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Amylase in human lungs and the female genital tract

Histochemical and immunohistochemical localization *

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Summary. We investigated the localization of amylase in normal human lungs and the female genital tract using immunohistochemical and histochemical methods. Immunohistochemical procedures were applied to formaldehyde-fixed, paraffin-embedded specimens as well as to cryostat sections of periodate/lysine/paraformaldehyde (PLP)-fixed tissues. The starch-substrate-film method was used for the histochemical investigation of unfixed frozen sections. Amylase immunoreactivity was observed in ciliated epithelial cells of the bronchus and in serous cells of the bronchial glands but not in the alveolar epithelium. Immunoreactive amylase was also found in the cytoplasm of the ciliated epithelium of the fallopian tubes, especially in the apical part of the cytoplasm and in ciliary vesicles. Immunoreactive amylase was also found to be present in the surface epithelial cells and glands of the uterine cervix, as well as in the superficial part of the endometrial glands. The distribution of amylase activity revealed using histochemistry was similar to that observed in cryostat sections of PLP-fixed tissues after immunohistochemical staining. Amylase antigenicity was better preserved in cryostat sections of PLP-fixed materials than in formaldehyde-fixed, paraffin-embedded specimens. The results are discussed in relation to pulmonary and female-genital-tract diseases.

Introduction

The presence of salivary-type amylase has been demonstrated in various tumor tissues, particularly in tumors of the lung (Harada and Kitamura 1971; Ammann et al. 1973; Gomi et al. 1976; Otsuki et al. 1977; Morohoshi et al. 1980; Maeda et al. 1982) and ovary (Nakayama et al. 1976; Corlette et al. 1978; Hayakawa et al. 1984; Hodes et al. 1985). In neoplasms of organs other than the pancreas and the salivary gland, the presence of amylase has been considered to be an example of the 'ectopic production' of enzymes in cancer tissues. On the other hand, amylase activity has been found in bronchial secretions (Clarke et al. 1981), in tissue extracts of the lungs (Otsuki et al. 1977; Maeda et al. 1982), in secretions of the fallopian tubes (Green 1957; Nakayama et al. 1976), and in homogenates of the female genital tract (Robles et al. 1972; Skude et al. 1976). However, the histological localization of amylase-containing cells in normal lungs and the female genital tract has not been examined in detail (Bruns et al. 1982).

In the present study, we investigated the localization of amylase in normal lungs and the female genital tract using both histochemical and immunohistochemical methods. Precise knowledge of the localization of amylase may extend our understanding of the ectopic production of enzymes in tumor tissues, and may also provide some insight into the as yet unknown function(s) of amylase.

Materials and methods

Tissue samples. Tissue samples were obtained from non-neoplastic portions of surgically resected specimens. The samples comprised lobar or segmental bronchi ($n=10$; age of subjects, 51–73 years), lungs ($n=5$; age of subjects, 55–72 years), endometrium and uterine cervical mucosa ($n=10$; age of subjects, 37–61 years), fallopian tubes ($n=9$; age of subjects, 40–54 years), and ovaries ($n=5$; age of subjects, 33–71 years). For female genital tract, the phase of the menstrual cycle was determined from the date of the last menstruation and from the histology of the uterine glands. Of the specimens of uterus and uterine cervix, four were in the proliferative phase, three were in the secretory phase, and three were at the postmenopausal stage. Of the specimens of fallopian tubes, three were in the proliferative phase, three were in the secretory phase, two were in the menstrual phase, and one was at the postmenopausal stage. Ovaries were obtained during proliferative phase ($n=2$), secretory phase ($n=1$), and postmenopausal stage ($n=2$).

Preparations of samples. Tissue samples were obtained within 15 min of surgical removal and were processed using three different procedures:

1. Tissues were placed in embedding medium (Tissue-Tek, OCT Compound; Miles Laboratories, USA), rapidly frozen in liquid nitrogen, and cut (thickness, 8 μm) using a cryostat (Pearse New Type; Bright Instruments, UK) before histochemical examination.

2. Tissues (thickness, 3 mm) were fixed in periodate/lysine/paraformaldehyde (PLP; McLean and Nakane 1974) at 4 °C for 18–24 h, washed in 10 m*M* phosphate-buffered saline (PBS), pH 7.2, for 24 h, and immersed in Holt's hypertonic gum sucrose solution for 24 h at 4 °C. The fixed specimens were embedded in Tissue-Tek (OCT Compound), frozen in liquid nitrogen, and sectioned (thickness, 5 μm) using a cryostat. The sections were kept at −20 °C and were subjected to immunohistochemical procedures within 2 weeks of their preparation.

3. Tissues were fixed in 10% neutral buffered formaldehyde for 1–3 days, dehydrated in ethanol, cleared in chloroform, and embedded in paraffin. The sections were cut at a thickness of 4 μm.

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Histochemistry. The starch-substrate-film method of Shear and Pearse (1963) was used to obtain histochemical staining for amylase. Cryostat sections were prepared according to procedure 1, and then mounted on glass slides coated with films of soluble starch (Merck, FRG). After incubation at 37 °C for 45 min, the slides were fixed in 10 min in methanol/acetic acid/distilled water (50:10:50), rinsed under running water for 1 min, and stained with Lugol's iodine for 1 min.

Immunohistochemistry. Amylase was extracted from human salivary glands and purified as previously described (Yamashita et al. 1980). An antiserum against salivary-type amylase was obtained by immunizing a rabbit with the purified amylase. The Ouchterlony test and immunostaining of human pancreas sections with this antiserum revealed the presence of cross-reactivity against pancreatic-type amylase. Swine antirabbit immunoglobulins and soluble peroxidase-antiperoxidase (PAP) complex were obtained from Dakopatts (Denmark).

The PAP method of Sternberger (1979) was applied to specimens processed according to procedures 2 and 3. Sections of formaldehyde-fixed, paraffin-embedded specimens were pretreated by being deparaffinized in xylene, rehydrated in a graded series of ethanols, and then immersed in methanol containing 0.3% hydrogen peroxide for 20 min in order to block endogenous peroxidase activity. The sections were then washed three times (5 min each) in PBS and were treated with 0.1% pronase (type VII; Sigma, USA) in 100 m*M* phosphate buffer, pH 7.4, for 1 min. The staining procedure used has been described elsewhere (Fukayama et al. 1986). In brief, pretreated formaldehyde-fixed, paraffin sections and PLP-fixed sections were incubated with the primary antiserum to amylase (1:200) for 1 h at room temperature. The sections were then incubated with swine antirabbit immunoglobulins (1:300) for 30 min, after which the PAP complex (1:200) was applied for 30 min at room temperature. Between each incubation, the sections were washed three times (5 min each) in PBS. The peroxidase reaction was developed by adding 0.02% 3-3′-diaminobenzidine, 0.005% hydrogen peroxide, and 10 m*M* sodium azide in 50 m*M* *Tris*-buffer (pH 7.6) for 10 min. The sections were counterstained with Mayer's hematoxylin.

Control experiments were carried out using nonimmune rabbit serum and antiamylase serum preabsorbed with the purified salivary amylase.

Results

The results concerning the localication of amylase as revealed by histochemical and immunohistochemical procedures are summarized in Table 1. The results obtained by immunostaining cryostat sections of PLP-fixed tissues were in agreement with those obtained by the histochemical staining of unfixed frozen sections, although morphological details were more sharply delineated in the sections stained using the immunohistochemical method. The formaldehyde-fixed, paraffin sections, in which the morphological features were best preserved, exhibited immunostaining of a low intensity. More amylase-positive cells were found in PLP-fixed sections than in formaldehyde-fixed, paraffin sections.

Bronchi and lungs

Bronchi. Immunohistochemical staining of PLP-fixed sections produced positive granular staining for amylase in the apical part of ciliated epithelial cells (Fig. 1A) and in the serous cells of the bronchial glands (Fig. 1B). However, in formaldehyde-fixed, paraffin-embedded sections, amylase immunoreactivity was not observed in ciliated epithelial cells. Furthermore, granular staining for amylase in serous cells of the bronchial glands was demonstrable in only three of the ten formaldehyde-fixed, paraffin-embedded samples. Fewer amylase-positive cells were observed in formaldehyde-fixed, paraffin sections than in PLP-fixed sections.

Histochemical staining showed the presence of amylase activity in the apical part of the surface epithelium as well as in the bronchial glands (Fig. 1C).

Alveoli. Amylase was not detectable in the alveolar epithelium using either the immunohistochemical or the histochemical methods.

Table 1. Reactivity for amylase observed using histochemical and immunohistochemical procedures

Organ	Histologic site	Staining procedure		
		Histochemical staining	Immunohistochemical staining	
			PLP/cryostat[a]	Formaldehyde/paraffin[b]
Lung				
Bronchus	Ciliated epithelial cells	+	+	−
	Serous glandular cells	+ ~ + +	+ ~ + +	− ~ +
Alveoli		−	−	−
Fallopian tube	Surface epithelial cells	±	+	−
	Ciliary vesicles	+ +	+ +	+ +
Uterus				
Endometrium	Surface epithelial cells	±	+	−
	Glandular cells	±	± ~ +	−
Cervix	Surface epithelial cells	± ~ +	+	−
	Glandular cells	+ +	+ +	− ~ ±
Ovary		−	−	−

The intensity of the staining was graded as follows: + +, strong; +, moderate; ±, weak; −, none

[a] Cryostat sections of periodate/lysine/paraformaldehyde-fixed tissue

[b] Formaldehyde-fixed, paraffin-embedded sections

Female genital tract

Fallopian tubes. The apical cytoplasm of the ciliated epithelium of the fallopian tubes was positive for amylase in PLP-fixed sections stained using the immunohistochemical method (Fig. 2A). In addition, dense immunoreactivity for amylase was observed in the contents of vesicular structures (Fig. 2A). In formalin-fixed, paraffin sections, cilia-like structures (Fig. 2C and D) were found in amylase-positive intracytoplasmic vesicules that were mainly located in the basal part of the epithelial cytoplasm (Fig. 2A and C).

Histochemical investigation revealed the presence of weak amylase activity in the epithelial cells of the fallopian tubes (Fig. 2B). Furthermore, intense amylase activity was also observed in vesicular structures of the epithelium (Fig. 2B).

Uterine cervix. Irrespective of the hormonal cycle, amylase was demonstrable in the surface epithelium, glandular cells, and lumen using both the immunohistochemical (PLP-fixed sections) and the histochemical procedures (Fig. 3). The cy-

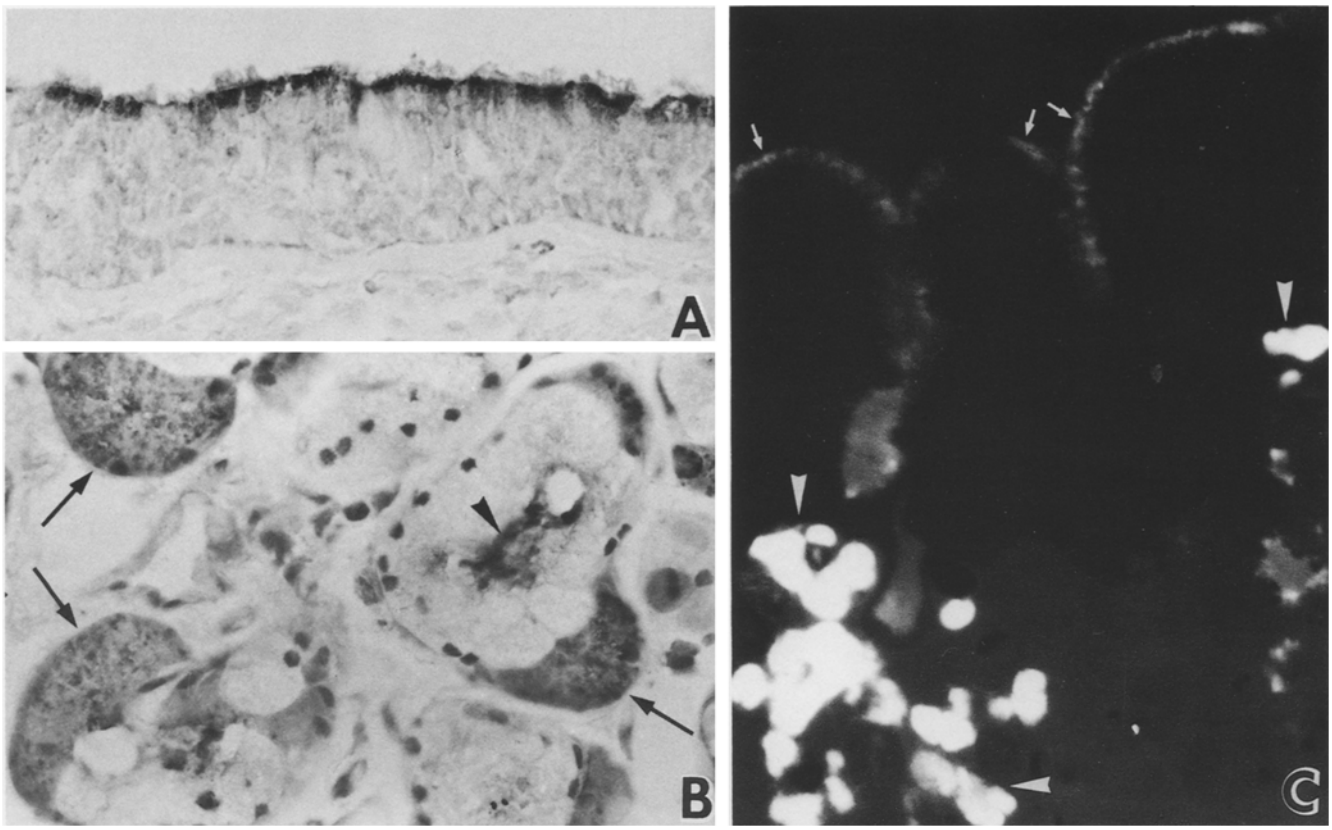

Fig. 1 A–C. Demonstration of amylase in bronchi stained using immunohistochemical and histochemical methods. **A** Immunohistochemical staining: the apical cytoplasm of the epithelial cells of the bronchus is positively stained. Cryostat section of PLP-fixed tissue (×390). **B** Immunohistochemical staining: serous cells of the submucosal glands exhibit granular cytoplasmic staining for amylase (*arrows*), while no staining is present in mucous cells. The lumen (*arrowhead*) also exhibits a positive reaction for amylase. Cryostat section of PLP-fixed tissue (×450). **C** Histochemical staining: the lining epithelial cells (*arrows*) and the glands (*arrowheads*) exhibit moderate to strong staining. Frozen section (×90)

toplasm of glandular cells exhibited strong and diffuse staining for amylase, while surface epithelial cells exhibited weak to moderate staining. The staining pattern was not influenced by the menstrual cycle.

In formaldehyde-fixed, paraffin sections, negative or weak immunostaining was observed in the cervical glands (Table 1).

Endometrium. In PLP-fixed sections, the superficial part of the endometrial glands exhibited positive immunoreactivity (Fig. 4A), but amylase-positive cells were rarely found in the deeper part of the endometrial glands. Immunoreactive amylase was observable in surface epithelial cells during all stages of the menstrual cycle as well as during the postmenopausal stage. Formaldehyde-fixed, paraffin sections exhibited no immunostaining.

The histochemical method used revealed the presence of weak amylase activity in surface epithelial cells, in luminal contents, and in the cytoplasm of the glandular epithelium (Fig. 4B).

Ovaries. Neither amylase activity nor amylase immunoreactivity was detectable in the ovaries.

Discussion

In the present study, we first evaluated the effects of fixatives and embedding procedures on the results obtained using the immunostaining procedure. We also performed histochemical staining for amylase activity to provide a standard with which to compare the sensitivity and specificity of the immunostaining technique. Immunostaining for amylase using PLP-fixed sections was almost as sensitive as the histochemical technique and was more sensitive than immunostaining using formaldehyde-fixed, paraffin-embedded sections. This finding may be useful for further immunohistochemical studies performed to investigate the presence of amylase in various tissues.

In the present study, we used immunohistochemistry to demonstrate the presence of amylase in normal human lungs and the female genital tract. In addition, histochemistry showed that the immunoreactive amylase present in these tissues was active as a glycosidase.

There have been many reports describing cases of hyperamylasemia accompanied by various pulmonary diseases such as pneumonia, tuberculosis, and carcinoma of the lung (Harada and Kitamura 1971; Ammann et al. 1973; Gomi et al. 1976; Nakayama et al. 1976; Otsuki et al. 1977; Morohoshi et al. 1980; Maeda et al. 1982; Roberts et al. 1982). In such patients, the amylase exhibiting increased levels was salivary-type isoamylase, which has biochemical features similar to those of salivary-type amylase (Hayashi et al. 1975; Nakayama et al. 1976). Moreover, we have previously demonstrated the presence of significantly higher serum amylase levels in the left atrial blood than in the right atrial blood in patients without lung disease (Na-

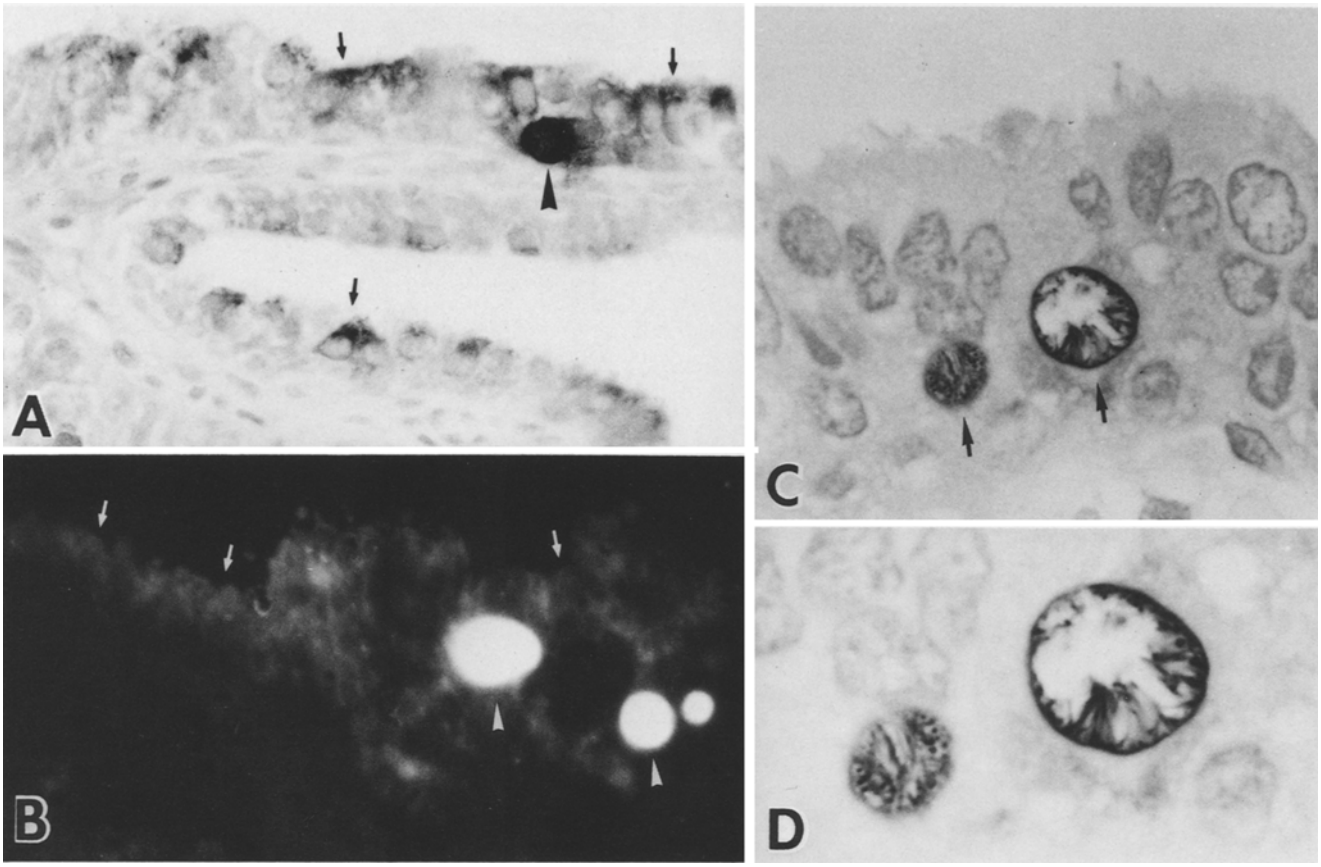

Fig. 2A–D. Demonstration of amylase in fallopian tubes stained using immunohistochemical and histochemical methods. **A** Immunohistochemical staining: an amylase-positive reaction is visible in the apical cytoplasm of the epithelial cells (*arrows*), and intense staining can be seen in a vesicle (*arrowhead*). Cryostat section of PLP-fixed tissue (×570). **B** Histochemical staining: the cytoplasm of epithelial cells exhibits weak staining (*arrows*). Round-shaped staining (*arrowheads*) is also present in epithelial cells corresponding to ciliary vesicles (see **C**). Frozen section (×450). **C** Immunohistochemical staining: the vesicles (*arrows*) in epithelial cells are intensely stained, while the cytoplasm is unstained. Formalin-fixed, paraffin section (×1,275). **D** Higher magnification of the specimen as shown in **C**. In vesicles, amylase-positive cilia are clearly visible. These ciliary vesicles (Jirsova et al. 1977) correspond to the round structures seen in **B** (×2,175)

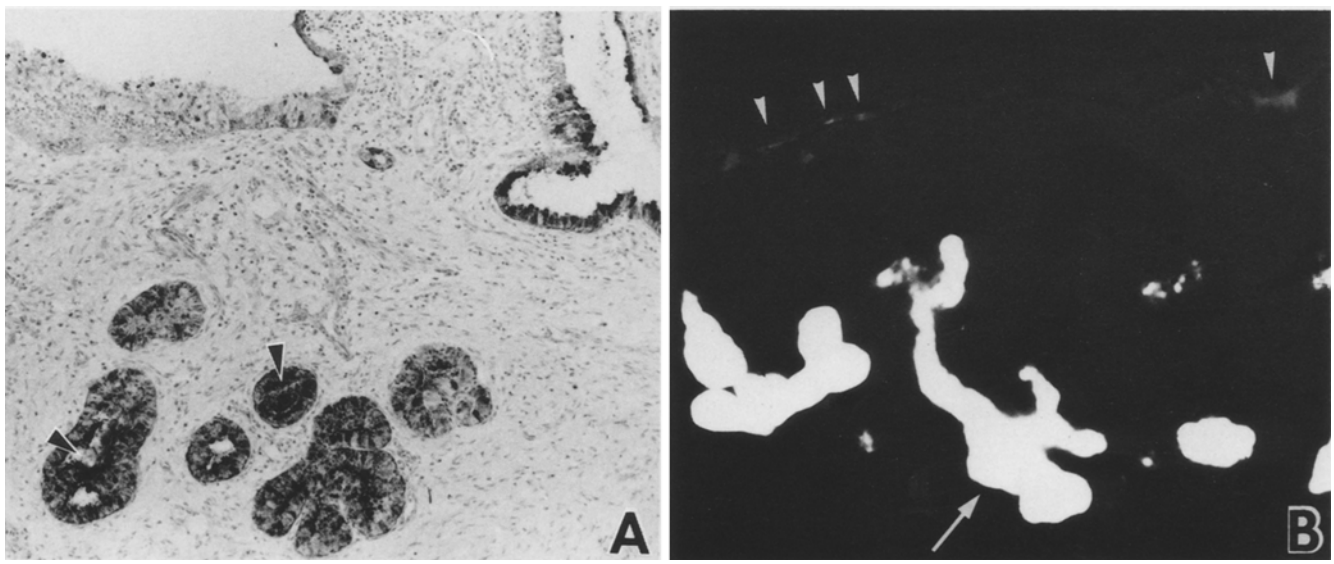

Fig. 3A, B. Demonstration of amylase in cervical mucosa stained using immunohistochemical and histochemical methods. **A** Immunohistochemical staining: the glands and luminal contents (*arrowheads*) are stained intensely and diffusely, and epithelial cells also show positive staining. Cryostat section of PLP-fixed tissue (×90). **B** Histochemical staining: strong amylase activity is visible in the glands (*arrow*), and weak activity is also present in epithelial cells (*arrowheads*). Frozen section (×55)

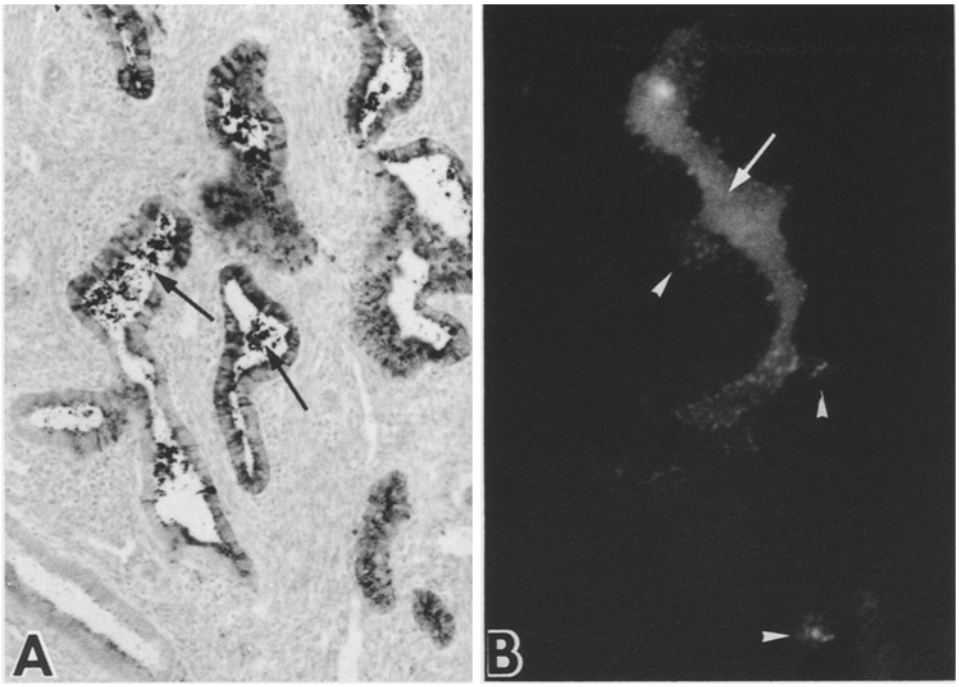

Fig. 4A, B. Demonstration of amylase in the endometrium stained using immunohistochemical and histochemical methods.
A Immunohistochemical staining: the cytoplasm of the endometrial glands and luminal contents (*arrows*) during the secretory phase contain immunoreactive amylase. Cryostat section of PLP-fixed tissue (×120).
B Histochemical staining: amylase activity is visible in the luminal contents (*arrow*) and in the cytoplasm (*arrowheads*) of the endometrial gland during the secretory phase. Frozen section (×280)

kayama et al. 1976). These results suggest that the lungs may be one of the sources of salivary-type amylase in both normal and pathological conditions. In fact, Otsuki et al. (1977) and Maeda et al. (1982) have observed amylase activity in tissue extracts from noncarcinomatous lung as well as in lung carcinomas. However, the cellular localization of amylase in normal lungs has not previously been investigated in detail. In the present study, immunohistochemical staining revealed amylase to be present in the serous glandular cells and ciliated epithelial cells of the bronchus, as well as in the luminal content of the bronchial glands. The staining pattern in serous cells was granular, suggesting that amylase is localized in secretory granules. The exact subcellular localization of amylase in these cells requires further ultrastructural study using immunoelectron-microscopic techniques. The type of amylase present in the studied tissue could not be defined due to the cross-reactivity (with pancreatic-type amylase) of the antiserum applied in the immunohistochemical staining procedure. Histochemical staining confirmed the presence of amylase activity in these cells. Recently, Clarke et al. (1981) found increased levels of amylase activity in the bronchial secretions of patients with chest infections, and they concluded that this was a consequence of the aspiration of saliva. Our results suggest that the amylase present in bronchial secretions is derived from serous cells of the bronchial glands and/or from ciliated epithelial cells of the bronchus. The physiological role of amylase, which occurs in the ciliated epithelial cells and glandular cells of the bronchus, remains unclear. Amylase secreted from bronchial cells may exert an antibacterial effect (Mellersh et al. 1979) in the bronchial lumen. Increased levels of amylase in the bronchial secretions (Clarke et al. 1981) and lung extracts (Otsuki et al. 1977) of patients with pulmonary infections may reflect this antibacterial effect.

Our data are in accordance with the view that, in lung cancers, the production of amylase may be eutopic and an expression of differentiation to bronchial cells. However, amylase-producing lung carcinoma cells do not necessarily originate from cells capable of producing amylase under normal conditions, because such tumors are of diverse cell types, e.g., small cell carcinomas (Ammann et al. 1973; Roberts et al. 1982), adenocarcinomas (Ammann et al. 1973; Gomi et al. 1976; Otsuki et al. 1977; Morohoshi et al. 1980), and squamous cell carcinomas (Maeda et al. 1982).

Our immunohistochemical and histochemical study demonstrated the presence of amylase in ciliated cells of the fallopian tubes. Recently, Bruns et al. (1982) have used immunohistochemistry to show the presence of amylase in epithelial cells of the fallopian tubes. We were able to identify the precise cell type of these amylase-containing cells. It is of interest that amylase staining was observed not only in the apical part but also in the ciliary vesicles of ciliated epithelial cells. Ciliary vesicles are considered to be the products of an abortive form of ciliogenesis (Jirsova et al. 1977). The intense staining for amylase in ciliary vesicles sugests that this enzyme is condensed after being secreted from the surrounding cytoplasm into the vesicles. The hyperamylasemia observed in cases of ruptured ectopic pregnancy (Flege 1966) may be due to the presence of amylase in fallopian epithelial cells.

We were able to demonstrate the presence of amylase activity in uterine cervical mucosa and in the endometrium. Our immunohistochemical procedure revealed more intense staining for amylase in the superficial part than in the deeper part of the endometrial glands. This histological arrangement of amylase-containing cells in the endometrium may indicate that amylase secreted from cells in the superficial part contributes to menstrual-cycle-related changes in amylase levels in endometrial tissues (Robles et al. 1972), because the growth of the superficial part of the endometrium is menstrual cycle dependent.

In the present study, amylase activity was not detected in ovarian tissues. The possibility that the procedures used are not sensitive enough to detect low levels of amylase in normal ovaries cannot be ruled out. On the other hand, it is widely accepted that the surface cells of the ovary fre-

quently transform into cells resembling those that line the fallopian tubes, endometrium, and endocervix (Blaustein 1981). The present study demonstrated that amylase is present in the normal female genital tract. Thus, it is possible that surface-cell-derived ovarian carcinomas are able to produce amylase. If so, the production of this enzyme by such carcinoma cells would be eutopic rather than ectopic.

In conclusion, amylase is present in a definite set of cells in normal human lungs and the female genital tract. Although the role of amylase in these organs is not fully clear, precise knowledge of the distribution of amylase in these tissues may provide new insights into the mechanism of the hyperamylasemia that accompanies some pulmonary and female-genital-tract diseases.

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