear at stage 19, a little later (22 mm C.-R.) according to Pearson (1941). On the peripheral side, the fibres of the terminal nerve originate from the whole olfactory mucosa, and often pass beyond this to the floor of the nasal cavity. The vomeronasal fibres are arranged in the form of 2 or 3 threads coming from the corresponding organ, and always in a caudal location. From stage 21 on, the peripheral arrangement is similar to that described by Pearson (1941) in a 45 mm C.-R. fetus.

On the central side, the vomeronasal fibres are in close relationship with the terminal ganglion and end in a medial area of the olfactory bulb (Pearson, 1941). The fibres of the terminal nerve seem scarcely bound to the terminal ganglion, and proceed towards the medial septal area, corresponding to Pearson's (1941) description at the beginning of the fetal period, but no fibre seems to return from the septal area to the accessory olfactory bulb. According to Larsell (1950), who seems to group terminal and vomeronasal fibres together as the terminal nerve, the autonomic fibres end in the supra-optic area, and the sensory fibres in the septal nuclei, olfactory lobe and posterior precommissural area (Calleja's island).

The terminal ganglion poses a problem. Its first appellation "olfactory ganglion" has rightly been rejected (van Campenhout 1936; Pearson 1941). Is it a sensory ganglion attached to the vomeronasal system (Lazorthes 1944), or an autonomic ganglion annexed to the terminal system, or both of these (Larsell 1950; Pearson 1941)? Observations in human embryos of excellent quality favour the last hypothesis, with a quantitative preponderance of the sensory vomeronasal over the terminal units. Thus, as O'Rahilly (1965) has suggested for the neural crest, the term terminal-vomeronasal ganglion is preferable. However, its origin seems double: a great participation of terminal-vomeronasal Anlage (O'Rahilly 1965), and a central participation comparable to that described by Hamburger (1961) for the trigeminal ganglion in the chick embryo.

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