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Microscopic Studies on the Digestive Gland of the Scallop, *Patinopecten yessoensis* (Jay).

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Summary

The digestive gland of the Japanese scallop, *Patinopecten yessoensis* (Jay), was studied microscopically. The seasonal changes of the tubule were followed in detail. The epithelium of the tubule consists of three types of cells; basophil cells with basic dye positive cytoplasm, secretory cells containing intracellular granules and fat cells with lipid droplets and granules of a special type.

During the period from October to March, the epithelial cells did not show any distinct change. However, when the sea water temperature rises, a swelling of the fat cells occured and atrophy of the basophil cells and secretory cells was observed.

The scallop is a commercially important species in the shallow inshore waters in northern Japan. But the biological references on the species have been scarece as compared to other bivalves. So far as we are aware, the morphology as well as the physiological function of digestive gland have never been studied in detail.

The present study was undertaken to study the structure of digestive gland in detail by means of the histological and histochemical techniques. In addition, the seasonal changes of the epithelial cells were observed.

Methods

Adult scallops were sampled once every month in Mohne Inlet, Miyagi Prefecture, during the period from December of 1966 to November of 1967. For histological and histochemical observations, the digestive gland was excised in 10% formalin, Helly's, Gendre's and Carnoy's fixatives. After fixation, the tissue was dehydrated in a graded series of ethanol and embedded in paraffin. Sections were made 6 μ in thickness and stained with hematoxylin-eosin, iron-hematoxylin,

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periodic acid-Schiff (PAS), acrolein-Schiff, Mallory azan, Feulgen and Unna Pappenheim. For the detection of lipid in the tissue, the frozen section was stained with sudan III and nile blue. The epoxy resin sections, 1 to 2 μ in thickness, were also prepared and were stained with toluidine blue for the observation of the fine structure.

Results of observations

Microscopic Structure of the Digestive Gland.

The digestive gland is made of a large number of blind-ending tubules which communicate with the stomach by several ducts (Figs. 2, 3).

In the digestive gland, the connective tissues are rater thin. The blood vascular system is well-developed and the capillaries are distributed around the basement membrane of the epithelium. The duct is circular in cross section and it is lined with ciliated epithelial cells. The lumen, however, is irregular in shape owing to the variations in the height of the ephithelial cells. The duct can be divided into two parts in cross section by different form of epithelial cell. The one part consists of ciliated columner epithelial cells and contains PAS-positive substances in the peripheral region of the cell, while the other part consists of more slender cells than the former and the apical cytoplasm of the cells reacts to acrolein-Schiff staining (Fig. 3).

The tubule, in cross section, is usually round in shape with a diameter from 55 to 100 μ. The epithelial cells of the tubules are of three types differing in shape, staining reactions to the basic dyes and character of intracellular granules. They can be distinguished as basophil cells, secretory cells and fat cells (Figs. 1, 4).

i) *Basophil cell*:

The cell is pyramidal in shape with a height from 15 to 25 μ. The cytoplasm contains large quantities of basophilic substances which are well stained by the basic dyes such as hematoxylin and toluidine blue, except in the apical region of the cell. The basal cytoplasm reacts to pyronin. The affinity to pyronin is lost when RNase is used. The apical portion of the cytoplasm contains a few PAS and acrolein-Schiff positive substances. The nucleus is oval and lies in the basal region. It can be seen distinctly under a light microscope (Fig. 5).

ii) *Secretory cell*:

The cell is cylindrical with brush border on the free surface and the size is 15–35 μ in height. The nuclei of secretory cells, oval or round in shape, are seen in the basal region. The supranuclear region is filled with intracellular granules which vary in number and size. These intracellular granules are stained pinkish by eosin and acrolein-Schiff uniformly when Carnoy's fixative is used. These

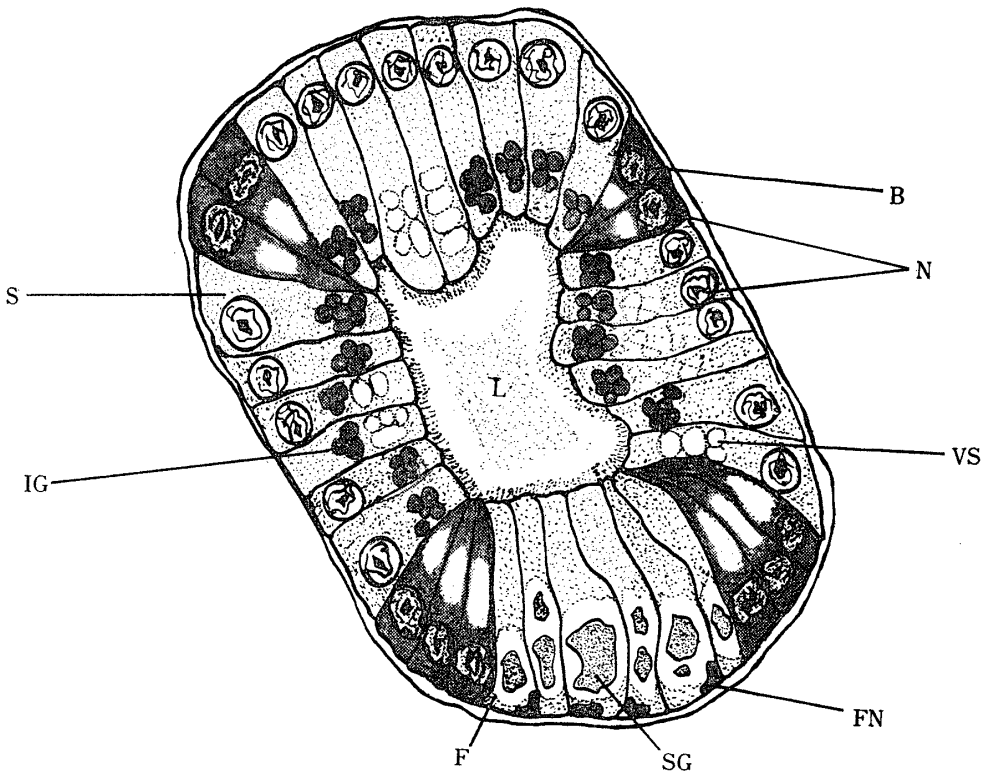

FIG. 1. Diagram of the tubule of the digestive gland. B, Basophil cell, the cytoplasm is stained with basic dyes; S, Secretory cell containing many intracellular granules and vacuole-like structures; F, Fat cell with granule of special type; N, Nucleus of basophil cell and secretory cell; VS, Vacuole-like structure of secretory cell; FN, Nucleus of fat cell, pressed against the basal portion; SG, Granule of special type of fat cell; IG, Intracellular granule of secretory cell; L, Lumen;

granules are well preserved if Helly's solution is used as a fixative. In the preparation of Mallory azan staining, the red-brown granules are distributed widely in the cells and are also stained with iron-hematoxylin. These granules are always surrounded with a limiting membrane and they are conspicuous in the spawning season. The blue granules which are less than 3 μ in size, are distributed in the apical region, but the limiting membrane is not seen distinctly. The light-green granules, 0.5–1.5 μ in size, are also observed (Fig. 6). The extrusions of the apical portion filled with these granules and their separations from the cell occur frequently and the pieces of cell can be observed in the lumen (Fig. 7). In the secretory cells with very few or no granules, the vacuole-like structures are observed in the supranuclear region (Figs. 1, 6). In the perinuclear region of secretory cell, a few lipid droplets are observed. The secretory cells are the most numerous in the tubule.

iii) *Fat cell*:

The fat cell is characterized by the massive accumulation of fat in the cyto-

plasm. The cell is cylindrical, 15–35 μ in height, but has a swollen appearance as compared to the secretory cells. The fat is in droplets and is abundant in the basal region. The nuclei are pressed close at the base of the cells and are flat in shape. In the preparation fixed by Carnoy's or Helly's solution, one or a few granules of a special type are recognized. The granules have wide variations in shape and size, and they are positive to PAS and acrolein-Schiff reactions. It was noticed that the granule reacts to toludine blue metachromatically and turns red-purple in color (Fig. 8). The granules of a type described in the secretory cells were not obserbed. But numerous fine granules of another type which are stained with PAS-reaction and aniline blue are observed in the apical region. The free surface of the fat cells show brush border.

Seasonal Changes in the Epithelial Cells of Digestive Gland.

The tubule of digestive gland shows morphological changes seasonally. The change is conspicuous in the summer season when the sea water temperature rise above about 18°C. While during a period from October through March, when the sea water temperature is lower than 17°C almost no change was observed.

In the individuals sampled on the 8th of April, when the sea water temperature was around 8°C, an increase of intracellular granules in the secretory cells, particularly the black granules which are stained with iron-hematoxylin, was observed. After the spawning, as the sea water temperature rises, the fat cells began swelling gradually. In June, the area of the fat cells of tubule in the cross section was about 53% in average as against about 25–30% in the winter season. On the 30th of August, when the sea water temperature was 23.5°C, the fat cells swelled markedly and its area covered about 75% in cross section. Atrophy of the basophil cells and secretory cells was observed in parallel with the extreme swelling of fat cells. The peak shrinkage of the cells was 5–13 μ and 3.5–12 μ respectively. Intracellular granules of the secretory cells almost disappeared in this season (Fig. 9). In the basophil cells, the reduction in affinity to the basic dye was also noticed. The diameter of the tubules in cross section, however, did not show change, and remained at 55–100 μ year round.

Discussion

The results of our microscopic observation confirm that there are three types of cells in the tubule epithelium of the digestive gland of the scallop. Summer (7) distinguished two types of cells in the tubules of *Anodonta anatina*, namely the basophil cells and the digestive cells. Eble (1) named two types of cells as generative cells and secretory-absorptive cells in his study on the oyster, *Crassostrea virginica*. According to Owen (6), Guardabassi and Ferreri (2) have classified three types of cells in the tubules of *Helix pomatia*: calcium cells, secretion cells containing yellowish-brown bodies and absorption cells with yellow granules.

Summer (7) noted that the basophil cells of *Anodonta anatina* have the fine structure typical of protein-secreting cells, such as pancreatic exocrine cells in mammalia. Sugawara* stated in his electron microscopical study that the dark cells of the Japanese oyster, *Crassostrea gigas*, is characterised by the presence of well-developed lamellae, rough-surfaced endoplasmic reticulum. The basophil cells of the scallop resembles in appearance the dark cells of the Japanese oyster under the light microscope.

It is clear that the intracellular granules of the secretory cells are secreted by the apocrine style secretion of apical cytoplasm, although it remains to be solved whether the granule contains enzymes or not. The digestive cells of the lamellibranchs have been shown to take part in absorption and phagocytosis (8, 10). Therefore, it may be considered that the development of the bursh border and the appearance of vacuole-like structures in the secretory cells of the scallop is related to the absorptive and phagocytic functions.

The fat cells seem to have never been described in lamellibranchs before. Judging from an accumulation of lipid in the cytoplasm, the fat cell is considered to take part in the lipid metabolism of the digestive gland of the scallop. In the summer season, the massive accumulation of lipid results in the swelling of the fat cells which, in turn, cause the atrophy of the basophil and secretory cells. The present study could not make clear whether this swelling of the fat cells during the summer is a normal cyclic change or not. But there seem to be no doubt that the atrophy of the basophil cells and the secretory cells, the reduction of stainability in the basophil cells and also the disappearance of intracellular granules in the secretory cells are an indication of a slow-down in the function of the digestive gland of the scallop in warmer season.

Recently, the Oyster Research Institute has succeeded in breeding and cultivation of scallop up to marketable size in Mohne Inlet, Miyagi Prefecture (3). The cultivations of scallop from juvenile to commercial size are widely practiced in the northern coast of Japan. During the cultivation, an adverse effect of high sea water temperature during summer on the scallop shell growth has been noticed. It often resulted in the formation of disturbance rings in the shells (5). Maru and Obara (4) concluded that the summer ring formation in the adult scallop was due to the physiological disturbance caused by breeding in Lake Saroma. The growth disturbance in Mohne Inlet seems to be due to the high sea water temperature rather than to the physiological disturbance caused by breeding, because the water is much warmer in Mohne Inlet and the scallop spawns in early spring. While the scallop spawn most actively in early summer in Lake Saroma (9).

We have no direct evidence to explain the cause of the disturbance of shell growth during the summer, but it can be assumed that the reduction in functional

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activity of the digestive gland in the warmer water is responsible for the disturbance in growth of the animal in the Tohoku area.

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Plate 1

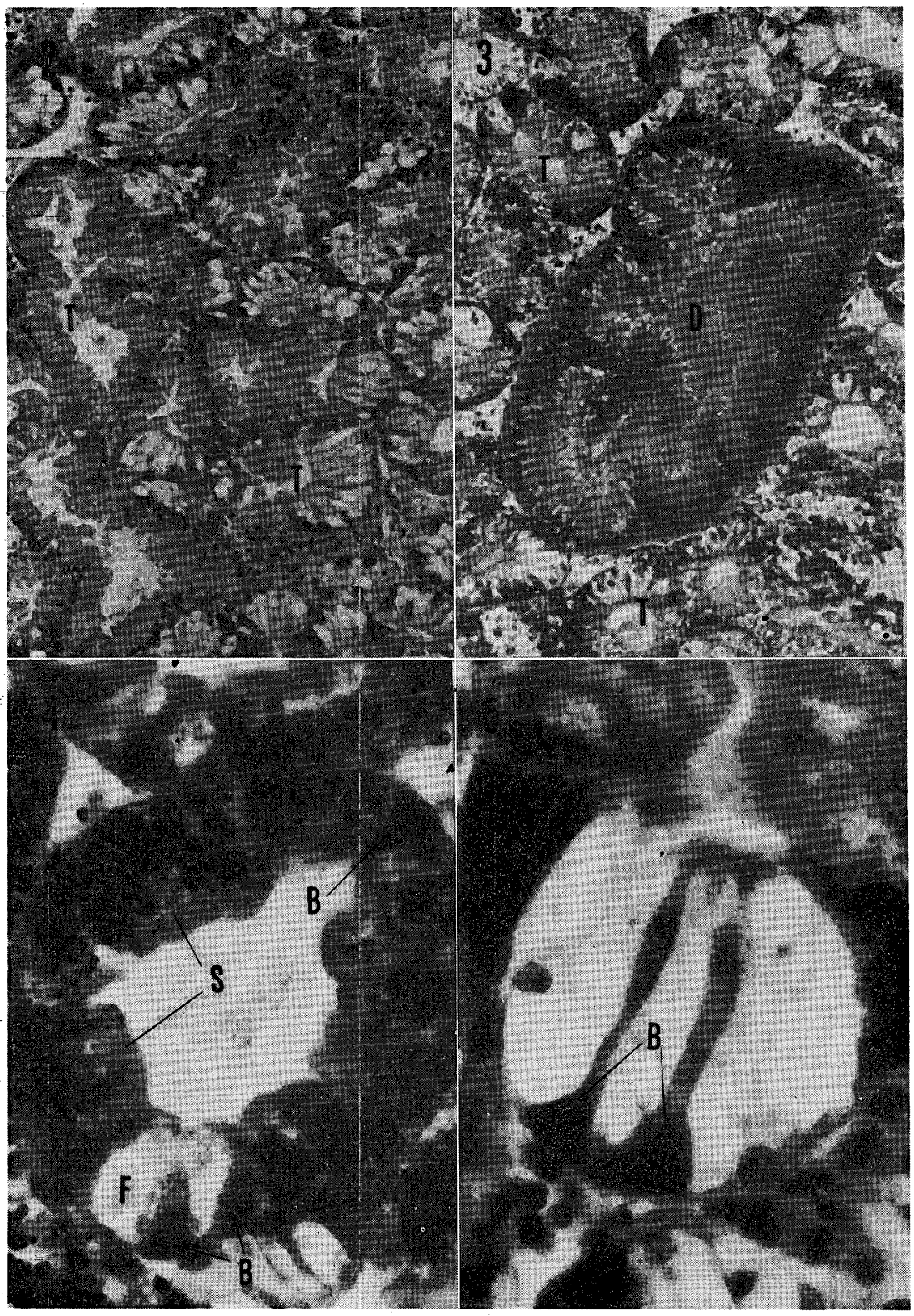
Explanation of Figures

Fig. 2. A micrograph showing a section of digestive gland, smapled on May. T, tubule; Carnoy, hematoxylin-eosin × 150

Fig. 3. A micrograph showing a section of digestive gland, tubule (T) and duct (D) are seen. Duct is divided into two parts in cross section by the form of epithelial cells, sampled on Feburary. Carnoy, heamtoxylin-eosin × 150

Fig. 4. Cross section of tubule. Tubule is consisted of three types of cells; basophil cell with cytoplasm stained with basic dyes (B), secretory cell with intracellular granules (S) and fat cell (F), sampled on April. Helly, Mallory azan × 600

Fig. 5. A micrograph showing basophil cells (B). The perinuclear region of basophil cell is well stained with basic dyes, sampled on May. Carnoy, hematoxylin-eosin × 1,200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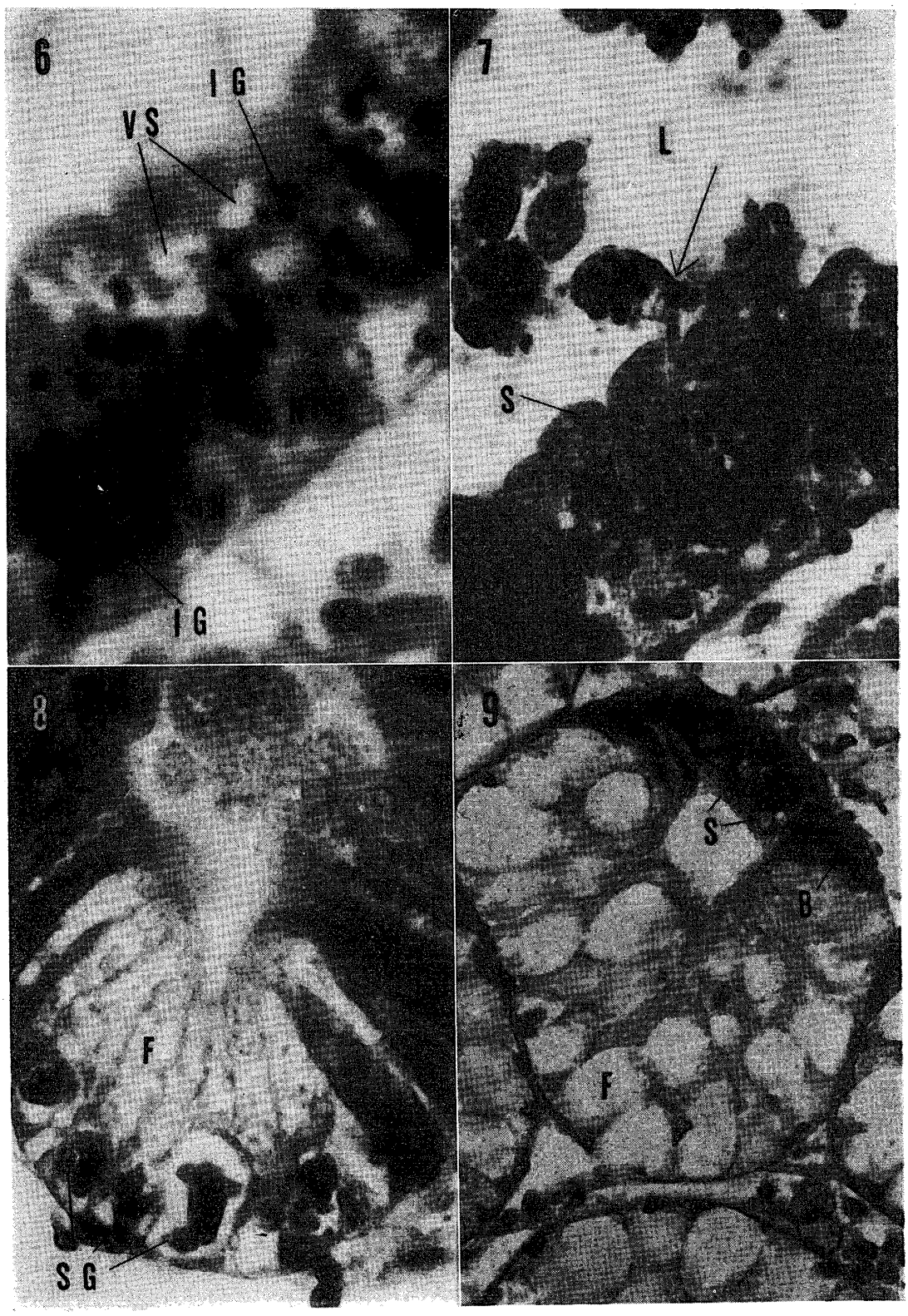
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PLATE 2

Explanation of Figures

FIG. 6. Higher magnification of secretory cells. Vacuole-like structure (VS) and intracellular granules (IG) are seen. Nuclear (N) of secretory cell is round at the basal region, sampled on April. Helly, iron-hematoxylin × 1,500

FIG. 7. A micrograph of a secretion of intracellular granules in the secretory cell, showing apocrine style secretion (arrow), sampled on April. S, secretory cell; L, lumen; Helly, iron-hematoxylin × 1,200

FIG. 8. Higher magnification of fat cells (F). Granules of special types (SG) are conspicuous in the micrograph, sampled on Feburary. Carnoy, toluidine blue × 1,200

FIG. 9. A micrograph showing a tubule of digestive gland sampled in August, extreme swelling of fat cells (F) and atrophy of basophil cells (B) and secretory cells (S) are observed. Carnoy, hematoxylin-eosin × 600