

# The Effect of Methionine on the Digestive Gland of the Japanese Scallop, *Patinopecten yessoensis* (JAY), with Special Reference to the Total Lipid Content and the Morphological Changes in the Digestive Gland

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the Japanese Scallop, *Patinopecten yessoensis*  
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Lipid Content and the Morphological  
Changes in the Digestive Gland**

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**Summary**

The total lipid content and morphological changes in the digestive gland of the Japanese scallop, *Patinopecten yessoensis* (JAY), were investigated after injecting methionine into the scallop in situ.

The total lipid content in the digestive gland of the group sampled at the beginning of the experiment, the methionine dose group and the control group were 16.2–24.5 per cent, 16.1–22.7 per cent and 29.1–41.3 per cent respectively.

The ratio of the cross section area occupied with fat vacuole in the digestive gland of the three groups was 34.6 per cent, 33.9 per cent and 42.3 per cent respectively. Furthermore, in the methionine dose group, the recovery of the functional activity of the secretory cell and the activation of amoebocyte were observed.

Fatty infiltration of the liver may be induced in the experimental animals by many different toxic substances, metabolites or nutritional deficiencies (1, 2). In the Japanese scallop, as the water temperature rises after spawning in the spring, the accumulation of lipid in the tubule epithelium of the digestive gland was noticed in the specimens collected from the Mohne Inlet or Onagawa Bay (3). On the other hand, it was observed by means of histological technique that the experimental high water temperature induced the fatty infiltration in the digestive gland of the scallop and methionine was effective on the decrease of the lipid and the recovery of physiological activity of the digestive gland (4).

In this article the authors report on the total lipid content and the morphological changes in the digestive gland after administering methionine into the scallop in situ.

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### Material and Methods

This experiment was carried out from the 25th of July to the 23rd of August in 1969 at the Mohne Laboratory of the Oyster Research Institute. Young scallops, with a shell length of about 7 cm, were put in a wireframed net and hung in the sea to a depth of 3 m. Then 3 mg of L-methionine, dissolved in Herbst's artificial sea water, was administered to these scallops by injection into the adductor muscle 9 times at an interval of 72 hours. For injection, methionine was given in a concentration of 1 mg/0.1 ml.

At 72 hours after the final injection, the total lipid content of the digestive gland was measured by the following method. The procedure for extraction of lipid from the digestive gland was based on the method of Bligh and Dyer (5). The tissue, for instance 10 g in weight, was blended mechanically for 2 minutes in 30 ml of chloroform/methanol (2:1) solution. To the mixture was added 10 ml of chloroform and 10 ml of distilled water, and then blending was continued for 1 minute. The homogenate was filtered then allowed to separate into chloroform and aqueous layers. The tissue residue was reextracted with 15 ml of chloroform, and then filtered again. The chloroform-lipid layers were combined and chloroform was taken off by the evaporator.

The digestive gland was excised in Carnoy's fixative. After fixation, the tissue was dehydrated in a graded series of ethanol and embedded in paraffin. Sections of 6  $\mu$  thickness were made and stained with hematoxylin-eosin and Heidenhain's iron-hematoxylin.

The scallops injected with only Herbst's artificial sea water and not treated were used as the control.

### Results and Discussion

#### *Total Lipid Content in the Digestive Gland*

The results of quantity of total lipid content are presented in Fig. 1. At the beginning of the experiment, the total lipid content in the digestive gland of the scallop was 16.2–24.5 per cent and in the individuals sampled on the 23rd of August (control group), it was 29.1–41.3 per cent. The total lipid content in the digestive gland showed a natural increase of about 100 per cent during the experimental period. It is clear from the previous experiment that the high water temperature over 20°C induces the lipid accumulation in the digestive gland (4), and it seems likely that the total lipid content made a rapid increase because of the high water temperature in August. The sea water temperature, in Mohne Inlet, in July of 1969 was lower than normal being 13–20°C at 3 m in depth. Also, in August, there was no normal temperature change. In the methionine dose group, the total lipid content was 16.1–22.7 per cent, the same as in the beginning of experiment. There is no biochemical or physiological data on the fatty infiltration in the

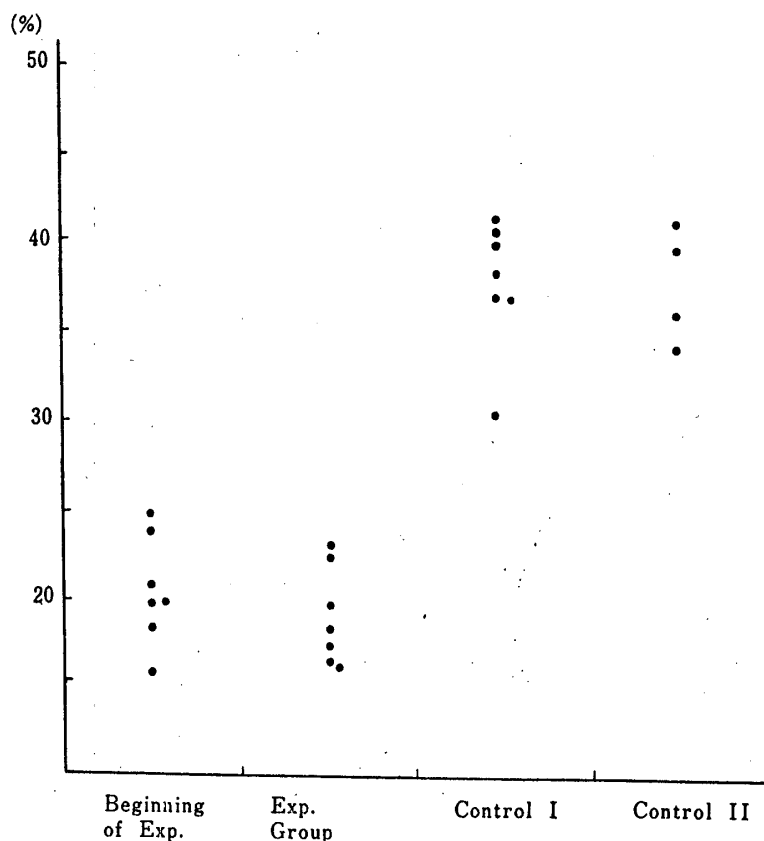


FIG. 1. Total lipid content in the digestive gland.  
 (Total lipid content/Digestive gland wet weight  $\times 100$ )  
 Exp. Group: methionine administered    Control I: not treated  
 Control II: only artificial sea water injected.

digestive gland of the scallop, it seems clear that methionine was effective on the obstruction of the increase of lipid.

In mammalia, it is well known that methionine participates in the synthesis of phospholipid by the transmethylation and secondary methionine effective as the lipotropic factor on the fatty infiltration (1, 2).

Meanwhile, in marine invertebrates, Bilinski observes that methionine-methyl-C was utilized for the formation of phospholipid-bound choline in the lobster, *Homarus americanus* (6). However, Shieh in his paper on the sea scallop, *Placopecten magellanicus*, stated that, when methionine-methyl-C was used as the precursor, phospholipid was not found (7).

There seems to be no stress by injection into the adductor muscle because the shell growth of scallop administered with methionine was normal. Namely, the growth of the shell length was 5-6 mm in the experimental period. On the other hand, that of the control group during the same period was 4-6 mm.

#### *Morphological Changes in the Digestive Gland*

At first, the area of fat vacuole in the digestive gland was measured; the

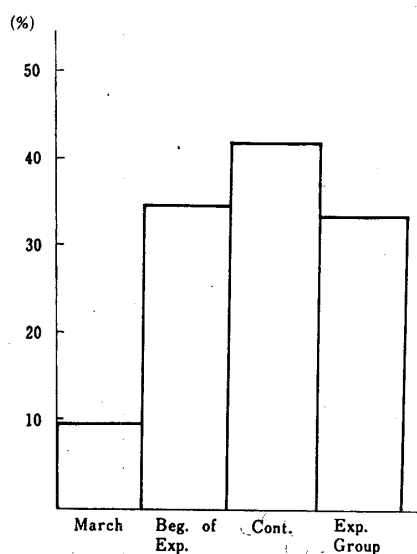


FIG. 2. Area occupied with fat vacuole in the digestive gland in cross section.  
Control: not treated Exp. Group: methionine administered

results are presented in Fig. 2. At the beginning of the present experiment, the fatty infiltration was considerably advanced and the ratio of the area occupied with the fat vacuole was 34.6 per cent as against 9.8 per cent in March. In the control group, the area of fat vacuole increased and the ratio was 42.3 per cent. In the scallops sampled at the beginning of the present experiment and in those of the control group, the swelling of the fat cell and the atrophy of the basophil cell and secretory cell were observed. In addition, the cell boundaries of the basophil and secretory cells were indistinct, and desquamation of the supranuclear region of the secretory cell was noticed (Fig. 3). These changes seem to be in parallel with the increase of fat vacuole as shown in the previous papers (3, 4). On the contrary, the ratio of the area occupied with the fat vacuole in the methionine dose group was 33.9 per cent, this agrees with the result of the total lipid content in the digestive gland.

Previously the authors reported on the experimental fatty infiltration in the digestive gland and on the effect of methionine (4). In the present experiment, the morphological changes of the basophil cell and secretory cell after administering with methionine were not so clear as in the previous study. Concerning the secretory cell, however, an aspect similar to the previous study was observed. Namely, the secretory cells were cylindrical in shape and their boundaries were distinct (Fig. 4).

Further, in the scallop dosed with methionine, the amoebocytes were activated in the connective tissue of the inter-tubule (Fig. 4). It has frequently been stated that in the oyster the amoebocytes have a part in assimilation, bearing nutrition and excretion (8,9). The pathophysiological role of the amoebocyte was also discussed in the oyster, *Crassostrea gigas* (10). It may be suggested that the amoe-



FIG. 3. A micrograph showing a tubule of the digestive gland of the control group.

In the epithelial cell, the cell boundary is indistinct and desquamation of the supranuclear region is observed (arrow). Connective tissue (Ct) is also loose.

FIG. 4. A micrograph showing a tubule of the digestive gland of the methionine dose group. Secretory cells are cylindrical in shape and activation of amoebocytes (A) is noticed. Furthermore, the connective tissue (Ct) is compact.

bocyte has relation with the decrease of fat in the digestive gland after injecting methionine. The authors plan to give a detailed account about the functional significance of the amoebocyte at another opportunity.

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