

# Early Development of Olfactory Organ in White Rat

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白鼠嗅覺器의 初期發生

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## 摘 要

本研究는 白鼠嗅覺器의 發生過程을 細胞組織學的으로 糾明 報告하는 바이며 使用된 stage는 stage 20 부터 32 까지는 Christe(1964)의 것을 引用하였으며 그 外에 明示 안된 stage 33, 34, 35는 著者가 便宜上 區分하였다. 材料는 白鼠 50匹을 室溫서 充分한 榮養으로 飼育後 妊娠시켜 要求되는 胚를 stage別로 얻어 Bouin's solution에 固定시켜 paraffin 法을 따랐으며 5~7 $\mu$ 의 두께로 切片을 만들어 Delafield's hematoxylin, eosin, thionin에 染色하였다.

Stage別 嗅覺器의 發生은 stage 20에 嗅樞, stage 22A에 嗅窩 및 鋤鼻器(Jacobson's organ), stage 23A에 口鼻腔膜(oro-nasal membrane), stage 23B에 嗅神經纖維와 原始內鼻孔, stage 24에 鋤鼻器神經纖維, stage 27에는 顎骨甲介上皮에 嗅細胞와 呼吸上皮에 纖毛 또한 顎骨甲介上皮에서 seromucinous 腺들이 最初로 나타났다.

本實驗結果 白鼠嗅覺器의 形成過程은 人體와 생쥐(mouse)에서와 類似하나 鋤鼻器의 成長, 鼻甲介의 數, 및 嗅域의 範圍에 있어서는 極히 差가 甚한 것 같다.

## INTRODUCTION

The early development of the olfactory organ in human embryo has been studied by Humphrey(1964), Dawcs and Prichard(1953), and Alphonser(1964).

In white rat, developmental features from 20th to 35th stages; i.e., from 10 and 3/4th day after fertilization up to delivery have been studied. Christe(1964) reported the appearance of olfactory placode at the stage 21, olfactory pit at the stage 22 A and primitive choana at the stage 23B; and Hiraiwa(1941) olfactory pit at the stage 22B.

As the scope of the previous studies did not cover any further development beyond the stage 23B, present paper will cover the cyto-histological changes from the conception at 10 and 3/4th day to the time of delivery; i.e., from 20th to 35th altogether 16 stages in continuous succession.

## MATERIALS AND METHODS

White rat embryos from 50 pregnant rats bred at shade temperature, 25°C, with sufficient nutritious food were used for experiment. The females' mating period was determined by vaginal smearing method and the embryos of respective stages were obtained accordingly. The method of dating the embryo is based on Christe's scale which divides the period from 10 and 3/4th day after conception, to the date of delivery, evenly into 18 stages, and sets the periods in series; i.e., from the stage 20th up to 35th. Christe's study was limited up to 32nd stage. As the olfactory organ grows beyond the 32nd stage, it has been extended in the present study up to the 35th stage.

They were fixed in Bouin's solution, embedded in paraffin, sectioned serially 5-7  $\mu$  in thickness, and then stained

with Delafield's hematoxylin, eosin Y, and thionin.

The crown-rump length of the embryos was measured before fixation and the length of the sectioned sample, with micrometer under the microscope. The measured lengths were averaged in six or eight embryos at a litter.

The percentage error in measurement was 0.5% in each length.

## OBSERVATION

Table 1 shows the measured length of the olfactory region and Jacobson's organ in each stage.

**Stage 20 :** The olfactory placode appears in the oval-form tilting outward on the lateral side fore-brain. The distance from placode to prosencephalon is 122  $\mu$ . The base of the placode is in direct contact with mesenchymal cells without mediation of the basement membrane. The placode is composed of a single layer of ectodermal cells. They stand vertically to the surface, while the other ectodermal cells lie horizontally. The nuclei of the placode cells are located at the bottom of the cell.

**Stage 21 :** The olfactory placode is thickened into a pseudostratified layer. Due to the increase of mesenchymal cells, the distance between placode to prosencephalon increased to 185  $\mu$ .

**Stage 22A :** The olfactory placode is caved into the olfactory pit. Lower lateral part of the pit remains open to be connected with lacrimal duct. The lateral and median nasal processes are not yet fused. On the lateral sides of the median nasal process Jacobson's organs begin to appear and nasal cavity enlarges and deepens toward the dorso-lateral sides. The epithelium of Jacobson's organ and dorso-lateral part of the olfactory pit undergoes active cell divisions so as to be embedded into the connective tissue, and becomes thicker than the epithelium of other parts of the olfactory pit.

**Stage 22B :** The distance from external nares to the deepest point of dorso-lateral wall of nasal cavity is 466  $\mu$  and the diameter of the external nares is 155-200  $\mu$ . Under the olfactory epithelium the capillaries begin to appear, and mesenchymal cells proliferate under the epithelium of Jacobson's organ.

**Stage 23A :** The length from external nares to the roof of nasal cavity is 550  $\mu$ . The height and width of external nares are 116 and 83  $\mu$ . The nasal cavity now grows dorso-caudal-ward; it begins to be separated from oral cavity by oro-nasal membrane which is made of single layer of epithelium, 10  $\mu$  in thickness and 133  $\mu$  in diameter. The capillaries begin to appear under the epithelium of Jacobson's organ and capillaries under olfactory epithelium proliferate more than Stage 22B.

**Stage 23B :** The length from external nares to the roof of nasal cavity is 630  $\mu$  and primitive choana appears between the nasal and oral cavities. Its diameter is 129.5  $\mu$ . A vertical groove of crescent form about 216 $\mu$  at the deep point, appears on the wall of the lateral nasal process. At the middle of the vertical process another shallow horizontal groove is formed pointing backward, which is going to separate the wall into ethmo(upper)-and maxillo(lower)-turbinal.

Epithelium of nasal cavity is thicker than that of its opening, and it becomes thickest at the roof.

**Stage 24 :** The length from external nares to the roof of nasal cavity is 863  $\mu$ ; the diameter of external nares is 100  $\mu$ ; primitive choana is 146  $\mu$  in width. The length from primitive choana to the roof of nasal cavity is 717 $\mu$ . A new vertical groove of 160  $\mu$  extending upward from the middle point of the previous horizontal groove appears to separate the ethmo-turbinal into 1st and 2nd ethmo-turbinal. Jacobson's organ is elongated into a blind sac on the dorsal side of the base of the nasal septum, and under its epithelium, nerve fibers stretch out toward prosencephalon.

**Stage 25 :** The length from the external nares to the roof of nasal cavity is 1333  $\mu$ , primitive choana is 200  $\mu$  in diameter, and the length from primitive choana to the roof of nasal cavity grows to 807  $\mu$ . A new vertical groove is formed near the external nares on the lateral nasal process. The grooves between the turbinals become deeper and they widen towards their ends. As the result, the bases of the turbinals become thinner, and their tips become thicker.

**Stage 26 :** The length from external nares to the dorsal wall of nasal cavity is 1467  $\mu$  and the nasal cavity lies almost horizontally. It is 882 $\mu$  in length between the primitive choana and the roof of the nasal cavity, and the groove

Table 1. Size of olfactory region and Jacobson's organ.

St.	Age(day)	No. of somites	C.R. length (mm)	No. of somites	C.R. length (mm)	Olfactory region			Jacobson's organ				
						Length ( $\mu$ )	Width ( $\mu$ )	Thickness ( $\mu$ )	Length ( $\mu$ )	Width ( $\mu$ )	Thickness ( $\mu$ )		
*20	10 3/4-11 1/2	18	2.0	15/16-22	1.6-3.0	135	95		19				
21	11 1/2-12	26	3.2	23/24-28	3.0-4.1	578	378		50				
						Length ( $\mu$ )	Depth ( $\mu$ )	Width ( $\mu$ )					
**22A	12-12 1/2	30	4.5	29-32	4.1-4.6	200	164	69	55	77	35	56	
22B	12 1/2-13	34	5.0	33-37	4.6-5.8	422	222	111	58	115	40	60	
23A	13-13 1/2	40	6.7	38/39-41	5.8-7.1	600	256	160	65	147	55	63	
23B	13 1/2-13 3/4	42	7.5	42-44	7.1-7.9	667	300	200	65	163	56	66	
											In***	Out***	
24	14-14 1/4	50	9.5	48	9.4-10.3	784	566	333	67	249	58	42	68
25	14 1/4-14 3/4		10.5		10.3-11.5	803	599	407	67	331	66	37	68
26	14 3/4-15 1/2		11.8		11.5-12.1	845	626	483	67	432	71	36	70
27	15 1/2-16		12.7		12.1-12.7	1430	692	523	68	626	88	31	71
28	16-16 1/2		13.0		12.7-14.5	1588	762	572	68	793	93	28	73
29	16 1/2-17		16.0		14.5-16.0	2070	816	678	67	910	134	25	74
30	17-17 1/2		17.0		16.0-17.6	2380	881	854	66	1190	164	24	75
31	17 1/2-18		18.0		17.6-19.1	2500	950	954	65	1310	173	20	76
32	18-18 1/2		20.5		19.1-22.0	2750	1090	1005	65	1435	187	19	78
33	18 1/2-19		22.5			3350	1438	1260	63	1916	190	17	80
34	19-19 3/4		26.0			3470	1600	1310	63	2005	200	15	83
35	19 3/4-20 1/2		35.0			3700	1900	1550	62	2150	214	15	82

\* Olfactory placode

\*\*Olfactory pit

\*\*\*In: Inner epithelium toward nasal septum. Out: Outer epithelium toward nasal cavity.

between maxillo-turbinal and 1st ethmo-turbinal is 217  $\mu$  in depth. Another slanting groove appears on the lateral wall of 2nd ethmo-turbinal to separate 2nd from 3rd ethmo-turbinal. The little olfactory cells are about well differentiated into olfactory terminal cells, and the epithelium of Jacobson's organ and the roof of the nasal cavity begins to be differentiated. The basal cells appear beneath the olfactory epithelium, and the goblet cells also observed under the epithelium of the lower nasal septum.

**Stage 27:** The length between the external nares and the dorsal wall of the nasal cavity is 1947  $\mu$  and from the palatine to the roof of nasal cavity is 1073  $\mu$ . Under the respiratory and the maxillo-turbinal epithelia seromucinous glands begin to appear. On both lateral walls of the oral cavity, the palatine processes extend toward the median line so as begin to be fused from its caudal end toward its front. As the palatal fissure is going to be closed the nasal septum extends and meets the palatine junction, in a T-shape. From this stage, the olfactory bulbs extend toward the direction of external nares right above both nasal cavities. The olfactory terminal cells on the maxillo-turbinal are well differentiated and the olfactory vesicle appears on the tip of the olfactory cells. The cilia appear on the epithelium of the nasal septum and the roof of the nasal cavity. The respiratory epithelium between the external nares and nasal cavity consists of two layers. The cells of the outer layer lie horizontally and the cells of the inner layer stand up vertically. The goblet cells begin to appear in the epithelium of the mid-nasal septum and deeper part of the turbinals.

**Stage 28:** The length between the external nares and the dorsal wall of nasal cavity is 2380  $\mu$  and between the palatine and the roof of nasal cavity is 1190  $\mu$ . A new groove is formed on the dorso-lateral wall of the 3rd ethmo-turbinal to build up the 4th ethmo-turbinal. Two processes appear on the upper and lower lateral walls of the nasal cavity right in front of the vertical groove. As the primitive palatine fuses with nasal septum two secondary internal nares were left right open below Jacobson's organ.

**Stage 29:** The length from the external nares to the dorsal wall of the nasal cavity is 2980  $\mu$ , and 1290  $\mu$  be-

tween the palatine and the roof of nasal cavity. The groove is 588  $\mu$  in depth between the maxillo-turbinal and 1st ethmo-turbinal. From this stage on, the olfactory epithelium becomes gradually elongated and spread thin.

**Stage 30:** The length from the external nares to the dorsal wall of the nasal cavity is 3500 $\mu$ , and 1330  $\mu$  between the palatine and the roof of nasal cavity. Seromucinous glands appear in the epithelium of the 1st ethmo-turbinal.

**Stage 31.** The length from the external nares to the dorsal wall of the nasal cavity is 3920  $\mu$ , and 1430  $\mu$  between palatine and the roof of the nasal cavity. A new groove is formed at the end of the dorso-lateral wall of the nasal cavity and it helps to form the 5th ethmo-turbinal. Olfactory cells appear in the upper and lower processes of epithelium which lies in front of first vertical groove (cf. stage 28).

**Table 2.** A list of comparative studies on the embryological development of olfactory organs in white rat, mouse and human embryos.

Stage	Feature in present research	White rat		Mouse		Human	
		Age(day)	Researcher	Age(day)	Researcher	Age(day, mm)	Researcher
20	Olfactory placode	11 1/2-12	Christe	9	Gruneberg	28	Streeter
				10	Streeter	21	Eggston
						4 CR length	Arey
22A	Olfactory pit	12.625	Hiraiwa	10	Gruneberg	29	Streeter
				12-12 1/2	Christe	11 1/2	Streeter
	Jacobson's organ			11 1/2	Streeter	37	Streeter
22B	Capillaries under olfactory epithelium					35	Boenig
23A	Oro-nasal membrane					12CR length	Arey, Schaeffer
	Capillaries under Jacobson's organ epithelium						
23B	Primitive choana	13 1/2-13 3/4	Christe				
	Olfactory nerve fiber			11 2/1	Pearson	16CR length	Pearson
	Maxillo- and 1st ethmo-turbinal					49	Frazer
24	Nerve fiber under Jacobson's organ epithelium						
26	Basal cells						
	Goblet cells						
27	Olfactory cells with olfactory vesicle at maxillo-turbinal					49	Arey
	Palatine processes on later wall of oral cavity					30	Boenig
	Cilia on respiratory epithelium						
	Seromucinous gland under maxillo-turbinal epithelium and respiratory epithelium						
28	4th ethmo-turbinal						
	Lateral processes on lateral wall of ventro-nasal cavity						
	Secondary internal nares					70CR length	Boenig
	Palate					70	Arey
29	Olfactory cells at ethmo-turbinal epithelium						
30	Seromucinous gland under 1st ethmo-turbinal epithelium						
31	5th ethmo-turbinal						
	Olfactory cells at lateral processes on upper and lower wall of ventro-nasal cavity						
32	Seromucinous gland at nasal septum and whole nasal cavity and Jacobson's organ						
33	Chitin layer on the respiratory epithelia						

**Stage 32 :** The length from the external nares to the dorsal wall of the nasal cavity is  $4050 \mu$ , and  $1527 \mu$  between the palatine and the roof of the nasal cavity. Seromucinous glands appear under the epithelium of the nasal septum and the roof of the nasal cavity. The glands at the lower end of the nasal septum empty the secretion into Jacobson's organ.

**Stage 33 :** The length from the external nares to the dorsal wall of the nasal cavity is  $4420 \mu$ , and  $2025 \mu$  between the palatine and the roof of nasal cavity. The nasal bone and the maxillary bone are well ossified and 18 ethmo-foramina appear in the ethmoidal bone for the passage of the nerves from brain to olfactory terminals. Olfactory cells of turbinals are well differentiated and developed into its characteristic form. The olfactory vesicle of the Jacobson's epithelium are not yet formed.

**Stage 34 :** The length from the external nares to the dorsal wall of the nasal cavity is  $4910 \mu$ , and  $2175 \mu$  between the palatine and the roof of nasal cavity. Many seromucinous glands are observed under the epithelium of all turbinals. The chitin layer, which is found on external skin, covers the respiratory epithelium from the external nares to the nasal cavity.

**Stage 35 :** The length from the palatine to the dorsal wall of the nasal cavity is  $5500 \mu$ , and  $2330 \mu$  between the palatine and the roof of nasal cavity. An average of one olfactory cell can be found in every  $19.6 \mu^2$  of the turbinal epithelium; an average of six olfactory cells in every  $19.6 \mu^2$  epithelium of Jacobson's organ, and the roof of the nasal cavity. Olfactory vesicle at all turbinals is  $2 \mu$  in diameter and  $2.5 \mu$  in length, and at the roof of the nasal cavity it is  $1.5 \mu$  in diameter and  $1 \mu$  in length. However, no vesicle appears on the epithelium of Jacobson's organ.

#### DISCUSSION

Concerning the olfactory placode in the human embryo, Gage(1904) reported the H-form or butterfly-like appearance, and Arey(1965) the oval form at 4mm C.R. length.

In this study on white rat embryo, the olfactory placode appeared in oval form at the stage 20(cf. Table 2).

In the human embryo, Arey reported that the olfactory pit growing to the dorsal part, makes an olfactory sac and Jacobson's organ to the nasal septum. In this study the olfactory pit also makes them at the stage 22A.

Olfactory cells appeared at the olfactory region on 7th week in the human embryo by Arey. In this study the olfactory terminal cells with the olfactory vesicle on its tip, begin to appear on the maxillo-turbinal at the stage 27 (for other features, confer Table 2).

Comparative studies of olfactory organs of the human, mouse and white rat embryos, indicate that the order of developmental differentiation is alike, but the degrees of the functionality of Jacobson's organ, the numbers of turbinals and the area for the olfactory perception differ widely among the various species of mammalia.

#### SUMMARY

1. The olfactory organ appears as an olfactory placode at the stage 20, as olfactory pit at the stage 22A, capillaries under the olfactory epithelium at the stage 22 B, and nerve fibers at the stage 23B.
2. Maxillo- and 1st ethmo-turbinal appear at the stage 23B, 2nd ethmo-turbinal at the stage 24, 3rd ethmo-turbinal at the stage 26, both the 4th ethmo-turbinal and upper and lower processes in front of the 1st vertical groove of the lateral wall of the nasal cavity at the stage 28 and 5th ethmo-turbinal at the stage 31.

Olfactory cells and seromucinous glands of maxillo-turbinal appear at the stage 27, seromucinous glands of the 1st ethmo-turbinal at the stage 30, and of the nasal septum and the roof of the nasal cavity at the stage 32.

3. Jacobson's organ appears at the stage 22A, and capillaries under its epithelium are formed at the stage 23A, and nerve fibers at the stage 24. Nerve cells of the organ begin to be differentiated at the stage 27.
4. The oro-nasal membrane separates the nasal and oral cavities at the stage 23A, and primitive choana linking the two cavities is formed at stage 23B. Palatine processes begins to fuse at the stage 27, and the palatine with the secondary internal nares is formed at the stage 28.

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### Explanation of Figures

- Fig. 1. Olfactory placode of a single cell layer at stage 20. 400X2. H-E stain.
- Fig. 2. Olfactory pit with Jacobson's organ on the nasal septum at stage 22A. 100X2. H-E stain.
- Fig. 3. Oro-nasal membrane and Jacobson's organ on the nasal septum at stage 23A. 100X2. H-E stain.
- Fig. 4. Nerve fibers of Jacobson's organ and olfactory nerve fibers at stage 24. 100X2. H-E stain.
- Fig. 5. I-III ethmo-turbinals at stage 25. 100X2. H-E stain.
- Fig. 6. Palatine process and Jacobson's organ at stage 27. 100X2. H-E stain.
- Fig. 7. Seromucinous gland and olfactory cells at maxillo-turbinal, and cilia on the nasal septum epithelium at stage 27. 400X2. H-E stain.
- Fig. 8. I-IV ethmo-turbinals and later process on upper and lower wall of ventro-nasal cavity at stage 28. 100X2. H-E stain.
- Fig. 9. Secondary internal nares under nasal septum and Jacobson's organ at stage 28. 100X2. H-E stain.
- Fig. 10. Seromucinous gland at 1 ethmo-turbinal and olfactory cells at stage 30. 400X2. H-E stain.
- Fig. 11. Bowman's gland and olfactory cell at nasal cavity roof at stage 32. 400X2. H-E stain.
- Fig. 12. Seromucinous gland opened Jacobson's organ at stage 33. 100X2. H-E stain.

