Seasonal Variations in the Metabolism of Lipids and Glycogen in the Scallop, Patinopecten yessoensis (JAY) II. Histochemical Studies

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Seasonal Variations in the Metabolism of Lipids and Glycogen in the Scallop, *Patinopecten yessoensis* (JAY)

II. Histochemical Studies*

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Summary

1. The seasonal variations in the metabolism of lipids and glycogen in the Japanese scallop, *Patinopecten yessoensis* (Jay), were histochemically analized. Scallops, cultivated in Onagawa Bay, Miyagi Prefecture, Japan, by the hanging method, were used as the materials for the study.

2. Lipids in the digestive diverticula were observed as numerous droplets in the epithelia of the tubules, though they were also detected in the epithelia of the ducts. Glycogen in the adductor muscle was found to be localized in the cells of the connective tissue.

3. In the epithelia of the tubules of the digestive diverticula, lipids (mainly neutral fats) were detected in large quantities in summer, but they showed a marked decrease with the beginning of sexual maturation. However, their total amount proved to increase rapidly as sexual maturation proceeded.

4. The glycogen amount in the adductor muscle increased as sexual maturation proceeded. This increase was not more marked than that of the lipid amount in the digestive diverticula.

5. These histochemical findings on the seasonal change in the amount of lipids in the digestive diverticula of the scallops cultivated in Onagawa Bay agree well with those obtained by us for scallops from Mutsu Bay, Aomori Prefecture.

There have been many reports concerning the seasonal variations in the chemical composition of the entire soft body of bivalves, but relatively little is known about that of the separate body components, namely, parts that can be conveniently separated from one another by dissection. As previously reported (1), we investigated biochemically the chemical composition of the separate body components of the scallop, *P. yessoensis*, in connection with the growth and reproductive cycles. However, it is uncertain whether the seasonal variation in the chemical

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composition of each body component is relative or absolute. Accordingly, we attempted to follow the seasonal variation in the histochemical distribution of lipids and glycogen.

The purpose of this study was to follow histochemically the seasonal variations in the metabolism of lipids and glycogen in the scallops, *P. yessoensis*, cultivated in Onagawa Bay, and to clarify the correlation between the aforementioned variations and the growth and reproductive cycle. In addition, scallops from Mutsu Bay were investigated for comparison.

Materials and Methods

The experiments were carried out each month from July in 1967 to August in 1968. One or two year old scallops cultivated at a depth of 6 m in Onagawa Bay, Miyagi Pref., by the hanging method, were used as the experimental materials. The juveniles of these scallops which had been hatched out of fertilized eggs in May, 1966, at the Mohne laboratory of the Oyster Research Institute near Kesennuma City, Miyagi Pref. (2), were transfered to Onagawa Bay at the end of April in 1967 for this study.

The fresh tissues of the gonad, digestive diverticula, gill, mantle and adductor muscle of 8 to 10 scallops were sampled at every experiment. For demonstration of glycogen, pieces of the tissues were fixed in Carnoy's fixative, then embedded in paraffin according to the usual manner and sectioned at 6 microns in thickness. After deparaffinization, the sections were stained with the periodic acid-Schiff (PAS) with or without the counterstaining of Delafield's haematoxylin or Heidenhain's iron haematoxylin. In order to more precisely substantiate glycogen, the saliva-treatment was made on some of the sections before the above stainings. For demonstration of lipids, the tissue pieces were fixed in Baker's fixative (formol-calcium solution), then embedded in gelatin and sectioned by a freezing microtome at a thickness of 15 microns. The sections were stained with Sudan black B, Sudan III and Nile blue (3). For detection of phospholipids, pieces of the tissues were treated with a bichromate solution containing calcium chloride after fixing with Baker's fixative for six hours and then embedded in gelatin. The sections were 10 microns in thickness. They were stained with Baker's acid haematein solution and then counterstained with a ferricyanide-borax mixture (4). As a negative control, the tissue was fixed in weak Bouin solution, then the lipids were successively extracted with hot pyridine before the bichromate treatment mentioned above. In some cases, the 6 micron paraffin sections fixed in Carnoy's fixative were stained by the Acrolein-Schiff reaction for detection of protein.

Results

1. Tissue Distribution and Main Component of Lipids

In the digestive diverticula exhibiting a marked seasonal variation in the

lipid content as shown in Fig. 16 of the previous report (1), a substance positive to Sudan III and Sudan black B was localized in the epithelia of the tubules and ducts but was not detected in the connective tissue (Fig. 1). In the tubules, it was dyed pink by Nile blue stain. In the ducts, it was dyed blue by the same stain and further it was positive to Baker's acid haematein. From these results, it is concluded that the main components of the lipids localized in the epithelia of the tubules are neutral fats, and in the epithelia of the ducts they are phospholipids. The main components of the lipids distributed in the ciliary epithelia of the crystalline style sac and intestine are assumed to be phospholipids, because they were dyed blue by Nile blue stain and were positive to Baker's acid haematein (Fig. 2).

There were no significant differences in the tissue distribution of lipids between the female and the male. However, a large quantity of lipids were always observed in the cytoplasm of the eggs (Fig. 3), while there were few lipids detectable in sperm. The lipids in the eggs were dyed blue by Nile blue stain and were positive to Baker's acid haematein and Acrolein-Schiff (Fig. 3). From this observation, it seems probable that the bulk of the lipids in the yolk are lipoprotein, the main component of the lipids constituting the lipoprotein being phospholipids. There were also neutral fats in the yolk (5, 6).

2. Seasonal Variation in the Tissue Distribution of Lipids

The results are given in Table 1. From late July to late August, 1967, when high levels of environmental temperatures were observed (1), it was found that a large quantity of neutral fats were stored in the epithelia of the tubules of the digestive diverticula and in some cases they were widely distributed in all parts of the tubules (Fig. 4). After that, they decreased markedly, as the temperature fell. And, in the latter part of October when sexual maturation had just commenced (1), only a small quantity was observed (Fig. 5). However, they increased in amount again as sexual maturation proceeded rapidly (Fig. 6). The trend to increase was also found during and after spawning, and again they could be detected in large quantities in the summer of 1968 (Figs. 7 and 8).

There were no significant seasonal variations in the phospholipids distributed in the epithelia of either the ducts of the digestive diverticula or the crystalline style sac. But, the phospholipids detected in the epithelia of the intestine showed an increase after spawning.

3. Seasonal Variation in the Tissue Distribution of Glycogen

The results are given in Table 2. In the adductor muscle, glycogen was observed to be localized in the connective tissues of the epimysium and endomysium. In the muscle cell also, it was sporadically detected as large granules (Fig. 9). In the ovary, it was found in large quantities in the ciliary epithelia of the

genital canal, but we could not detect a clear seasonal variation because there were great extremes of the amount even among the scallops sampled at the same time (Fig. 10). In the muscle of the mantle, glycogen was observed to be stored after May (Fig. 11).

In summer, a large quantity of glycogen was detected in the connective tissue of the adductor muscle. After that, however, it showed a decrease in amount until the beginning of sexual maturation (1). During the stage of sexual maturation it showed an increasing trend, and at the spawning period it again reached the level of the previous summer. The seasonal change in the distribution of glycogen in the adductor muscle was almost in parallel with that of the lipids in the epithelia of the tubules of the digestive diverticula, but the degree of change was more marked in the latter than in the former.

Discussion

From Fig. 16 of the previous paper (1), it is evident that the seasonal changes of the lipid content in the ovary and the spermary were considerably different. It seems probable that an increase in the lipid content found in the spermary from the spawning period on was due to two factors, namely, an increase in the amount of phospholipids distributed in the epithelia of the intestine running through the gonad (Table 1) and the relative increase caused by a decrease in the protein content attendant upon the discharge of sperms (Fig. 18 of the previous paper (1)). On the other hand, it is considered that an increase in the lipid content in the ovary during sexual maturation was due to an increase in yolk storage (Table 1). That no marked fall in the lipid content was found in the ovary even after spawning may be due to an increase in the amount of phospholipids localized in the epithelia of intestine running through the gonad.

It is of interest that, from the spawning period on, phospholipids were detected in large quantities in the epithelia of the intestine almost in parallel with the marked storage of neutral fats in the epithelia of the tubules of the digestive diverticula (Table 1). It is not known whether in marine invertebrates the lipids of the blood stream or body fluid are resynthesized from the products of digestion on entering the body fluid or whether they are mobilized from the gut as droplets of lipids (7). According to Noma (8), on the other hand, it appears most probable that in albino rats, lecithin is supplied from the liver through the bile duct into the intestinal lumen, where it is converted to fatty acid, choline and glycerophosphoric acid, which are taken up by mucosal cells and utilized for the resynthesis of lipids. The net synthesis of lecithin is therefore increased in the mucosal cells during fat absorption, and the increased lecithin, together with newly synthesized fat, contributes to the formation of chylomicron, which is then transported through the circulation, and taken up and utilized by the liver and

TABLE 1. *Seasonal Variation in the Tissue Distribution of*

Tissue		Date of		
		Jul. 29 '67	Aug. 30	Oct. 7
		?	?	?
Digestive diverticula	Tubule	⫲, ⫲	⫲	⫲
	Duct	+	+	±, +
	C. tissue	−	−	−
Gonad	Egg	/	/	/
	Sperm	/	/	/
Crystalline style sac		/	+	+
Intestine		/	⫲	⫲

Tissue		Date of			
		Mar. 4		Apr. 11	
		F	M	F	M
Digestive diverticula	Tubule	⫲, ⫲	⫲, ⫲	⫲, ⫲	⫲, ⫲
	Duct	⫲	⫲	⫲	⫲
	C. tissue	−	−	−	−
Gonad	Egg	⫲	/	⫲, ⫲	/
	Sperm	/	−	/	−
Crystalline style sac		+	+	+	+
Intestine		+	+	+, ⫲	+, ⫲

F: female M: male ?: The sexes were not identifiable. C. tissue: connec

TABLE 2. *Seasonal Variation in the Tissue Distribution of PAS-positive Substance*

Tissue		Date of			
		Aug. 30 '67	Oct. 1	Oct. 29	Dec.
		?	?	?	F
Adductor muscle	Epimysium	⫲	⫲	⫲	⫲
	Endomysium	⫲	⫲	+	+
Genital canal		⫲	⫲	⫲	⫲

Tissue		Date of		
		Apr. 1		May
		F	M	F
Adductor muscle	Epimysium	⫲, ⫲	⫲, ⫲	⫲
	Endomysium	⫲, ⫲	⫲, ⫲	⫲
Genital canal		+, ⫲	+, ⫲	+, ⫲

F: female M: male ?: The sexes were not identifiable.

Sudanophilic Substances, Namely, Total Lipids in Scallop.

Sampling						
Oct. 29	Dec. 4		Dec. 29		Jan. 30 '68	
?	F	M	F	M	F	M
+	+, ++	+, ++	+, ++	+, ++	++, +++	++
+	++	+	++	+	++	++
−	−	−	−	−	−	−
/	+	/	+, ++	/	+++	/
/	/	/	/	−	/	−
+	+	+	+	+	+	+
++	++	++	++, +++	++, +++	+	++

Sampling								
May 22		Jun. 14		Jul. 30		Aug. 22	
F	M	F	M	F	M	F	M
+++	+++	+++	++, +++	+++	+++	+++	+++
++	++	++	++	++	++	+	+
−	−	−	−	−	−	−	−
++, +++	/	++, +++	/	+	/	/	/
/	−	/	−	/	−	/	/
+	+	+	+	++	++	++	++
++, +++	++	++, +++	+++	+++	++, +++	+++	+++

tive tissue

Which Can Be Digested by the Saliva-treatment, Namely, Glycogen in Scallop.

Sampling						
4	Dec. 29		Jan. 30 '68		Mar. 4	
M	F	M	F	M	F	M
++	++	++	+++	++, +++	+++	+++
+	+	+	++	+, ++	+, ++	++
+++	++, +++	++, +++	++, +++	+, ++	+, ++	+, ++

Sampling						
22	Jun. 14		Jul. 30		Aug. 22	
M	F	M	F	M	F	M
+++	+++	+++	+++	+++	+++	+++
++	+++	+++	+++	+++	++	++
+, ++	+, +++	+, +++	++, +++	+∼+++	+∼+++	+, ++

other tissues. Accordingly, it seems likely that the external factors such as feeding are more important than the internal factors such as lipid synthesis from carbohydrate stores, concerning the marked storage of neutral fats in the epithelia of the tubules of the digestive diverticula of scallops. However, further investigations will be required in this connection.

The seasonal chnages in the contents of lipids in the digestive diverticula and glycogen in the adductor muscle mentioned in the previous report (1) were also histochemically observed (Table 1). However, it is not obvious whether these changes are characteristic of the scallops cultivated in Onagawa Bay or whether they are to be regarded as a physiological phenomena proper to *Patinopecten yessoensis* itself. In order to make this point clear, scallops from Mutsu Bay were also histochemically investigated for comparison concerning lipids in the digestive diverticula.

In the scallops from Mutsu Bay, it was observed that a large quantity of neutral fats were stored in the epithelia of the tubules of the digestive diverticula in summer (Fig. 12). The result of this observation agrees well with that from Onagawa Bay. From this fact together with that from the chemical analysis (1), it is evident that the seasonal change in the amount of lipids in the digetive diverticula is a physiological phenomenon proper to *P. yessoensis* itself.

Mori et al. (9) carried out histochemical studies on the distribution of total lipids and glycogen in the Japanese common oyster with special reference to the sexual cycle, and reported that sudanophilic substances, namely, total lipids, showed a drop in amount in the connective tissue surrounding the digestive diverticula and intestine during the stages of sexual maturation and spawning, while they were found to deposit in the epithelia of the digestive diverticula and intestine during these periods and to decrease in amount after spawning. They also reported that the amount of glycogen in the connective tissue decreased in parallel with total lipids as sexual maturation proceeded.

In the scallops cultivated in Onagawa Bay (Tables 1 and 2), neutral fats distributed in the epithelia of the tubules of the digestive diverticula showed an increase in amount with sexual maturation. In summer after spawning they were found to be stored in very large quantities in the epithelia, though there were no considerable seasonal variations in the phospholipids distributed in the epithelia of both tubules and the ducts of the digestive diverticula exclusive of those localized in the intestinal epithelia. These results indicate that oysters and scallops differ in the tissue distribution of lipids and glycogen during the stages of sexual maturation and spawning. In addition, they seem to indicate that the mobilization of stored lipids in scallops during these periods is not so marked as in oysters.

In scallops, high concentrations of glycogen were detected in the adductor muscle (Fig. 17 of the previous paper (1)), and they were found to be localized especially in the cells of the connective tissue such as the epimysium and endomys-

ium (Table 2). Furthermore, glycogen in this organ showed a marked seasonal variation in content (Fig. 17 of the previous paper (1)). These findings are vastly different from those of oysters in which the glycogen content in the adductor muscle is lower than that in other organs such as the mantle or gill, and shows no significant seasonal change (10). From this fact, it would be of special interest to investigate the questions of how much of the glycogen in the adductor muscle of the scallop is part of the locking or catch mechanism and how much of it is reserved for the energy metabolism of other organs or for gonad formation.

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Plate 1

Explanation of the Figures

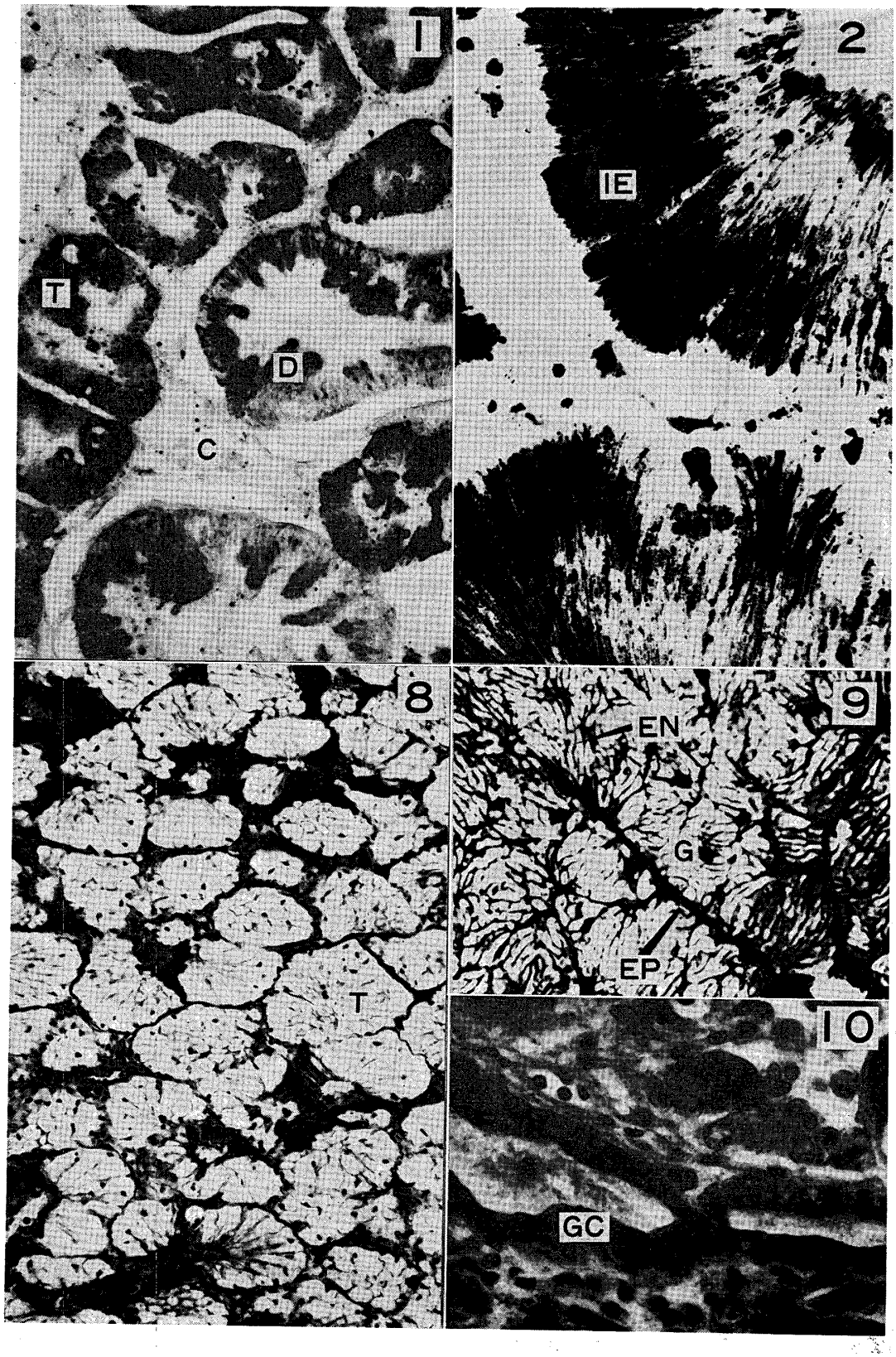
FIG. 1. Digestive diverticula (late January, 1968). A substance positive to Sudan black B is localized in the epithelia of the tubules (T) and ducts (D) but is not detected in the connective tissue (C). ×150.

FIG. 2. Intestine (late June, 1968). Phospholipids are dyed blue in the epithelia (IE). Nile blue stain. ×150.

FIG. 8. Digestive diverticula (late August, 1968). A marked artificial vacuolization is observed in all parts of the epithelia of tubules (T), because lipids were extracted with organic solvents used during the embedding and staining of tissue. Paraffin embedding. H-E stain. ×150.

FIG. 9. Adductor muscle (late August, 1967). Glycogen is observed to be localized in the connective tissues of the epimysium (EP) and endomysium (EN). In the muscle cell also, it is sporadically detected as large granules (G). PAS stain. ×600.

FIG. 10. Ovary (late August, 1968). Glycogen is found in large quantities in the ciliary epithelia of the genital canal (GC). PAS stain. ×600.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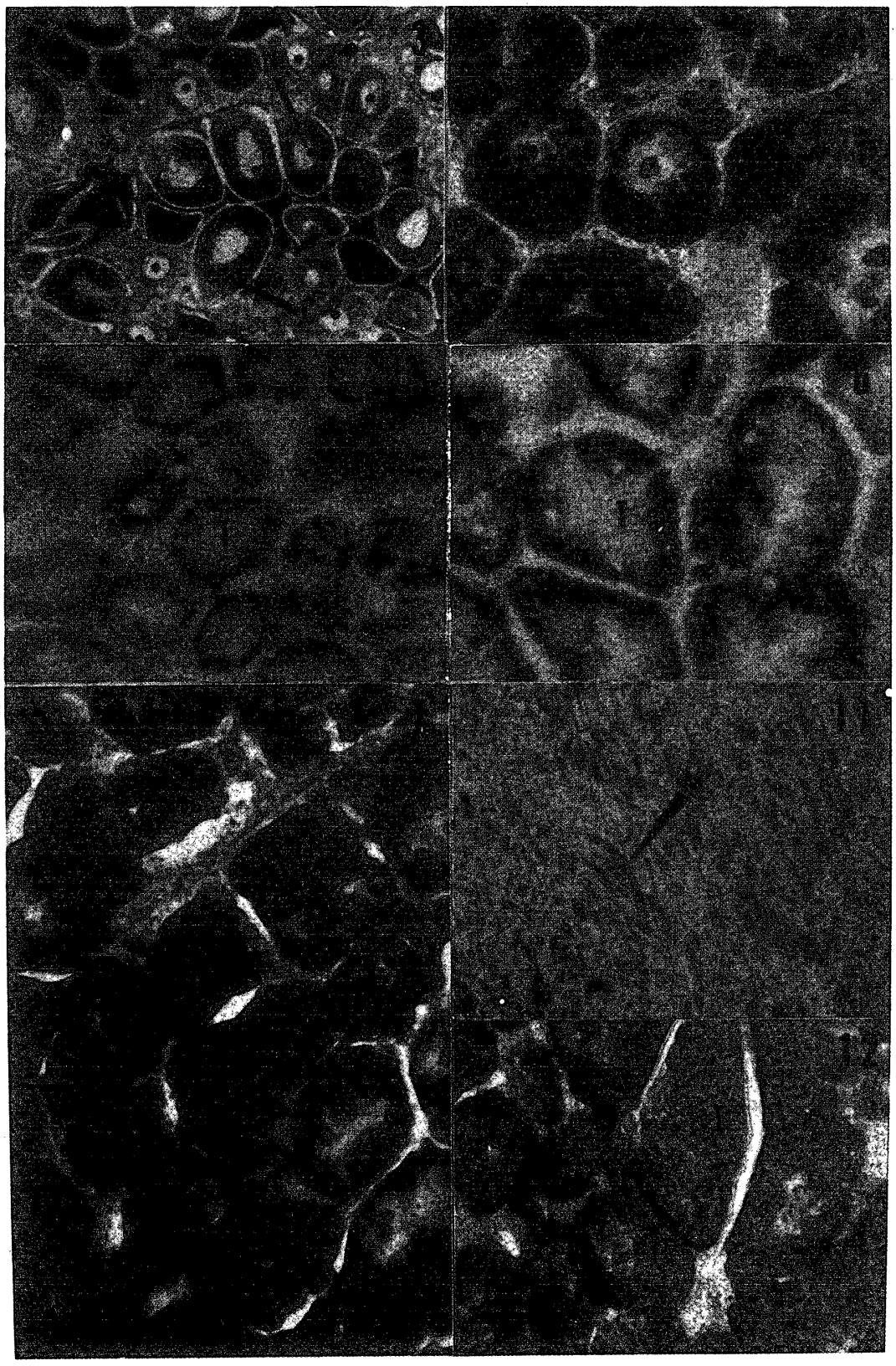

Plate 2

Explanation of the Figures

FIG. 3. Ovary (late February, 1968). The cytoplasm (C) and nucleolus (N) of egg are positive to Baker's acid haematein. After the extraction with hot pyridine, the cytoplasm is negative to this stain, but the nucleolus is still positive. ×150.

FIG. 4. Digestive diverticula (late August, 1967). A large quantity of total lipids (mainly neutral fats) are detected in the epithelia of tubules (T). Sudan III stain. ×150.

FIG. 5. Digestive diverticula (late October, 1967). Only a small quantity of total lipids (mainly neutral fats) are detected in the epithelia of tubules (T). Sudan III stain. ×150.

FIG. 6. Digestive diverticula (late January, 1968). It is observed that total lipids (mainly neutral fats) in the epithelia of tubules (T) increased in amount again as the sexual maturation proceeded rapidly. Sudan III stain. ×150.

FIG. 7. Digestive diverticula (late August, 1968). Again in the epithelia of the tubules, total lipids (mainly neutral fats) are present in large quantities. Sudan III stain. ×150.

FIG. 11. Mantle (late May, 1968). Glycogen is detected in the muscle (M). PAS stain. ×150.

FIG. 12. Digestive diverticula (early August) of a scallop cultivated in Mutsu Bay. A large quantity of neutral fats are detected in the epithelia of the tubules (T). Sudan III stain. ×150.