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Ultrastructure of metaplastic ciliated cells in human stomach

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Summary. Intestinal metaplasia of the gastric mucosa occurs commonly in aged Japanese patients and has been discussed in relation to the high incidence of gastric cancer in Japanese. Ciliated cells in the gastric mucosa have frequently been found in association with intestinal metaplasia in the pyloric gland and rarely in the cardiac gland in many Japanese patients, and exceptionally in one Chinese and in one Swedish patient. Electron microscopic examination of 12 Japanese patients has revealed that these structures are not metaplastic stereocilia, but true cilia. Ciliated cells have been found in the basal part of the gastric glands and never in the surface epithelium. The fine structure of the gastric cilia was almost the same as that of normal respiratory cilia. However, in the gastric cilia, most dynein arms were inconspicuous even after tannic acid fixation, indicating that ciliary beating of the gastric cilia is problematic. Abnormal cilia and basal bodies also were found. Ciliated cells have always occurred in association with intestinal metaplasia, therefore this phenomenon might be a type of metaplasia and is named "ciliated metaplasia" of the gastric mucosa.

Key words: Gastric mucosa – Intestinal metaplasia – Ciliated cell – Ciliated metaplasia – Ultrastructure

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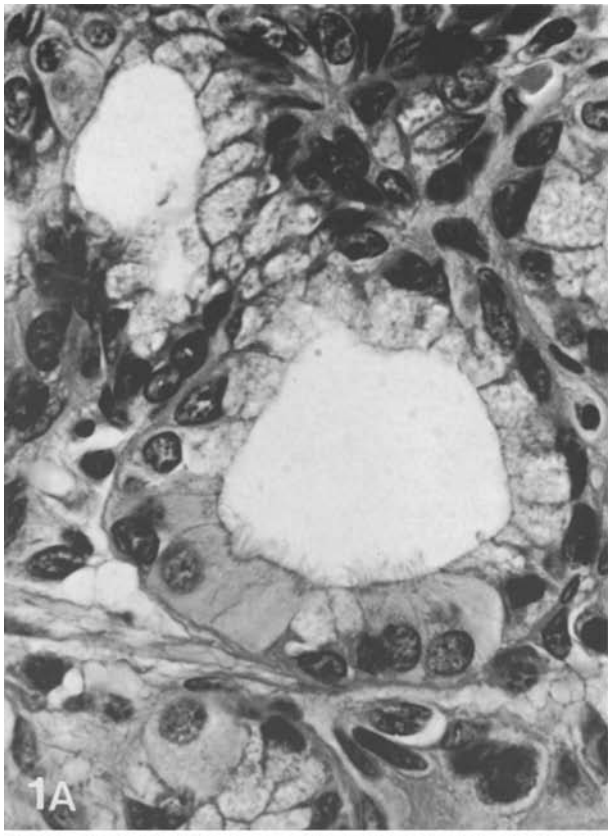
Introduction

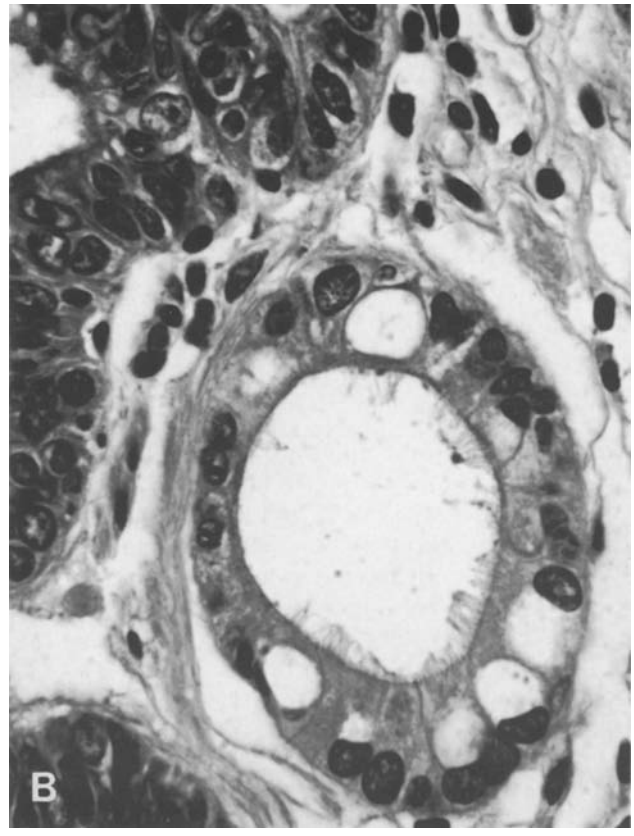
The presence of ciliated cells in human gastric mucosa in association with intestinal metaplasia, mainly in Japanese patients, has been reported (Kodaira et al. 1974; Okuda and Ogata 1976; Yamashiro et al. 1977; Rubio and Kato 1986; Torikata et al. 1986; Kawakita 1987), and a new term, "ciliated metaplasia" in the gastric mucosa has been proposed. Some reports have been based on light microscopic observations or only reported the presence of cilia observed by electron microscopy.

Table 1. Metaplastic gastric cilia: cases and clinicopathological data

No	Age/Sex	Main lesion	Intestinal metaplasia	Ciliated cell
1	72/F	Oesophagus ca, SCC	mod, complete, P(−)	cardiac + +
2	73/M	Gastric ca, Tub	mod, complete, P(−)	cardiac + + +
3	63/M	Gastric ca, Tub	mod, complete, P(+)	pyloric + +
4	67/M	Gastric ca, Sig	mod, complete, P(+)	pyloric + +
5	68/M	Gastric ca, Tub	mod, complete, P(+)	pyloric + +
6	79/F	Gastric ca, Pap	mod, complete, P(+)	pyloric + +
7	57/M	Gastric ca, Tub	mod, complete, P(+)	pyloric + + +
8	63/M	Gastric ca, Tub	mod, complete, P(+)	pyloric + +
9	72/M	Gastric ca, Tub	mod, complete, P(+)	pyloric + +
10	73/M	Gastric ca, Sig	mod, complete, P(+)	pyloric + + +
11	73/M	Gastric ca, Sig	mod, complete, P(+)	pyloric + + +
12	77/M	Gastric ca, Tub	mod, complete, P(+)	pyloric + +

SCC, Squamous cell carcinoma; Tub, Tubular adenocarcinoma; Pap, Papillary adenocarcinoma; Sig, Signet ring cell carcinoma; mod, moderate; complete, complete type of intestinal metaplasia; P, Paneth cell. Incidence of gastric glands containing ciliated cells: +, less or 5; + +, 6–20; + + +, more than 20





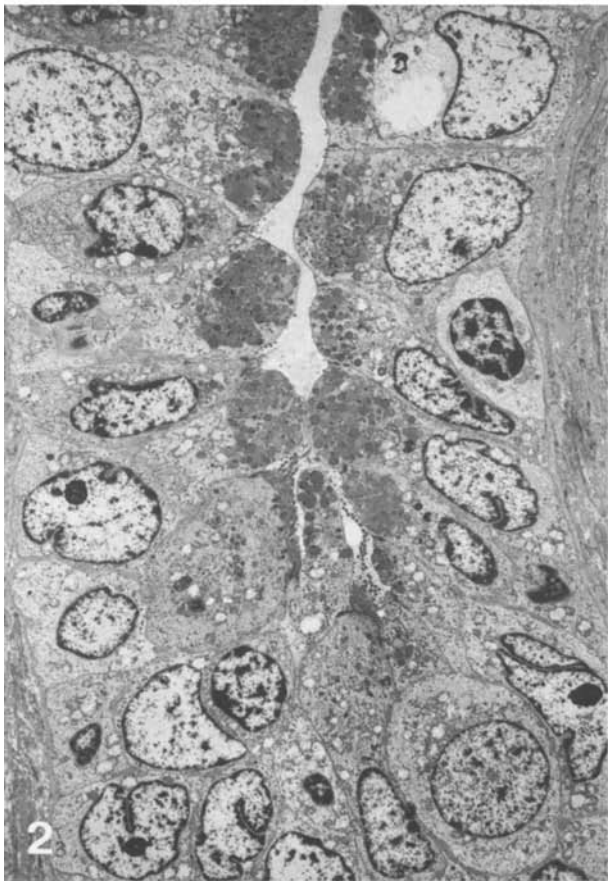
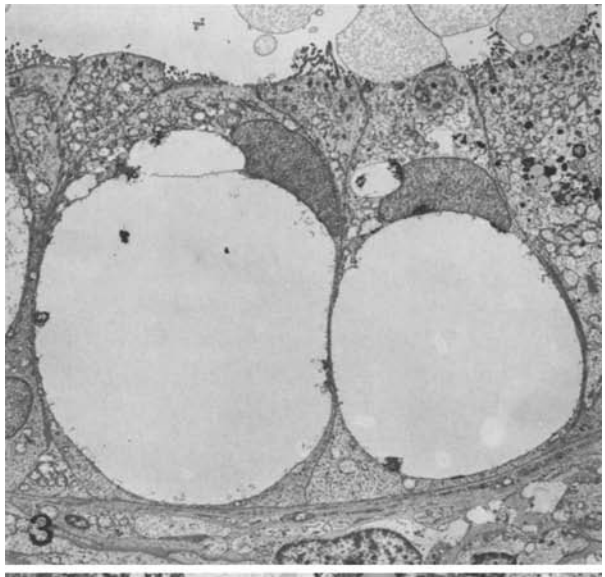


Fig. 1. Ciliated cells in human gastric mucosa. **(A)** In the cardiac gland, ciliated cells are noted in the basal part of the glands singly or in a cluster among normal-looking mucus cells. Ciliated cells have eosinophilic (*dark*) cytoplasm and numerous cilia on the apical surface. **(B)** In the pyloric gland, ciliated cells have large non-mucus vacuoles in the cytoplasm. In the *upper part* of the glands, intestinal metaplasia was noted. **(A)** Case No 1. **(B)** Case No 5. H–E, ×800

Fig. 2. Ciliated cells in the pyloric gland. Ciliated cells are noted in the basal part of the gland among normal-looking pyloric mucus cells. Ciliated cells have abundant filaments in the cytoplasm. Case No 10. ×2000



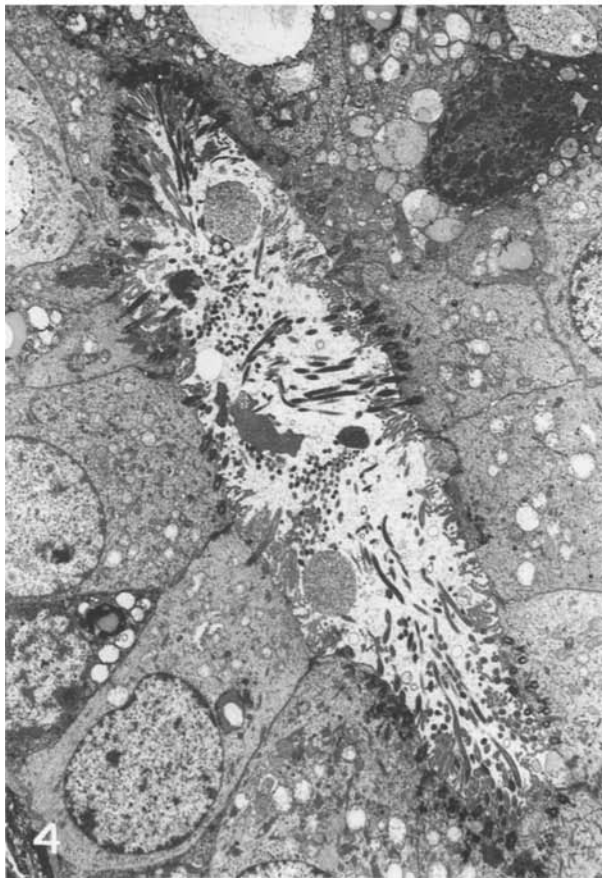


Fig. 3. Mucus-negative large vacuoles in the ciliated cells of pyloric gland. Large vacuoles are limited by a unit membrane and contain electron-lucent material. Cilia and basal bodies are note in the apical portion. Case No 9. ×2100

Fig. 4. Ciliated cells in the pyloric gland. This gland consists of numerous ciliated cells. Cilia, microvilli and basal bodies are noted in the apical surface of the cells. Unusual electron dense structures are noted in close relation to basal bodies. Case No 10. ×4200

The fine structure of metaplastic gastric cilia has been briefly reported by several authors (Kodaira et al. 1974; Okuda and Ogata 1976; Yamashiro et al. 1977; Torikata et al. 1986; Kawakita 1987). Additionally a few abnormal cilia have been presented (Kodaira et al. 1974) and 9+3 and 9+4 abnormal configuration in an axoneme (Yamashiro et al. 1977).

We have studied numerous gastric cilia found in 12 Japanese stomachs, some of which were fixed directly with 1% glutaraldehyde containing 1% tannic acid. Tannic acid fixation is useful for investigation of microtubules and dynein arms of the cilia (Mizuhira and Futaesaku 1972; Tilney et al. 1973; Torikata 1985).

In this paper, we will present the fine structure of the metaplastic gastric cilia in comparison with that of normal respiratory cilia and also some abnormal cilia and basal bodies found in the gastric mucosa.

Materials and methods

The human stomachs used in this study were obtained by surgical resection for gastric or oesophageal malignancies. Age and sex, clinical diagnosis and site of ciliated cells are summarzied in Table 1. Formalin fixed gastric mucosae in which considerable numbers of ciliated cells had been observed by light microscopy were cut and washed in water, postfixed with 1% osmium tetroxide, dehydrated with graded alcohols and acetone, and embedded in Spurr's resin. Ultrathin sections were cut and doubly stained with uranyl acetate and lead. Fresh gastric mucosae from male patients over 50 years old were directly fixed with 1% glutaraldehyde mixed with 1% tannic acid (Mizuhira and Futaesaku 1972) and then processed by the procedures described above. Stained ultrathin sections were observed under a JEOL100C electron microscope at 100 kV of accelerating voltage.

Results

Ciliated cells were found in the cardiac gland in 9 cases and in the pyloric gland in more than 50 cases in association with so-called intestinal metaplasia: the occurrence of absorptive cells, goblet cells and Paneth cells on light microscopy. Ciliated cells were located in the basal parts of the gland mixed with normal-looking mucus cells singly or in a cluster and never in the surface epithelium, however in the upper part metaplastic cells were commonly observed. Ciliated cells had eosinophilic cytoplasm and in the pyloric gland not infrequently had large mucus-negative vacuoles (Fig. 1).

By electron microscopy, ciliated cells were observed in 2 cases in the cardiac gland and in 10 cases in the pyloric gland. From No. 1 to No. 6, blocks were made from formalin-fixed tissues;

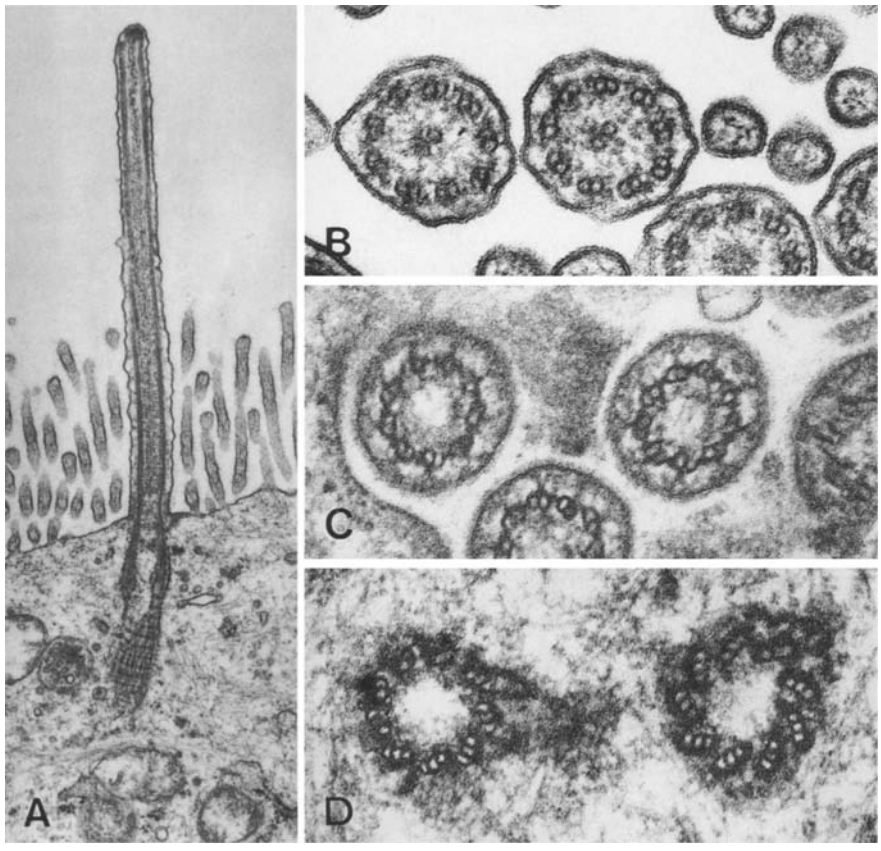
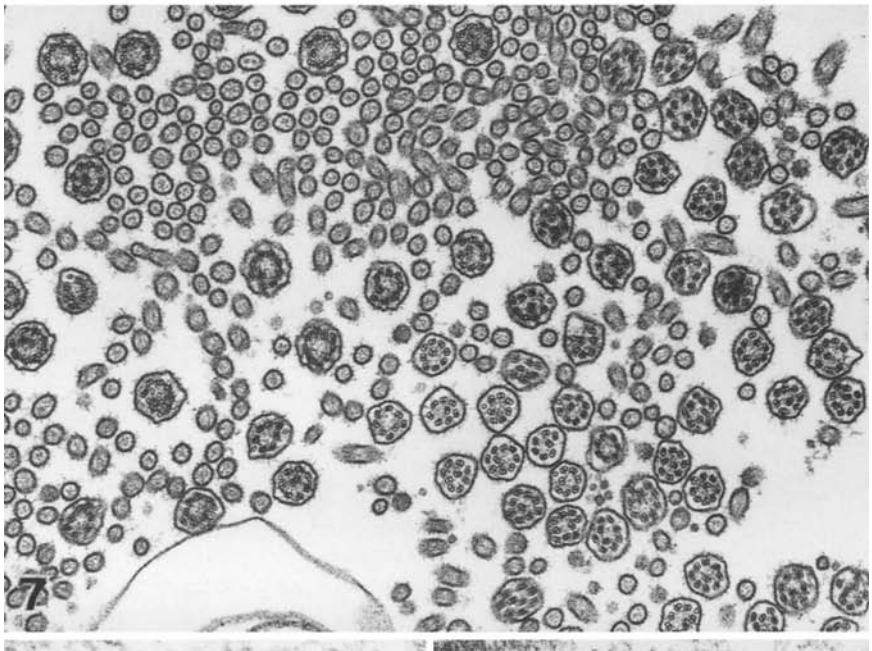


Fig. 5. Fine structure of metaplastic gastric cilia. **(A)** Longitudinal section of a gastric cilium. A longitudinal-sectioned cilium displays the ciliary shaft and the basal body with the ciliary rootlet. In the neck, microtubule-membrane structures and several intramembrane granules, corresponding to the ciliary necklace, are seen. In the basal body, alar spokes are noted between the upper part of a basal body and the apical cell membrane and the ciliary rootlet from the lower part into the cytoplasm. Case No 5. ×20000. **(B) (C) (D).** Cross sections of the ciliary shaft **(B)**, the neck **(C)** and the basal body **(D)**. Nine peripheral doublets in a ring and two central singlets (9+2 configuration) with dynein arms and radial spokes are seen in the ciliary shaft **(B)**. Nine peripheral doublets without a central pair (9+0) with Y-shaped microtubule-membrane structures are seen **(C)**. Nine triplets in a ring with a basal foot form the basal body. Electron-dense structures around the basal bodies are seen **(D)**. **(B)** Case No 10. **(C), (D)** Case No 4. ×94000

however tissue preservation was not bad and the fine structure of the cilia was observable. Among more than 10 specimens directly fixed with glutaraldehyde, we found considerable numbers of ciliated cells in the pyloric gland of 6 male patients. Electron microscopic observation revealed ciliated cells among mucus cells singly or in a cluster and they had abundant filaments in the whole cytoplasm. Autophagosomes were sometimes prominent in the supranuclear region (Fig. 2). In the pyloric gland, autophagosomes produced a cystic change and contained electron lucent material consisting of small amounts of lipid droplets and lamellar bodies (Fig. 3). Neither mucus nor neuroendocrine granules were noted in the ciliated cells. Numerous cilia were found on the apical surface and in the concave channels mixed with several microvilli; they were about 5 to 6 μ in length and 0.2 μ in width (Fig. 4).

The fine structure of the gastric cilia was almost the same as that of the respiratory cilia, consisting of a ciliary shaft, a neck and a basal body. In the cross section of the ciliary shaft, a 9+2 configuration with nexin link, radial spokes and a central sheath were noted. A few cilia had dynein arms but in most, these were inconspicuous even after tannic acid fixation. In the neck, 9+0 configuration of microtubules was noted and after tannic acid fixation intramembrane granules which corresponded to the ciliary necklace were found by transmission electron microscopy. In the upper part of the neck, a short basal plate was noted. In the basal bodies, nine triplets were arranged regularly and formed a short cylindrical structure like a centriole. Like the basal apparatus, the basal foot, the ciliary rootlet and the alar spokes they were found in connection with a basal body (Figs. 5, 6).



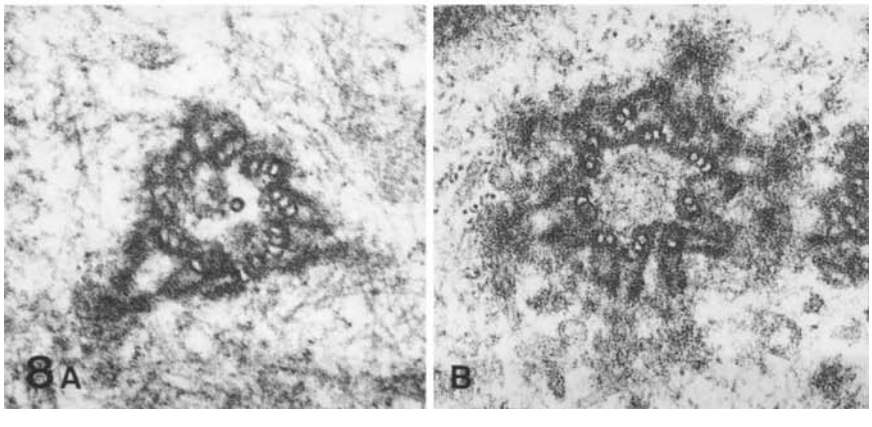


Fig. 6. An apical cross section of a ciliated cell in the pyloric gland. Cilia in the concave channels and basal bodies in the apical cytoplasm are arranged randomly. In the cilia, abnormal numbers and dislocation of the microtubules are seen. Alar spokes, basal feet and ciliary rootlets are noted in connection with the basal bodies. Unusual electron-dense structures are noted in close relation to the basal bodies. Case No 10. ×30000

Fig. 7. A transverse section of the gastric cilia. Electron microscopy reveals an anomalous axonal substructure, malposition of outer doublets and deletion of both outer and central tubules. Note the absence of compound cilias in this case. Case No 9. ×30000

Fig. 8. Abnormal basal bodies in the gastric cilia.
(A) This basal body has an abnormal singlet inside the nine triplets and also has two basal feet.
(B) This basal body has 7 basal feet.
(A) Case No 4. **(B)** Case No 9. Both ×94000

Table 2. Ultrastructural comparison of respiratory cilia and metaplastic gastric cilia

	Respiratory cilia	Gastric cilia
Normal configuration in the cross section		
In ciliary shaft	nine peripheral doublets in a ring and two central singlets; 9+2.	same
In neck	nine peripheral doublets, only; 9+0.	same
In basal body	nine triplets in a ring	same
Associated structures in ciliary shaft		
Nexin link	present	present
Radial spokes	present	present
Central sheath	present	present
Dynein arms	present	present?
Associated structures in neck		
Basal plate	present	present
Ciliary necklace	present	present
Associated structures in basal body		
Basal foot	present	present
Ciliary rootlet	present	present
Alar spokes	present	present
Autophagosomes	present	present
Non-mucus vacuole	not observed	present
Ciliary motility	motile	?
Abnormal cilia and basal bodies		
In ciliary shaft		
Lack of central pair	rare	frequent
Dislocation of doublet	rare	frequent
Excess singlet	a few	a few
Lack of dynein arms	common in congenital*	common?
Random arrangement	common in congenital*	common
Knob	frequent	not observed
Swollen cilia	rare	not observed
Compound cilia	not infrequent	not observed
Intracytoplasmic cilia	not infrequent	not observed
In basal bodies		
Excess singlets	rare	rare
Two or more basal feet	rare	a few
Random arrangement	common in congenital*	common

* in immotile cilia syndrome

In many cases, cilia were found in concave channels, possibly meaning that these ciliated cells were not fully developed. One of the common abnormalities observed was random arrangement of the cilia and the basal bodies (Fig. 6). Abnormalities found in the ciliary shaft were lack of a central pair and location of the doublets sometimes in the central area; 8 doublets in a ring and one centralized doublet. An excess number of singlets in an axoneme was occasionally found, such examples included 9 doublets in a ring and 4 central singlets, 9+4; and 6 central ones, 9+6 (Fig. 7). The most important finding was the lack of definition of the dynein arms. Definite arms were found only in a few cilia, and in most, the arms seemed to be absent or incomplete even after tannic acid fixation. Whether these gastric cilia are motile or immotile is a key point. Among the gastric cilia, no compound cilia of the type commonly found in abnormal respiratory cilia were found in our 12 cases.

In the basal bodies, an abnormal number of basal feet and rarely excess microtubules were found inside the 9 triplets (Fig. 8). All of the abnormalities mentioned above have been observed in the respiratory cilia; however additional unusual structures were seen in 2 cases, a 73-year-old man and a 63-year-old man both with gastric cancer. In the apical portion of the ciliated cells, electron-dense intracytoplasmic inclusions were found in close relation to the basal bodies. High magnification revealed that they consisted of electron dense granular and filamentous counterparts and were sometimes closely connected with the top of the basal foot. In longitudinal sections, they looked like paracrystalline structures showing even-spaced tubular structures with cross-bridgings between them (Figs. 4, 6). Details of their structure will be presented elsewhere (Table 2).

Discussion

Intestinal metaplasia occurs commonly in aged Japanese and this fact has been discussed in relation to the high incidence of gastric cancer in Japanese (Imai et al. 1971).

Ciliated cells in the stomach have been found associated with intestinal metaplasia, mostly in Japanese patients and in one Chinese (Chang et al. 1986) and one Swedish patient (Rubio and Serck-Hanssen 1986). The occurrence of ciliated cells in gastric mucosa is thought to be acquired, not a congenital heterotopia. In the pyloric mucosa of Japanese patients, ciliated cells were found in 23 cases out of 50 consecutive resected stomachs and in 19 out of 32 over 50-year-old patients in our preliminary study; this might be a common phenomenon in old Japanese. A new term "ciliated metaplasia" has been proposed (Torikata et al. 1986; Rubio and Kato 1986) and should be included in textbooks as a new type of metaplasia.

The fine structure of the respiratory cilia has

been fully studied and dynein arms are the most important structure in ciliary beating (Satir 1965; Fawcett 1981; Warner 1981). The fine structure of the metaplastic gastric cilia in both the pyloric and the cardiac mucosa was the same as that of the respiratory cilia: the basic composition of microtubules, nexin links, radial spokes, a basal apparatus and even a ciliary necklace (Gilula and Satir 1972). An exceptional but most important difference was the poor definition of the dynein arms of the gastric cilia even after tannic acid fixation. Dynein arms have a very important role in ciliary movement and thus beating of the gastric cilia is doubtful from ultrastructural findings. Although it is extremely difficult, direct observation of living ciliated cells is necessary to determine ciliary motility.

Several types of abnormal cilia were found in the gastric mucosa which have been found in respiratory cilia (Ailsby and Ghadially 1975; McDowell et al. 1976; Torikata et al. 1976; Afzelius 1979). The most curious phenomenon was the complete absence of compound cilia in the gastric mucosa. Compound cilia are frequently found in the respiratory cilia and fusion of the ciliary membrane (Ailsby and Ghadially 1975), probably due to virus infection, has been proposed as a formative mechanism. However, the gastric environment is clean and aseptic in contrast with the respiratory tract.

The electron-dense structures found in the apical portion of the metaplastic gastric ciliated cells in this study have never been reported in human ciliated cells. The highly organized internal structures and the close relation to the top of the basal foot suggest aggregates containing tubulin molecules which might be related to ciliogenesis. As to formation of the unusual aggregates, the effect of antitumour drugs and anaesthetics might be implicated; however, neither patient was administed any antitumour drug before the operation. Ethrane (enflurane) was used as an anaesthetic and has been reported to be toxic to the microtubules (Hinkley 1976).

Electron microscopy clearly demonstrated the presence of ciliated cells in the gastric mucosa and revealed the fine structure of the gastric cilia. Our interpretation of the occurrence of ciliated cells in the gastric mucosa is that it is a metaplasia, but the function of these gastric cilia still remains obscure.

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