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Ultrastructures of Glosso-Palatal Fusion after Treatment of Meclozine-Hydrochloride

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Summary. The behavior of the epithelial cells on the tongue and palatal processes during glosso-palatal fusion induced by Meclozine-hydrochloride was investigated with light and electron microscopes. Microscopical observations revealed that; at two or three days after the administration, the palatal processes were approximating to the tongue, and the superficial cells on the lateral sides of tongue became swollen and to have inclusion bodies, and in the epithelium on the medial sides of palatal processes, some inclusion bodies appeared too. At the time of contact, the superficial epithelial cells of tongue tended to degenerate markedly, and these degenerating cells were dissociated from the epithelium, and concurrently, superficial layer of the palatal epithelium became to have a lot of inclusion bodies.

Between the two tissues, attachment devices developed just after the contact, and then, the cells in the intervening epithelial lining fell into a degeneration gradually.

As well known, Meclozine-hydrochloride is one of important miner tranquilizers. From the findings mentioned above, it seemed to be possible that a heterotopic fusion caused with this drug is a result of a destruction of embryonic epithelium on tongue and palatal processes at a certain stage of development.

Key words: Meclozine-hydrochloride — Superficial Cells — Degenerating Changes — Glosso-Palatal Fusion.

Introduction

Ten years ago, the knowledge of secondary palate formation was not enough to discuss the details of it, and most of embryologists believed that the fusion of palatal processes was the result of approximating motion of each process pushed from lateral sides just after transposition.

On that time, the following question arose in the authors' minds whether "the location" of organs was only a principal factor for fusion of tissues or not. If the fusion was related only with the location of the organs, palatal processes might be expected to fuse with tongue at a certain stage of palatal formation. But, such abnormality had scarecely been known during a normal development.

Based on ultrastructural studies on the presumptive regions of fusion in palatal processes, the authors reported the specific epithelial changes—disarrangement and degeneration of epithelial cells—on the medial edges of palatal processes at the critical period of the palatal fusion (Mato, Aikawa and Katahira, 1966, 1967). The findings were confirmed later by Smiley and Dixon (1968), Shapiro and Sweney (1969), Koziol and Steffek (1969), Sweney and Shapiro (1970) and other investigators.

From these evidences, the authors postulated that, if the opposed epithelial cells covering on different organs were damaged by means of a treatment of drugs, they would fuse with each other.

Recently, it was also known that the administration of some tranquilizers brought about a heterotopic fusion in the oral region.

The purpose of this study is to elusidate the morphological changes in embryonic oral epithelium after the treatment of Meclozine-hydrochloride.

Materials and Methods

As the experimental materials, seven pregnant Wistar rats were employed. They were ingested 50 mg of Meclozine-hydrochloride (by the courtesy of Taito-Pfizer Co.) which was dissolved in 2 ml of physiological saline by oral intubation on the 12th, 13th and 14th day of gestation. The rats were killed at two or three days after the last dose, and the fetuses were removed from uterus.

For electronmicroscopical observation, after decapitation, oral region of the fetuses were fixed with 5% glutaraldehyde buffered with cacodylate-HCl, pH 7.4 for 24 hours, and post-fixed with 1% osmium tetroxide buffered with cacodylate-HCl for 3 hours, and they were dehydrated with a graded ethanol. After then, the specimens were embedded in Epon 812 as a routine procedure and cut frontally by Porter-Blum MT-2B ultramicrotome. Sections were stained with uranyl and lead acetate and examined with JEM-100B electronmicroscope.

For light microscopical observation, some of the fetuses were fixed with 10% neutral formalin and embedded in wax. Frontal sections were cut at 5 μ and stained with hematoxylin-eosin, Mallory's trichrome staining and periodic acid Schiff (PAS) solution.

For the control, the normal fetuses at the same day of gestation (that is, on the 17th day of gestation) were used. The preparation procedure of them was the same as in the experimental group.

In the present study, light microscopical observation was surveyed on whole area of tongue and palatal processes, but electronmicroscopical observation was limited to the lateral sides of the tongue and to the medial aspects of palatal processes.

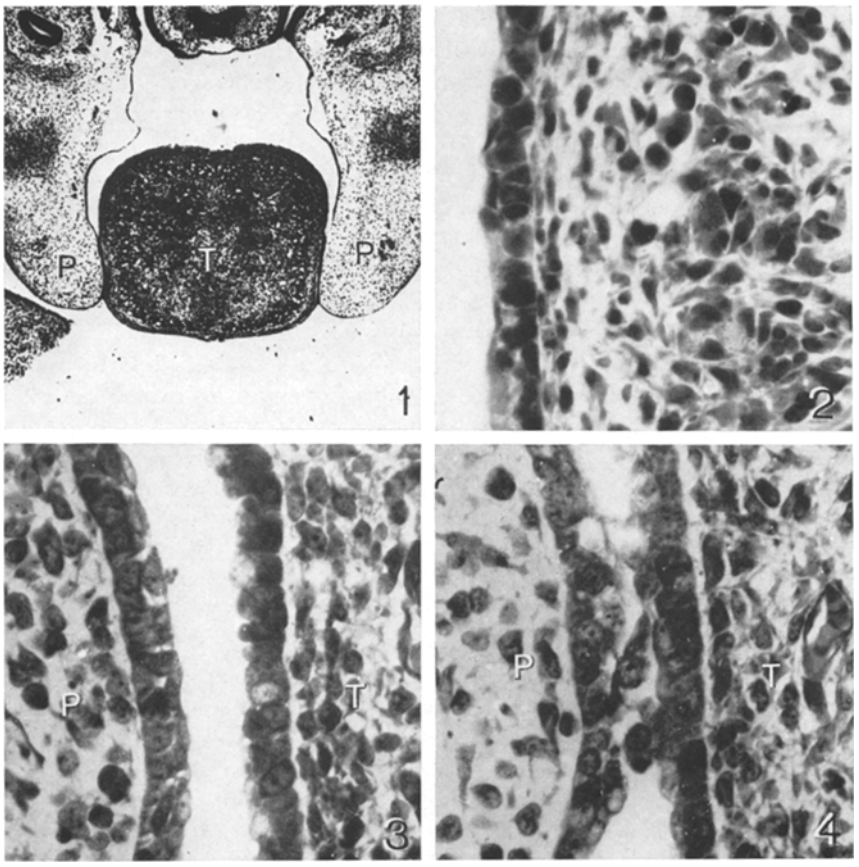
Result

Light Microscopical Observations

Under the authors' conditions, the glosso-palatal fusion including partial and complete fusion appeared in 95% of the fetuses from treated pregnant rats. In most of the fetuses, the palatal processes remained vertical, and the lateral side of tongue attached or fused with the medial aspects of palatal processes. These palatal processes looked swollen and somewhat retarded in growth (Fig. 1).

a) Tongue of the Control Group. The epithelium on the dorsal side of the tongue was made up a single layer of flat superficial cells, and three or four layers of oval basal cells. In general, their nuclei took an oval form and were provided with well defined nucleoli. The epithelium was various in thickness depending on its location, and the basement membranes was not always obvious at this developmental stage. Taste buds were developing in some areas, and the surface of tongue was not always smooth.

In the lateral and inferior sides of the tongue, the epithelium consisted of two or three layers of cells—flat superficial and cylindrical deep cells (Fig. 2). The surface of the epithelium was smooth, and the thickness of the epithelium kept constant in all areas. Their nuclei were oval and stained intensely with basic dyes and the cytoplasm were relatively pale. The epithelium was lined with obvious basement membrane, and subjacent to it, mesenchymal cells with irregular shapes were loosely distributed.



Figs. 1 to 4 are light micrographs of tongue and palatal processes. *P* palatal process, *T* tongue

Fig. 1. General view of glosso-palatal fusion induced with Meclozine-hydrochloride. Either sides of tongue are contacted with a medial side of palatal processes. The processes are somewhat swollen. Mallory's trichrome staining. × 40

Fig. 2. This is a high magnification of the lateral side of the control tongue excised from embryo at 17th day of gestation. Superficial cells are thin and flat. Nuclei of them are stained intensely. Hematoxylin and Eosin. × 600

Fig. 3. This figure shows the epithelia covering tongue and palatal process just prior to contact at the high magnification. Superficial cells in both epithelia — especially in the tongue epithelium—are swollen and have round nuclei. Epithelial cells are also round or irregular in shape. Mallory's trichrome staining. × 600

Fig. 4. This figure shows the early stage of glosso-palatal fusion. Several cells, which are locating between the opposed epithelia, connect both tissues. Mallory's trichrome staining. × 600

b) Palate of the Control Group. The palatal processes of the embryos at the same days of gestation were already fused, and epithelial remnants—epithelial pearls—were occasionally observed in the region of fusion. The medial side of the palatal processes constituted a floor of nasal cavity, and the epithelium on it became elongated and ciliated.

c) Tongue of the Experimental Group. The epithelium covering on the dorsal side of the tongue consisted of superficial slender cells and three to four layers of irregularly shaped cells. The cytoplasm of cells was swollen and stained intensely with eosin. The nuclei in those cells possessed dispersed chromatin and were relatively large. The mitosis appeared more frequently than that in controls. The basement membrane was not clearly seen.

The lateral side of the tongue was generally covered with two or three layers of cells (Fig. 3). The covering cells showed a swelling and took an irregular form, and their nuclei were relatively pale. Subjacent to this layer, one or two layers of deep cells with cylindrical or irregular shapes were present. The boundary between epithelium and mesenchyme was obvious. The lateral epithelium covering on the regions close to the palatal processes became less stained with eosin, and became to have relatively large and oval nuclei. Even in this region, mitosis was also observed, and the PAS positive granules were scattered in the cytoplasm.

The inferior surface of the tongue did not show a remarkable change, and this surface was covered with superficial flat and cuboidal basal cells. The nuclei of the basal cells were oval and contained a rich chromatin.

Summarizing the findings mentioned above, the whole epithelium of tongue was affected more or less with administration of Meclozine-hydrochloride, and the effect was discernible especially in the covering cells on the lateral side of tongue.

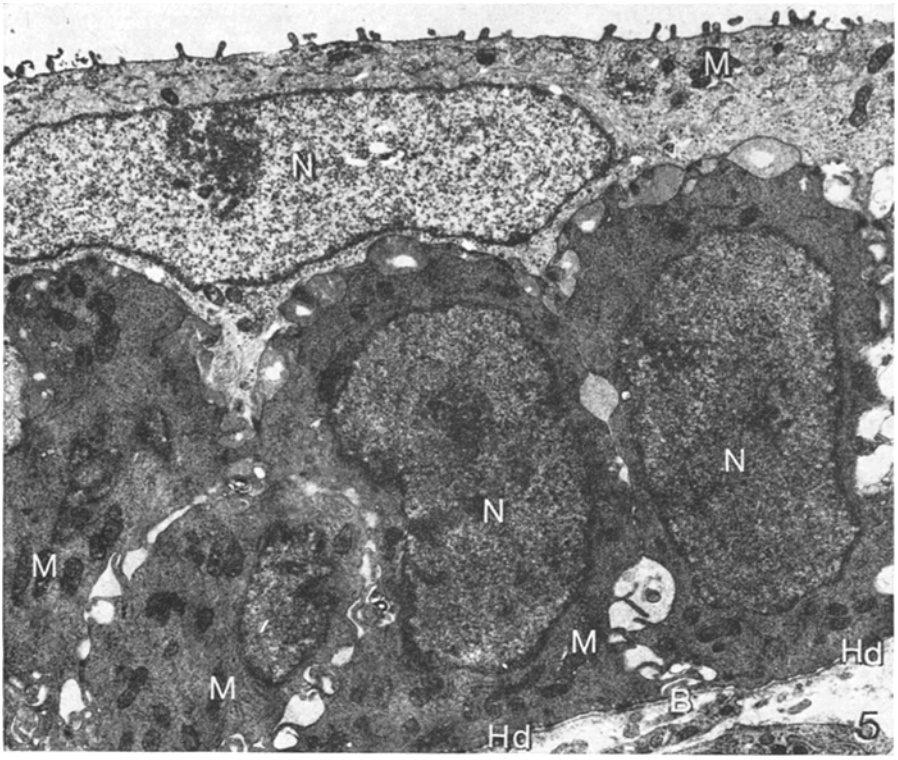
d) Palatal Process of the Experimental Group. The epithelium on the palatal processes consisted of superficial flat and two layers of cuboidal cells. Their nuclei were slender or oval. The basement membrane of the epithelium was obvious, and mesenchymal cells were loosely distributed. The epithelium was lined clearly by the basement membrane. However, the epithelium in the contact regions consisted of superficial swelling cells and oval or cuboidal basal cells (Fig. 4). Swelling cells were pale and their nuclei were stained faintly.

In the regions of contact or fusion, the intervening epithelium was seen between the lateral side of tongue and medial side of palatal process. It was composed of two or three layers of cells with irregular shapes. Most of nuclei took oval

Figs. 5 to 10 are electron micrographs of medial epithelium of palatal process and lateral aspects of tongue. Fig. 5 is obtained from the control specimen, and Figs. 6 to 10 from the experimental group. *B* basal lamina, *D* desmosome, *Db* dense body, *DeC* degenerating cell, *DeM* degenerating mitochondrion, *ER* endoplasmic reticulum, *F* fibrous structure, *G* Golgi apparatus, *Hd* half desmosome, *Ib* inclusion body, *M* mitochondrion, *N* nucleus

Fig. 5. This figure shows tongue epithelium of control group. Superficial cell is relatively light and forms cytoplasmic projections on the free surface. The cytoplasm of underlying cells are moderate in electron opacity and take a cylindrical shape. Occasionally, intercellular spaces are wide. The epithelium is lined with a defined basal lamina and provided with half desmosomes. × 5,700

Fig. 6. This is the tongue epithelium of experimental group. The superficial cells are various in shape and in electron opacity. Underlying cells look healthy. The basal lamina lines the epithelium. Inclusion bodies with various size appear in dark cell. The nucleus of the light cell is oval and contains dispersed chromatin. × 5,000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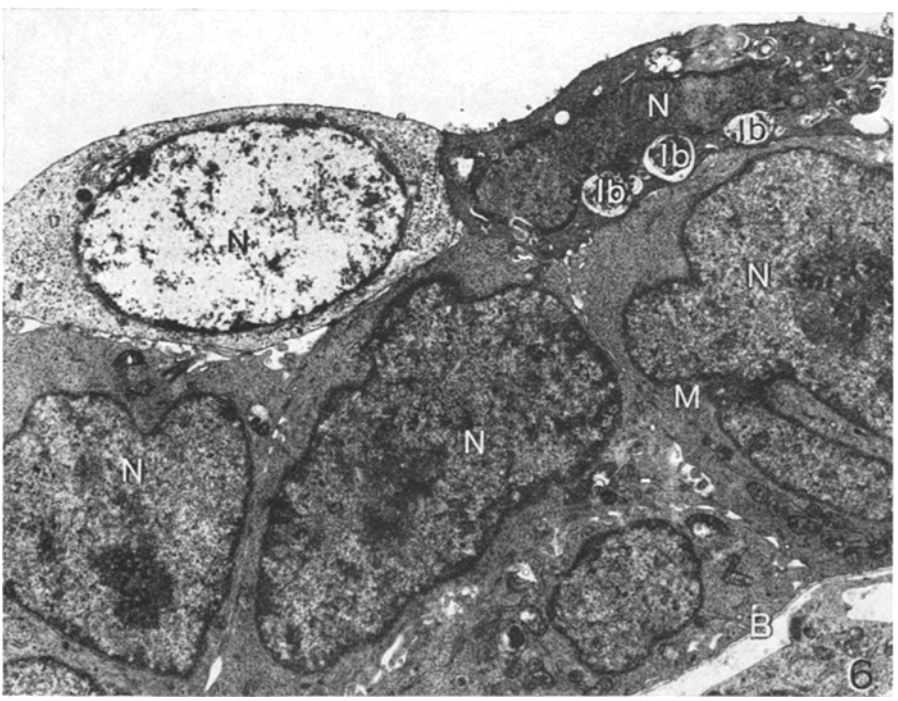
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forms, and some of them looked pycnotic. Often, PAS positive granules scattered in the cytoplasm. The boundary between the tongue and palatal processes became obscure, and along bilateral sides of the intervening epithelial seam, basement membrane looked faintly and fragmented. In a few cases, the epithelial seam broke down and penetration of mesenchymal cells occurred.

Electronmicroscopical Observations

a) Tongue Epithelium of the Control Group. The superficial flat cells were moderate in electron opacity and contained slender nuclei. Small projections were encountered on their free surfaces, and cytoplasmic organelles in these cells were chiefly distributed in peripheral parts (Fig. 5). The mitochondria were round and rod in shapes and were provided with distinct cristae. Endoplasmic reticula were very thin and small in number. Through the cytoplasm, some amount of polysomes, vesicles and fine fibrous structures were seen. Sometimes, Golgi apparatus consisting of lamellae and vesicles were prominent close to nuclei. The underlying cells were dark and cylindrical in shape. Their nuclei were relatively large and contained homogenous dense matrices. The mitochondria took oval or rod shapes, and the cisternae of endoplasmic reticula were somewhat dilated. In the cell boundaries between each cell, desmosomes developed. In some places, the cytoplasmic membranes were undulated and intercellular spaces became wide. The epithelium was clearly lined with basal lamina, and occasionally half desmosomes could be seen (Fig. 5).

b) As described afore, the palatal processes were already fused, and the palatal epithelium of the control group was differentiated into a nasal epithelium.

c) Tongue Epithelium of the Experimental Group. The profiles of the epithelium on the lateral side of tongue were rich in variety at the electronmicroscopical level too. That is, the cells varied in shapes with distance from the palatal process or the region of contact, and the superficial layer consisted of two kinds of dark and light cells (Fig. 6). The light cells were slender and had a scanty of mitochondria and thin endoplasmic reticula, whereas the dark cells looked swelling and contained several inclusion bodies of moderate size near nuclei. Within the bodies, degenerating cytoplasmic organelles and amorphous material were filled up. Their nuclei were dense and took irregular forms. The underlying cells looked rather normal, although their nuclei were irregularly shaped. Between the superficial and underlying cells, desmosomes or desmosome-like thickenings developed.

In the regions close to the contact, the superficial cells represented a marked degeneration and were finally detached from the epithelium (Fig. 7). That is, most of mitochondria in these cells showed a considerable swelling, and cristae of them were completely destructed. In the cytoplasm, a certain amount of fibrous structures were seen. The debrices of degenerating superficial cells were scattered on the cell surfaces.

d) Palatal Epithelium of the Experimental Group. The epithelium on the medial aspect of palatal processes consisted of two to three layers of cells (Fig. 8). In general, the profiles of cytoplasmic organelles in the superficial cells looked healthy, but frequently, the electron opacity increased moderately in superficial

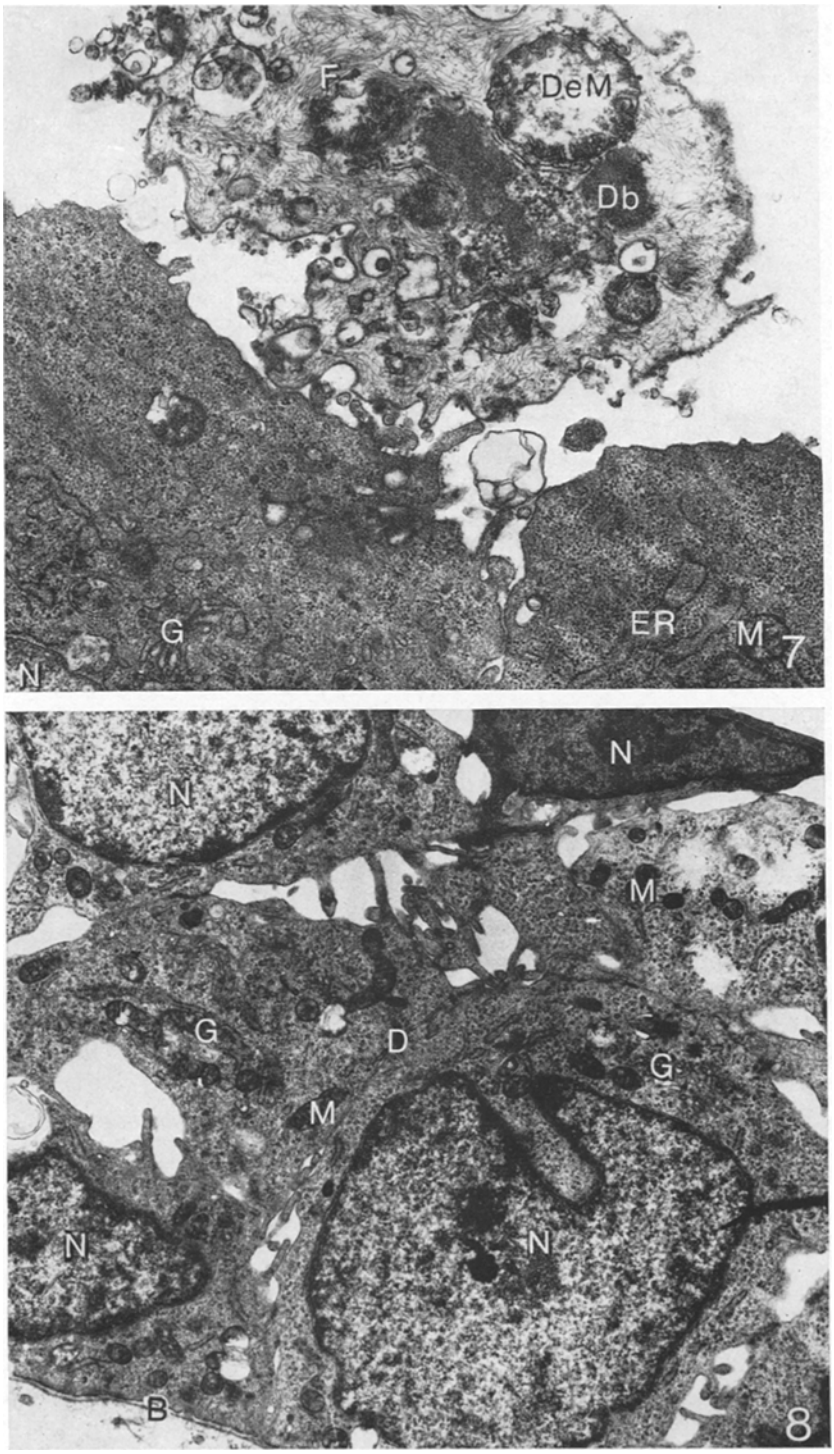


Fig. 7. This figure shows a dissociating cell from the tongue epithelium. The cell contains various shapes of degenerating signs and fibrous structures. × 16,000

Fig. 8. This is an epithelium on medial side of the palatal process. The superficial cells are dark and flat, and underlying cells are relatively light. Intercellular spaces are wide, and most of cells contact with cytoplasmic projections. × 7,600

cells, and their nuclei were irregular and pycnotic. The cells in the deep layer were oval or cuboidal in shape and were sometimes provided with well developed Golgi apparatus. The mitochondria were distributed through the cytoplasm and took round or rod shapes. The intercellular spaces became wide and the epithelium was lined with the obvious basal lamina. However, the epithelial cells close to contact area showed a swelling, and contained degenerating dark matrices (Fig. 9). Some cellular debrices were attached on the free surface of the cells.

In the region of fusion, the tongue epithelium was joining with the palatal epithelium. The mitochondria in those cells took on various forms and their cristae were destructed and obscure. Between two tissues, the epithelial lining was constituted. The intervening epithelial lining consisted of two or three layers of cells with various shapes (Fig. 10). Just after the contact, the cells kept the original features, and the discrimination of cells was not difficult. Generally, those cells in the lining possessed the prominent dense bodies and mitochondria with the obscure cristae (Fig. 10). Between the tongue and palatal epithelium, desmosomes appeared at the initial stage of contact. As the fusion proceeded, the intervening cells rearranged and degenerated. Along the epithelial lining, basal lamina were observed.

Discussion

As well known, the "fusion" appeared in the several portions of embryos—palatal, cervical, urethral, scrotal and other regions—during a normal development.

Microscopically, the process of fusion consisted of (1) epithelial contact of the opposed surfaces, (2) formation of intervening epithelium and (3) disintegration of epithelial lining between the two tissues. A complete fusion means a mesenchymal penetration through the two tissues.

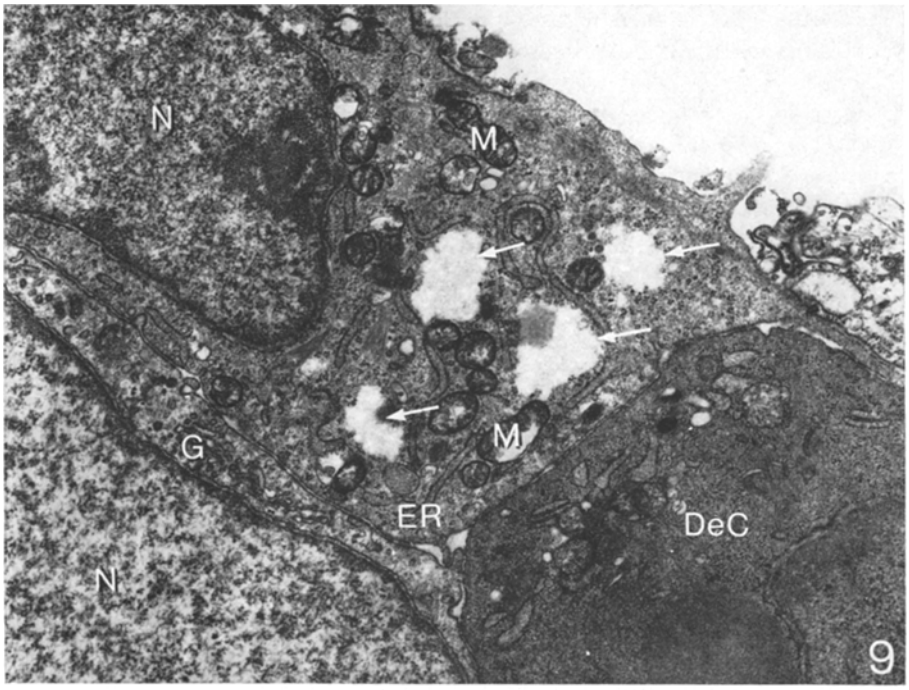
As reported previously by the authors (1966, 1967a, b, 1968 and 1972a, b) and others (Pourtois, 1968, Smiley and Dixon, 1968 and Smiley and Koch, 1971), in the epithelium on the medial edges of the palatal processes, specific changes occurred prior to a formation of secondary palate. It is of very interest that these cellular changes were localized only in the opposed surfaces which were physiologically destined to fuse.

On the basis of these evidences, the authors postulated a hypothesis that a kind of degeneration in the epithelial cells was one of essential events in the process of fusion.

However, some drugs could induce pathological heterotopic fusion, for instance, glosso-palatal, glosso-septal and glosso-alveolar fusions. That is, King (1963) observed a glosso-palatal fusion after treatment of Meclozine-hydrochloride, and Koziol and Steffek (1969) induced glosso-palatal fusion with an administration of

Fig. 9. This is a palatal epithelium close to a region of fusion. Cell debrices scatter on the free surface, and a degenerating cell (at the lower, right of this figure) is adjacent to an intact cell having elongated endoplasmic reticula and round mitochondria. Amorphous materials can be seen (arrows). × 13,500

Fig. 10. This figure shows an epithelial seam at the later stage. In it, degenerating cells having dense inclusion bodies are present randomly. Attachment devices are observed between the cells. Along the seam, basal lamina are clearly observed. × 7,300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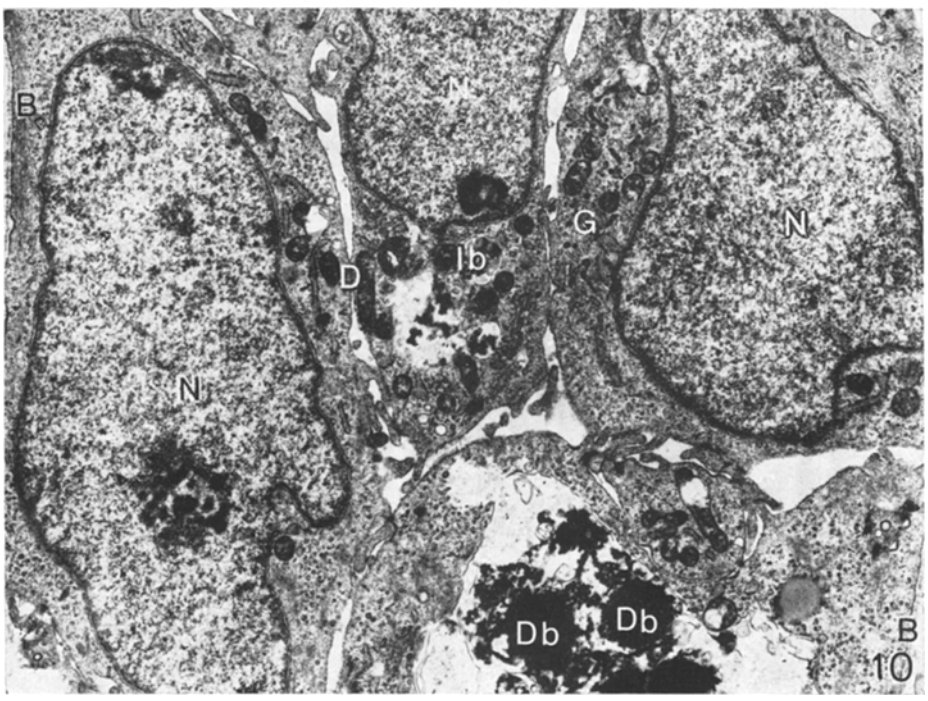
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Chlorcyclizine. But in their reports, the detailed description as to the epithelial behavior of tongue and palatal processes during glosso-palatal fusion could not be found.

Concerning heterotopic fusion, Goss, Bordner and Avery (1970) asserted that the glosso-palatal and the other heterotopic fusions were completed, if the two tissues kept contact with each other for an enough time. Their evidences were obtained from experiments using artificial culture-technics. However, according to unpublished data of us, the tongue was difficult to keep a living state and rather rendered to degenerate in vitro. Under such condition, the fusion between two tissues could easily occur.

In 1968, Vargas presented his hypothesis concerning a lytic factor in the fusion of palatal processes. By his opinion, the capacity of fusion between tongue and palatal processes was also depended on the presence of a lytic factor which was contained only in the palatal process. Therefore, the parallelly setted tongues could not fuse with each other even in vitro, but the fusion between tongue and palatal process was completed. However, in spite of this lytic factor, under physiological conditions, the palatal processes did not fuse with tongue except pathological conditions.

From the present study, it becomes clear that the administration of Meclozine-hydrochloride at a certain developmental stage enhanced a marked degeneration to the epithelia of developing tongue and palatal processes and subsequently induced a heterotopic fusion between the two.

According to pharmacological studies, most of miner tranquilizers were believed to suppress the production of histamine and biogenic amines. For the authors, it seems also to be significant that such tranquilizers have a possibility to make a strong influence to the differentiation of embryonic epithelium.

At present, the authors intend to know the role of histamine and biogenic amines during a morphogenesis of embryos.

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