*The Journal of Laryngology and Otology*
September 1984. Vol. **98.** pp. 879–886

# Further observations on nasal mucosal changes in scleroderma

## A histochemical and electron microscopic study

*by*

SAMY ELWANY, MAMDOUH TALAAT, NABIL KAMEL and WAHID STEPHANOS*
(Alexandria, Egypt and Alkhobar, Saudi Arabia)

Scleroderma (Progressive Systemic Sclerosis) is a chronic connective tissue disease which can affect many organs. Talaat *et al.* (1980) reported some of the ultrastructural changes in the respiratory nasal mucosa of patients with scleroderma. In this work additional ultrastructural features will be reported and the prominent histochemical changes will be described and discussed.

### Material and Methods

The material for this work consisted of seven patients (all females), aged 21–39 years, suffering from scleroderma. The duration of the disease varied from two to 12 years. A small punch biopsy was taken from the lower edge of the inferior turbinate, 1.5 cm. behind its anterior edge, using 4 per cent xylocaine without adrenalization. All patients accepted the biopsy procedure.

*Preparation of the specimen for transmission electron microscopy*

The tissues were cut into small pieces, 1 mm. in diameter, and were immediately fixed in Karnovsky's fixative at 4 °C. for four hours. They were then post-fixed in 1 per cent osmium tetroxide, and dehydrated in graded alcohol solutions. Using propylene oxide as an intermediate solvent, the specimens were impregnated with TAAB araldite (CY212), then polymerized at 60 °C. in TAAB embedding capsules. Ultra-thin sections were cut with an LKB ultramicrotome, stained with 4 per cent uranyl acetate and Reynold's lead acetate and examined with Hitachi H-600 transmission electron microscope.

*Preparation of the specimens for scanning electron microscopy*

The specimens were immediately fixed in Karnovsky's fixative at 4 °C. for one hour, washed with cacodylate buffer and then post-fixed in 1 per cent osmium tetroxide for one hour. The specimens were then dehydrated with the ethanol series, critical point dried with liquid carbon dioxide, coated with pallidum gold and examined with Joel JSM-35C scanning electron microscope.

*Preparation of the specimen for histochemical study*

The specimen was freshly cryostat-sectioned and stained for:

(1) Succinic dehydrogenase enzyme (Nachalas *et al.*, 1957);
(2) Alpha esterase enzyme (Malaty, 1969);

* From the Otolaryngology, Dermatology and Histology Departments, Alexandria Faculty of Medicine (Alexandria, Egypt) and Dr. Fakhry Hospital (Alkhobar, Saudi Arabia).



https://doi.org/10.1017/S0022215100147644 Published online by Cambridge University Press

(3) Acid and alkaline phosphatase enzymes (Malaty, 1971);
(4) Choline esterase enzyme (Gomori, 1952).

## Results

The ultrastructural features of the nasal mucosa of four patients were similar to those reported by Talaat *et al.* (1980). The most striking feature was the presence of numerous goblet cells (Fig. 1).

The remaining three patients showed some new ultrastructural findings. These included:

(1) Complete absence of cilia and microvilli and occasional exfoliation of the superficial epithelial layers (Fig. 2).
(2) Frank squamous metaplasia (Fig. 3).
(3) Increased number and activity of mucous acini. The acinar cells appeared distended with secretory granules. The mitochondria were numerous and the cisternae of the rough endoplasmic reticulum were generally dilated (Fig. 4).
(4) Increased deposition of thick interwoven collagenous bundles in the tunica propria (Fig. 5).

The histochemical reactions of the normal human respiratory nasal mucosa have been reported by Taylor (1958, 1961) and by Malaty *et al.* (1970). The main departures from the normal were increased activity of succinic dehydrogenase enzyme in the seromucinous glands and a moderately enhanced alkaline phosphatase activity in the endothelial cells of the periglandular blood vessels (Fig. 6). These changes were especially prominent in cases with squamous metaplasia.

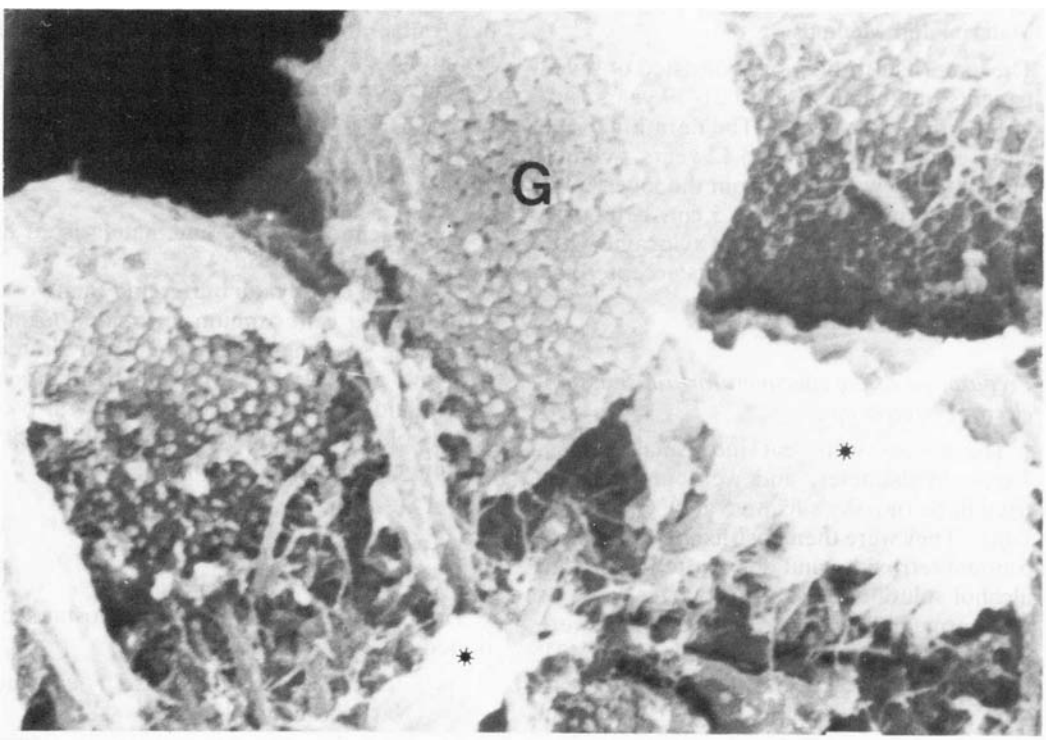



FIG. 1

A scanning electron micrograph showing a goblet cell (*G*). The surface of the cell is covered with microvilli and its upper part is markedly distended with secretion. Mucus (*) is seen around the cell. (Magnification ×6,000).

https://doi.org/10.1017/S0022215100147644 Published online by Cambridge University Press

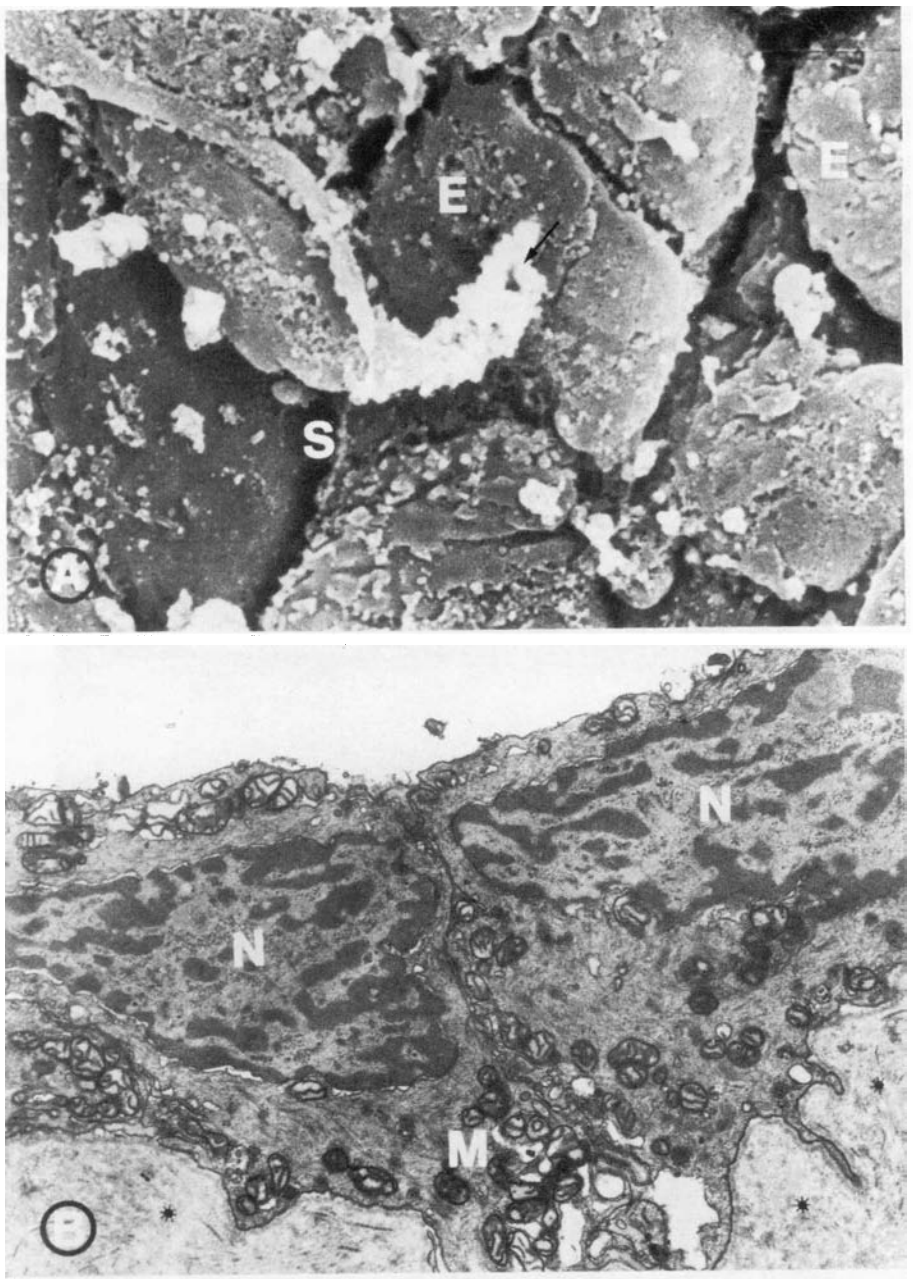



FIG. 2

(a) Complete loss of the cilia and microvilli of the epithelial cells (E). The intercellular spaces (S) are normal. The discharge (arrow) is not transported away but dehydrated *in situ*. (Magnification ×6,300).
(b) Only one line of epithelial cells is still in existence. The cells have relatively large nuclei (N) and numerous mitchondria (M). (Magnification ×12,000).

https://doi.org/10.1017/S0022215100147644 Published online by Cambridge University Press

## Discussion

Scleroderma is a systemic connective tissue disease of unknown pathogenesis. Talaat *et al.* (1980) reported several ultrastructural changes in the human respiratory nasal mucosa of patients with scleroderma. In the present work some additional ultrastructural features have been noticed. The epithelial changes seemed to depend on the duration of the disease. Squamous metaplasia was noticed only in patients who had the disease for relatively longer durations. On the other hand, goblet cell hyperplasia was observed in

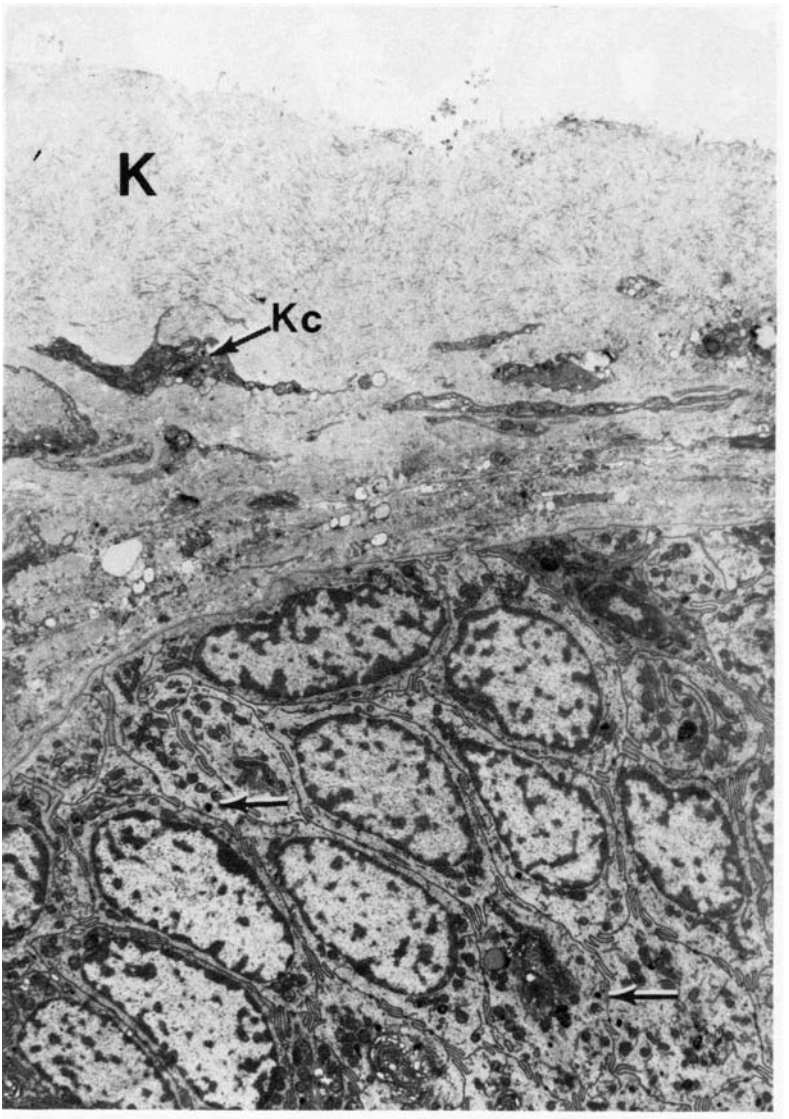



FIG. 3

Squamous metaplasia of the epithelium. The epithelium is covered with keratin (K) and keratinocytes (Kc) are seen in the deeper parts of the horny layer. Keratohyline granules (arrows) are present in the cells of the stratum granulosum. (Magnification ×3,500).

https://doi.org/10.1017/S0022215100147644 Published online by Cambridge University Press

patients who had had the disease for relatively short periods. Both squamous metaplasia and goblet cell hyperplasia probably represent defensive mechanisms, of different nature and strength, against the intermittent mucosal infection which is usually seen in these cases. This concomitant infection was also most probably responsible for the occasional exfoliation of the superficial epithelial layers. The complete loss of cilia and microvilli probably represents an intermediate stage prior to frank squamous metaplasia.

The glandular activity was directly related to the epithelial changes. Cases with many goblet cells exhibited normal glandular metabolic activity, as evidenced by the normal reaction of succinic dehydrogenase enzyme which is one of the members of Krebs' tricarboxylic acid cycle and is strongly related to carbohydrate metabolism and general metabolic activity (Thorpe *et al.*, 1964). On the other hand, cases with squamous metaplasia and absent goblet cells exhibited a striking increase in glandular activity. This was evidenced by:

(1) The presence of numerous secretory granules and mitochondria within the acinar cells. Also the cisternae of the rough endoplasmic reticulum were frequently dilated.
(2) The markedly enhanced reaction for succinic dehydrogenase enzyme.
(3) The moderately increased reaction of alkaline phosphatase enzyme in the acinar cells and the markedly increased one in the periglandular blood vessels. Alkaline phosphatase enzyme is one of the membrane-associated enzymes concerned with secretory functions (Pearse and Riecken, 1967). Its

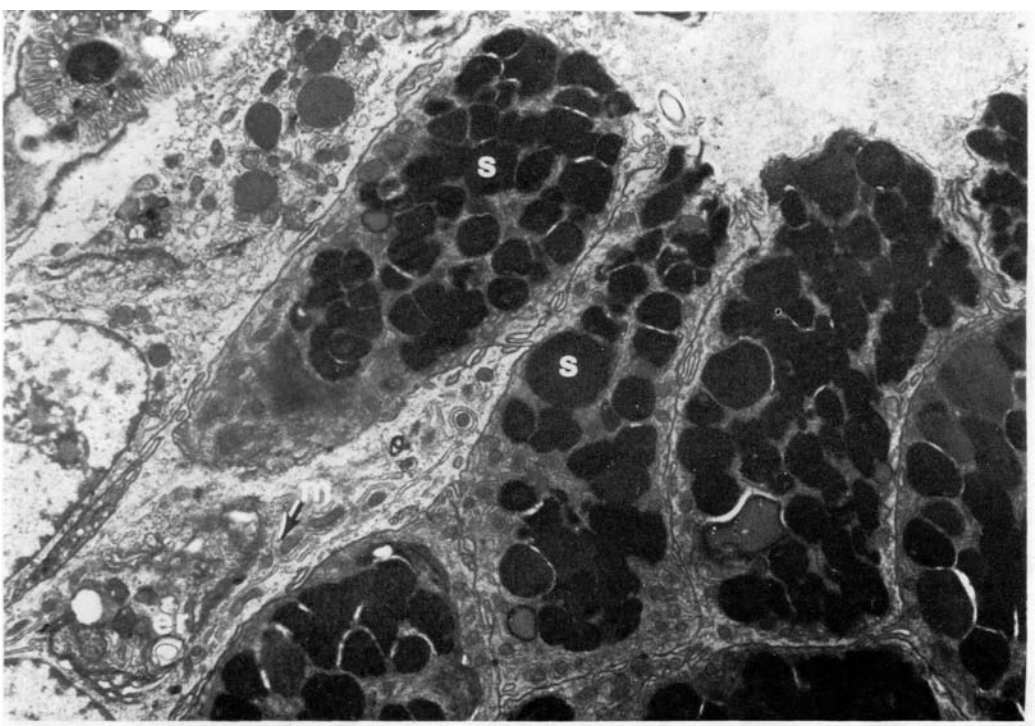



FIG. 4

A mucous acinus. The acinar cells are packed with secretory granules (s). The mitochondria (m) are numerous and some of the cisternae of the endoplasmic reticulum (er) are dilated. (Magnification ×6,000).

https://doi.org/10.1017/S0022215100147644 Published online by Cambridge University Press

increased activity indicates hyperactivity of the acinar cells and increased transfer of metabolites across the endothelial cells.

The normal reaction of the choline esterase enzyme in the cholinergic nerve fibers around the glands and blood vessels indicated that the previously mentioned changes were not dependent upon the parasympathetic reflex arc. The glandular hyperfunction, in association with squamous metaplasia, obviously represented a compensatory mechanism to replace the lost goblet cells.

Leroy (1972) suggested that the fibroblasts in scleroderma produce more collagen than normal. The fibromatogenic aspect of scleroderma was reflected in our cases in the greater number and thickness of the collagenous bundles. Again fibrous tissue proliferation was especially noticeable in long-standing cases. The increased activity of fibroblasts was also evidenced by the increased succinic dehydrogenase enzyme activity in the connective tissue stroma around the glands and blood vessels.

## Summary

The respiratory nasal mucosa of patients with scleroderma showed several ultrastructural and histochemical changes. These included initial goblet cell hyperplasia, loss of cilia and microvilli, exfoliation of the superficial epithelial layers, increased glandular activity in cases with squamous metaplasia and fibrous tissue deposition in the lamina propria. These changes seem to be related to the duration of the disease, whose nature and pathogenesis are still uncertain.

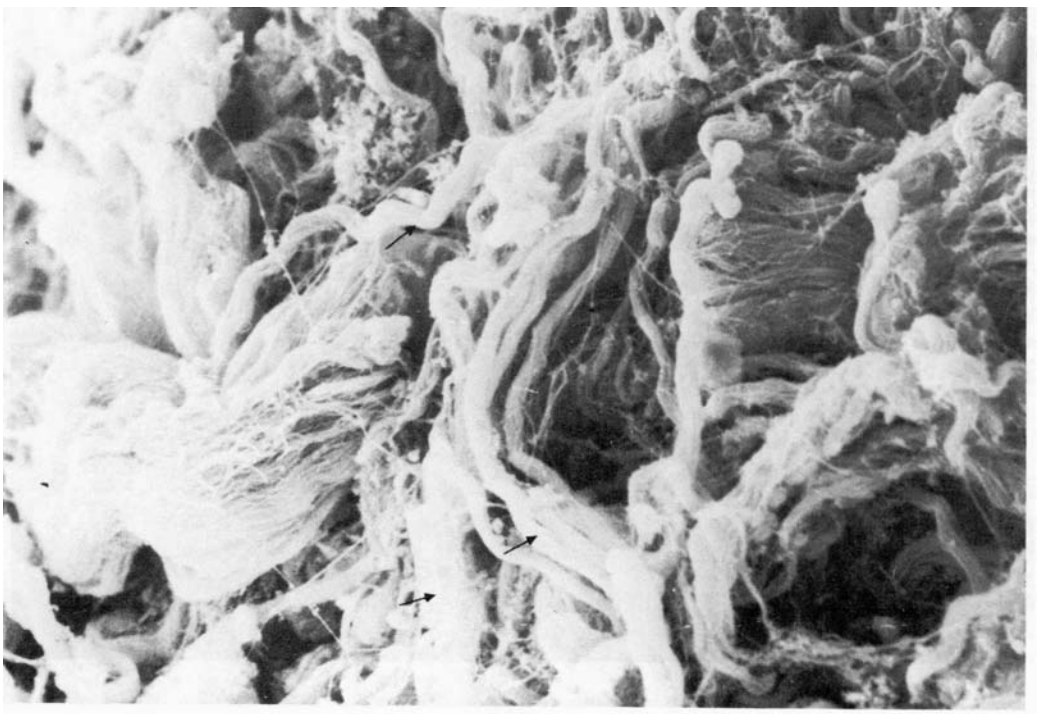


FIG. 5

Thick interwoven collagenous bundles (arrows) traversing the lamina propria. (Magnification ×3,000).

https://doi.org/10.1017/S0022215100147644 Published online by Cambridge University Press

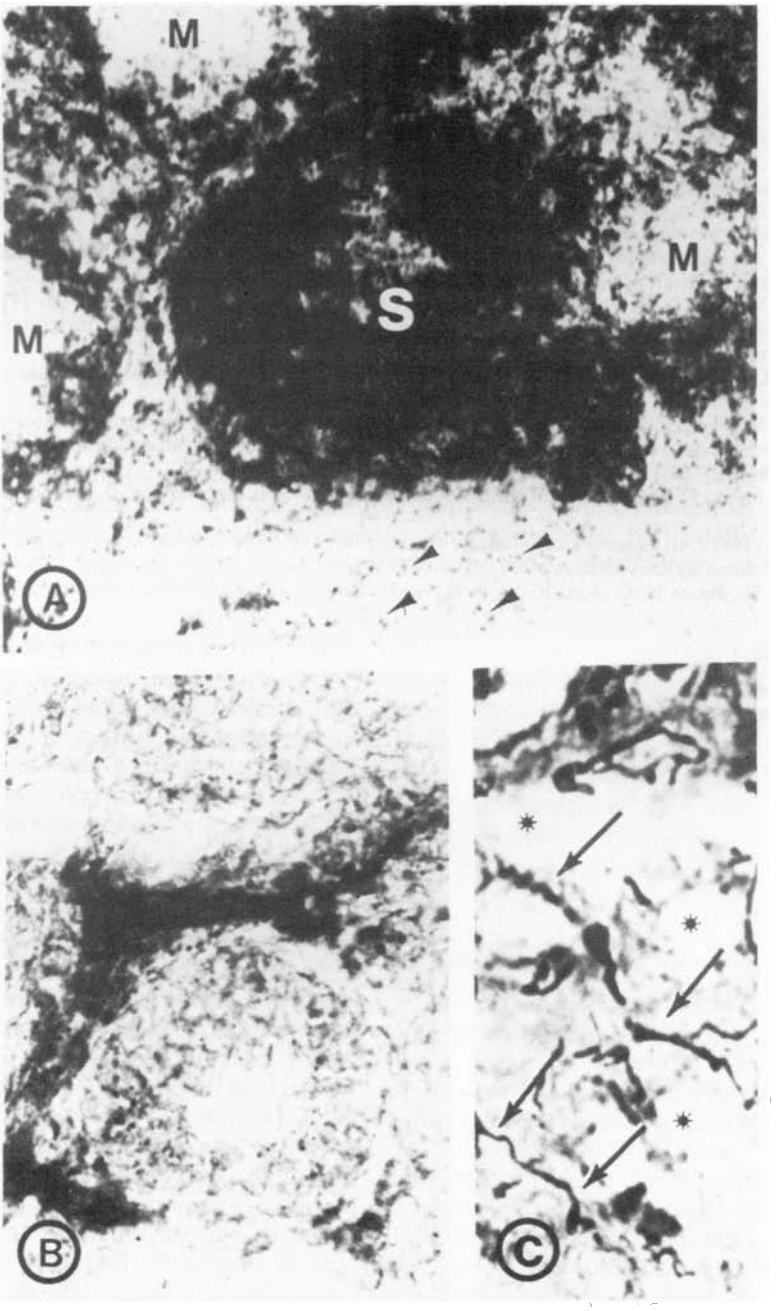



FIG. 6

(a) Increased activity of succinic dehydrogenase enzyme in the mucous (M) and serous (S) acini. The stromal cells (arrowheads) showed a moderately enhanced activity. (Magnification ×450).

(b) Moderately enhanced reaction for alkaline phosphatase enzyme in the endothelial cells of periglandular blood vessels. (Magnification ×450).

(c) Normal choline esterase enzyme activity in the cholinergic nerve fibers. (Magnification ×450).

https://doi.org/10.1017/S0022215100147644 Published online by Cambridge University Press

**References**

GOMORI, G. (1952) *Microscopic Histochemistry—Principles and Practice*. First edition. University Press, Chicago.

LEROY, C. E. (1972) Connective tissue synthesis by scleroderma skin fibroblasts in cell culture. *Journal of Clinical Investigation*, **135:** 1351–1362.

MALATY, H. A. (1969) The cytological localization of alpha and as esterases and their role in cell Metabolism. *Alexandria Medical Journal*, **15:** 271–274.

MALATY, H. A. (1971) A modified simultaneous coupling method for the demonstration of both alkaline and acid phosphatases enzymes. *Alexandria Medical Journal*, **17:** 292–297.

MALATY, H. A., SHEHATA, M. A., and SAFI, M. M. (1970). A histological and histochemical study of the human nasal mucosa in different ages. *Alexandria Medical Journal*, **16:** 487–497.

NACHALAS, M. M., TSOU, K. C., DESOUZA, E., CHENG, C. S., and SELIGMAN, A. M. (1957) Cytological demonstration of succinic dehydrogenase by the use of a new p-nitro-phenyl substituted ditetrazol. *Journal of Histochemistry and Cytochemistry*, **5:** 420–425.

PEARSE, A. G. E., and RIECKEN, E. O. (1967) Histology and cytochemistry of the cells of the small intestine in relation to absorption. *British Medical Bulletin*, **23:** 217–222.

TALAAT, A. M., KAMEL, M. N., AZIZ, M. T., and SHAABAN, N. A. (1980) Human respiratory nasal mucosa in progressive systemic sclerosis (scleroderma)—An electron microscopic study. *Journal of Laryngology and Otology*, **94:** 617–627.

TAYLOR, M. (1958) Histochemistry of the nasal respiratory mucosa. *Journal of Laryngology and Otology*, **72:** 365–376.

TAYLOR, M. (1961) An experimental study of the influences of the endocrine system on the nasal respiratory mucosa. *Journal of Laryngology and Otology*, **75:** 972–977.

THORPE, W. N., BRAY, H. G., and JONES, J. R. (1964) *Biochemistry for Medical Students*. J. and A. Churchill, London.

Address for correspondence:
Samy Elwany, M.D.,
503 El-Horreya Street,
Boulkeley,
Alexandria,
Egypt.

https://doi.org/10.1017/S0022215100147644 Published online by Cambridge University Press