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journal or publication title	Tohoku journal of agricultural research
volume	20
number	4
page range	204-211
year	1970-03-30
URL	http://hdl.handle.net/10097/29583

Tohoku Journal of Agricultural Research
Vol. 20, No. 4, Dec. 1969
Printed in Japan

The Effects of Methionine and Choline Chloride on the Digestive Gland of the Japanese Scallop, *Patinopecten yessoensis* (JAY)

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(Received, July 24 1969)

Summary

The effects of methionine and choline chloride on the digestive gland of Japanese scallop, *Patinopecten yessoensis* (JAY), which were kept at a temperature of 24±0.5°C for 200 hours were studied.

At this high temperature condition, the tubule epithelium of the digestive gland showed swelling of fat cells with lipid storage. Also physiological involution of the basophil cells and secretory cells was detected.

Methionine induced the decrease of lipid in the digestive gland and the recovery of basophil and secretory cells in the scallop kept at high temperature.

The effect of choline chloride was not as remarkable as that of methionine, but partial recovery of the basophil and secretory cells was recognized.

Previously, the authors studied the morphology of the digestive gland in Japanese scallop in detail, and found that the digestive gland is composed of a large number of blind tubules which are connected to the stomach by several ducts, and that the tubule epithelium consists of basophil cells, secretory cells and fat cells. Furthermore, it was found that, as the water temperature rises after spawning in the spring, the swelling of fat cells with lipid storage, the atrophy of basophil cells and secretory cells which indicate a slow-down of function in the digestive gland occured (1).

In Mohne Inlet, Miyagi Prefecture, an adverse effect of high water temperature during summer on scallop shell growth has been reported (2), and it was assumed that the reduction of functional activity of the digestive gland is connected with the disturbance in growth of the scallop in the summer season.

Up to now, fatty infiltrationin mammalia liver has been intensively studied and methionine, lecithin and choline chloride etc. are known as the lipotropic factors (3,4,5,6,7). In addition, in this, interference with "hepatic triglyceride secretory mechanism" has received some attentions (3,4,5,6).

The present study was undertaken in order to define the mechanism of fatty

infiltration of the digestive gland in the hot summer season and to observe the effects of the lipotropic factor in mammalia on the scallop. Here authors will report the experimental fatty infiltration of the degestive gland and the effects of methionine and choline chloride on it.

Method

The experiment was carried during the period from the 18th of March to the 7th of April in 1969 at the Laboratory of Fisheries, Tohoku University. Adult scallops, with shell length of 10 to 12 cm, were obtained from Onagawa Bay, Miyagi Prefecture. The gonad has matured fully in the scallops used in present study. The live scallops were maintained in a cistern (100 liters in volume) for 50 hours at 8°C supplied with circulating sea water filtered through glass wool and sand. And then, the water temperature was rised 3°C every 12 hours. The final temperature of the sea water was 24±0.5°C. The scallops were kept at this temperature for 400 hours. At 200 hours after the sea water temperature was rised to 24±0.5°C, 3 mg of L-methionine and 1 mg, 3 mg, 5 mg of choline chloride, solved in Herbst's artificial sea water, were administered to the scallops by injection into the adductor muscle 8 times, with an interval of 24 hours. Drugs were given in the same concentration of 1mg/0.1 mg for all injection. Five individual scallops were used for each injection.

Scallops injected with only Herbst's artificial sea water were used as a control.

At 24 hours after the final injection, the digestive gland was excised in 10% formalin and Carnoy's fixative. 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. For the detection of lipid in the tissue, the frozen section, embedded in gelatin, was stained with sudan III.

Results

At the beginning of the present experiment, the digestive gland showed the normal characteristics which are observed in winter. Namely, the fat cells are characterized by the accumulation of lipid in the cytoplasm, but the swelling of cells is not observed. The nucleus of fat cell is pressed close at the base and is flat in shape. The secretory cell is cylindrical and the supranuclear region is filled with numerous eosinophilic intracellular granules. In the basophil cell, the cytoplasm of perinuclear region is compact and basophilic. The nuclei of secretory cells and basophil cells are almost round at the basal portion and chromatin as well as nucleolus are distinct (Fig. 1).

The digestive glands of the individual scallops kept at 24±0.5°C for 200 hours showed certain remarkable changes as compared with that observed at the beginning of present experiment morphologically. First of all, the swelling of fat

cell with the increase of cytoplasmic lipid was observed. The lipid was stained in red with sudan III and the fat vesicles were conspicuous in the preparation fixed by Carnoy's solution. The other cell components except the fat vesicle were not clearly seen. In the basophil cell, the cytoplasm was rough and the cell boundary is indistinct. The atrophy of basophil cell was also noticed. In the secretory cell, the cell bundary was indistinct and the vacuolization of cytoplasm was recognized. Furthermore, the most of secretory cells showed the physiological involution which is represented by the vacuolization and the break-down in the cytoplasm of supranuclear region. Both the nuclei of basophil cell and secretory cell seemed to be pyknotic (Figs. 2, 3, 4).

On the other hand, in the methionine dose group, the decrease of lipid was recognized in the digestive gland and the area occupied by the fat cell was much reduced in the tubule. The cytoplasm of secretory cell was coarse and the intracellular granules were not recognized. But the cell boundary, cyrindrical in shape, was distinct and the nucleus was round at the basal portion. The basophil cells also showed the normal feature as seen at the beginning of experiment. In both the basophil cell and the secretory cell, no distinct indication of the physiological involution was observed (Figs. 5,6,7). No individual died in this group.

All of scallops administered with 3 mg and 5 mg of choline chloride died 5 hours after injection. The scallops administered with 1 mg of choline chloride showed violent action after injection, but none of them died during the experiment. In this group, the effect of choline chloride on the digestive gland was not so clear as in case of methionine. The secretory cells, however, were cyrindrical in shape and the cell boundaries were distinct. The nuclei of the basophil cell and the secretory cell were round at the basal portion, chromatin and nucleolus were also recognizable. The physiological involution in both cells was not shown (Fig. 8).

Discussion

By holding the scallop at the high temperature of $24\pm0.5°C$ for 200 hours, an increase of lipid was recognized and the reduction of functional activity which occures naturally in the hot summer season was observed in the digestive gland. It is assumed from the present investigation that the high temperature induce the lipid accumulation and the slow-down of functional activity in the digestive gland in hot summer season.

In methionine dose group, the elimination of lipid in the digestive gland of the scallop was confirmed just as known in the liver of mammalia. The effect of choline chloride on the elimination of lipid in the digestive gland was not so clear as methionine. The recoveries of function in the basophil cell and the secretory cell, however, were observed, and it can be expected that the administration which extends over longer period of time may be effective on the elimination of lipid.

In mammalia, it is known that methionine participate in the synthesis of phospholipid by the transmethylation, and secondary, it is effective as the lipotropic factor on the fatty infiltration of liver (7). But there is no reference on the fatty infiltration of the digestive gland in bivalvia. In the present study, the mode of action of methionine and choline chloride in the digestive gland of the scallop could not be difined. Furthermore, it remains to be solved whether the lipid accumulation and the effect of methionine and choline chloride on the scallop belong to the similar category with the fatty liver in mammalia or not. But it may be suggested that not only the physiological condition of the fat cell but also those of the basophil cell and the secretory cell are effected by these drugs, and that the recovery of function in both cells also have a part in the elimination of lipid in the digestive gland.

Acknowledgements

The authors are gratefully indebted to Dr. T. Imai and Dr. R. Sato for the supervisions and the valuable advices during the course of this study, and to Mr. R. Maeda for his assistance.

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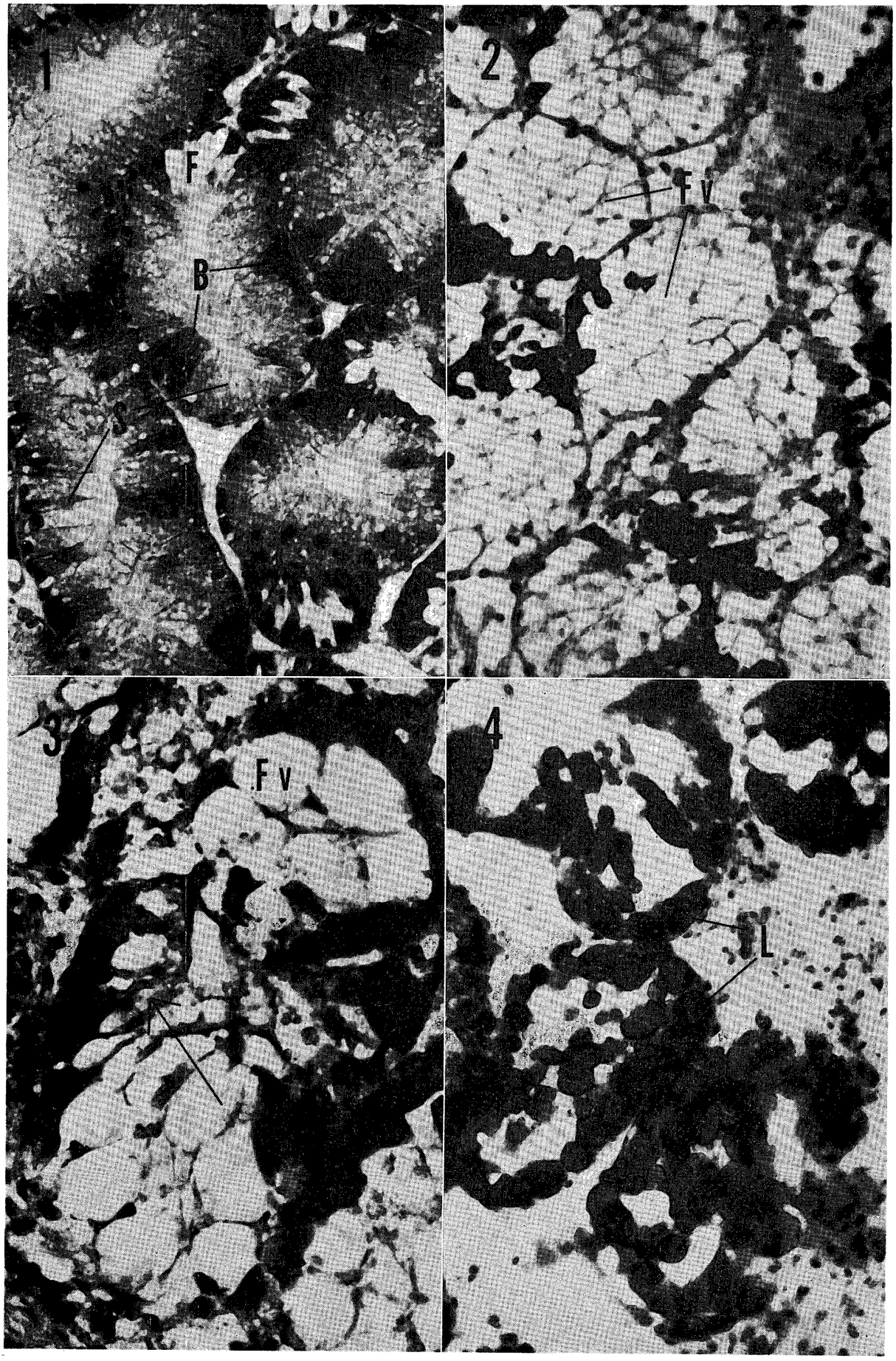
PLATE 1

Explanation of Figures

FIG. 1. A micrograph showing tubules of digestive gland at the beginning of experiment. The tubule shows the normal characteristics observed in winter and the swelling of fat cell is not recognized. B: Basophil cell, F: Fat cell, S: Secretory cell ×300

FIGS. 2–4. Micrographs showing tubules of digestive gland kept at high temperature of 24±0.5°C for 200 hours. The increase of lipid (L) is recognized and the fat vesicles (FV) are conspicuous in the tubule. The atrophy of basophil cell and the vacuolization of secretory cell (arrow) are observed. Figs. 2, 4. ×300, Fig. 3. ×600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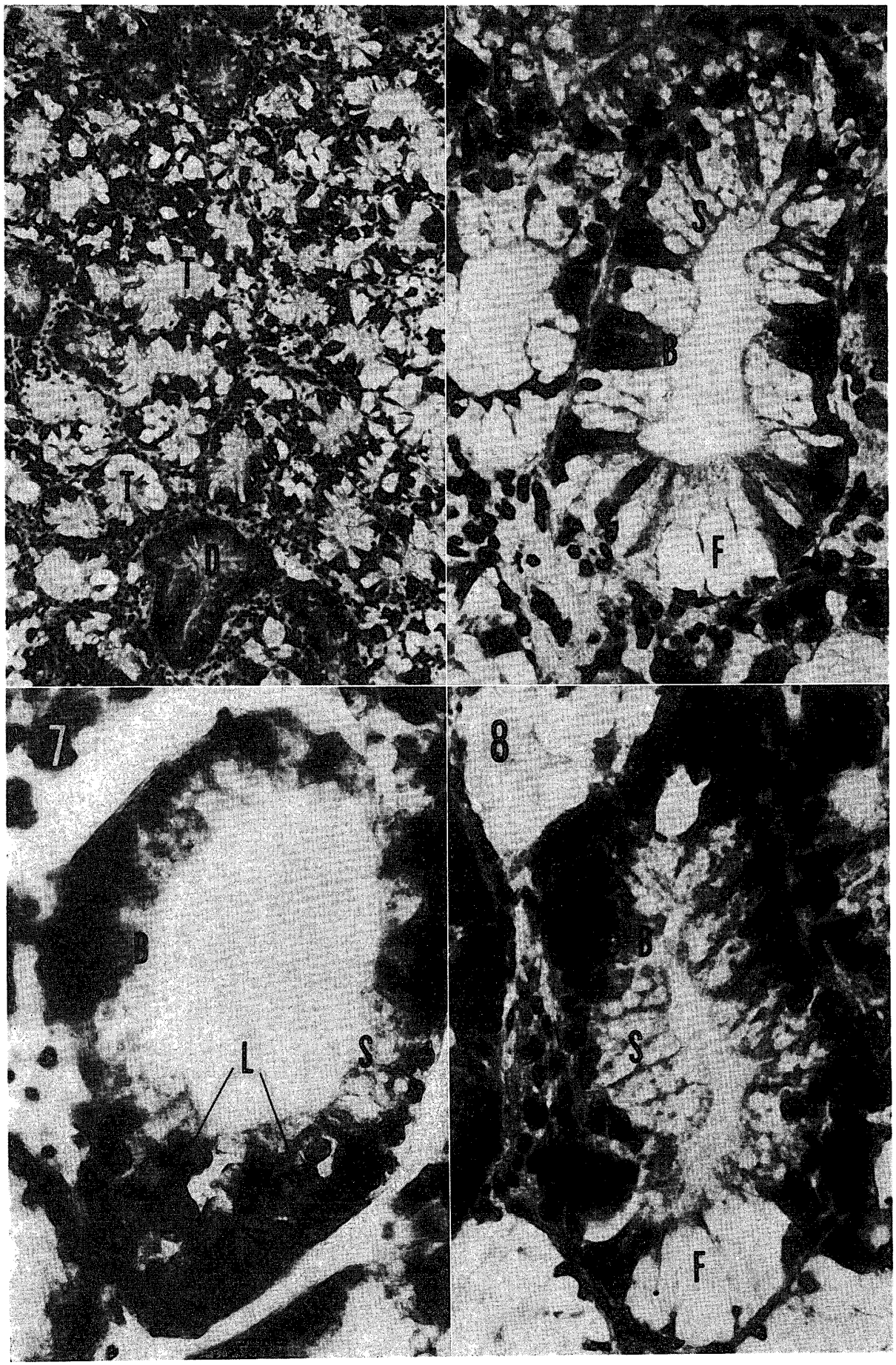
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PLATE 2

Explanation of Figures

Figs. 5–7. Micrographs showing tubules of digestive gland administered methionine. The decrease of lipid (L) is recognized and the cell boundaries of basophil cell (B) and secretory cell (S) are distinct. The swelling of fat cell (F) is not observed. D: duct, T: tubule
Fig. 5 ×150, Figs. 6, 7 ×600

Fig. 8. Micrograph showing tubule of digestive gland administered choline chloride. Basophil cell (B) and secretory cell (S) show normal characteristics. In the nuclei of both cells, chromatin and nucleolus are recognizable. F: fat cell ×600