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Eyestalk Hormones and P^{32} Incorporation of the Hepatopancreas Cells in the Crayfish, *Procambarus clarkii*

With 2 Text-figures

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ABSTRACT The incorporation of P^{32} into hepatopancreas tissue was studied by autoradiographic methods in connection with the influence of eyestalk hormones in the crayfish, *Procambarus clarkii*. $P^{32}O_4H_3$ dissolved in physiological saline was injected into two groups of crayfishes; the one, intact normal crayfishes, and the other consisted of destalked ones. After about 20 hours, the hepatopancreas was removed from each crayfish and fixed in 10% formalin. Tissue sections made by the usual paraffin method were stained with hematoxylin and eosin and then covered with photosensitive emulsion. The slides were examined under the microscope after exposure and development. The incorporation of P^{32} was observed only in the nucleoli of the hepatopancreas cells in the normal crayfishes, but was not observed in the destalked ones. The results may indicate that RNA synthesis in the nucleoli is active in the normal crayfishes but not in the destalked ones. The lack of eyestalk hormones causes the RNA synthesizing ability to be inactive in the hepatopancreas cell nucleoli. It seems clear that RNA synthesis in the hepatopancreas cells is directly dependent on the occurrence of the eyestalk hormones.

It is certain that the hepatopancreas of Crustacea plays very important roles in digestion and in the accumulation of many inorganic and organic substances. The occurrence of metal containing cytoplasmic granules has been demonstrated histochemically in the cells of the hepatopancreas for the present species (Ogura, 1959). Miyawaki, Matsuzaki and Sasaki (1961) reported that Ogura's (1959) iron granule containing cells (Fe cells) were extremely rich in ribonucleic acids (RNA). Miyawaki and Sasaki (1961) demonstrated by autoradiography using Ca^{45} that the Fe cells were very important for calcium metabolism in this species.

On the other hand, the results have been reported on numerous observations and experiments on the eyestalk hormones of Crustacea (hormones from sinus gland-X organ complex). The influence of the eyestalk hormones on a wide variety of physiological phenomena of Crustacea have also been published repeatedly. However, very meager reports have been published on the relationship

between eyestalk hormones and the hepatopancreas. Yamamoto (1960) pointed out that the removal of eyestalks brought about degeneration of hepatopancreas tissue and that implantation of the sinus gland into eyestalkless crayfish prevented the degeneration. Miyawaki and Tanoue (1962), Miyawaki and Ukeshima (unpublished) have shown that the removal of eyestalks results in transformation of ultrastructures of the hepatopancreas cells.

In the present paper, the author wishes to report on the results on autoradiographic study, using P^{32}, on the hepatopancreas tissue. Since phoshoric acid is one of the three main constituents of RNA, the results will be concerned with RNA synthesizing ability of the hepatopancreas cells and with the influence of the eyestalk hormones on it.

MATERIAL AND METHODS

Procambarus clarkii used in the present work were collected from rice fields in the suburbs of Kumamoto City. The crayfishes were divided into 2 groups; one destalked and the other intact. Each animal was kept separately in a small container with water in order to avoid preying one another. The crayfishes were fed fish meat every other day. Fifteen days after eyestalk removal, 0.08 mc of $P^{32}O_4H_3$ in 0.15 ml of M/4.6 NaCl solution was injected into abdominal muscles of each crayfish, both destalked and intact controls. On the next morning, about 20 hours after the injection, tissue fragments of hepatopancreas were taken out and fixed in 10 % formalin (buffered at pH 7.2 with phosphate buffer). Sections, 10 μ thick, were made by ordinary paraffin methods. After staining with hematoxylin and eosin the sections were covered with autoradiographic photosensitive emulsion (Sakura NR-M2, a product of Konishiroku Photographic Industry Co. Ltd.) by the dipping method. Exposure was made for 72 hours in a refrigerator. Developer for X ray film was used in development.

RESULTS

When the autoradiographs of intact crayfishes were compared with those of destalked ones the following fact was observed. The blackening of photosensitive grains was found at the sites of the nucleoli of hepatopancreas cells in the intact (normal control) crayfishes as shown in Figure 1, whereas, in the autoradiographs of the destalked animals, the condition of the nucleoli was quite different. As shown in Figure 2, the sites of the nucleoli remained blank.

The results just mentioned indicate that P^{32} injected into normal crayfishes has been incorporated in nucleoli of the hepatopancreas cells about 20 hours after the injection, while in eyestalkless animals the incorporation of P^{32} has not occurred at all. As mentioned above, phosphoric acid is one of the main constituents of RNA and it has been accepted generally that RNA synthesis actively takes place in the nucleolus. It is highly possible, therefore, that these results indicate that injected P^{32} is incorporated into newly synthesized RNA in nucleoli of the normal crayfishes. The negative autoradiography in the destalked animals indicates that the incorporation of P^{32} into the nucleoli has not taken place, and consequently RNA synthesis has not been carried out in the

hepatopancreas cells in the destalked animals. It can be concluded, therefore, that the synthesis of RNA in the hepatopancreas cells is controlled under the influence of the eyestalk hormones. More precisely, it must be stated that the

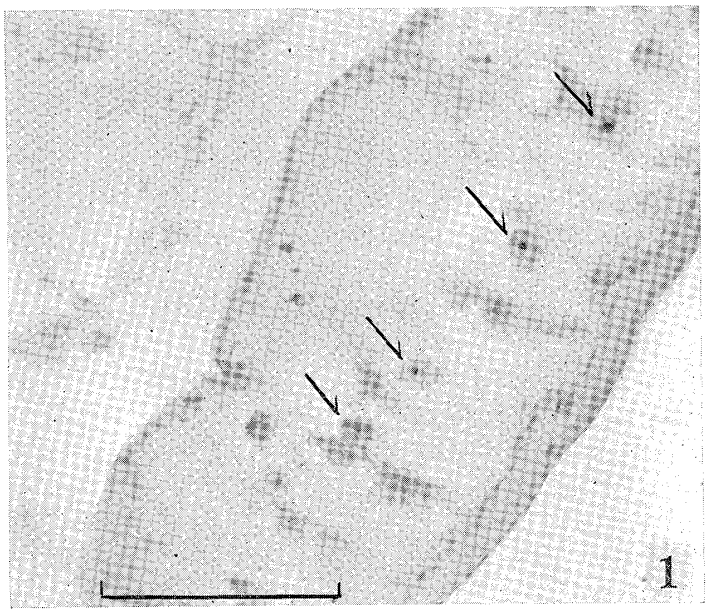

Fig. 1. Autoradiograph of the hepatopancreas tissue of an intact crayfish injected P^{32}; arrows indicate the nuclei of hepatopancreas cells with P^{32} incorporated nucleoli. Scale indicates 50 μ.

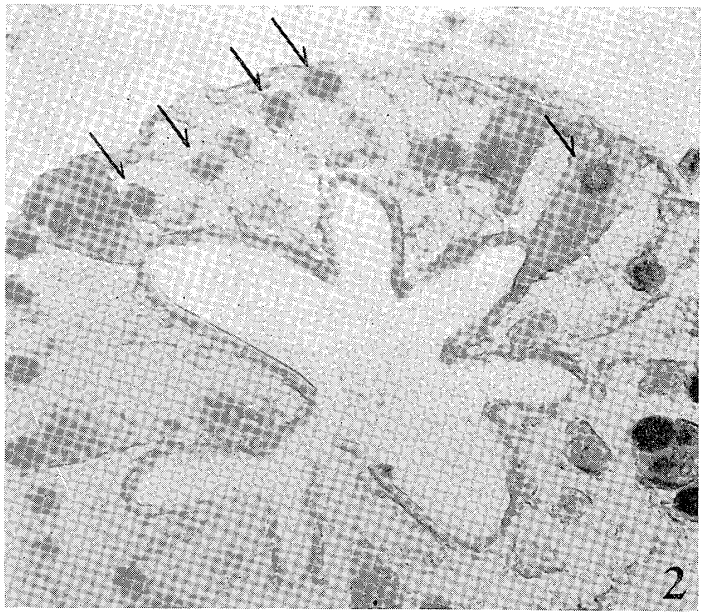

Fig. 2. Autoradiograph of the hepatopancreas of a destalked crayfish; arrows indicate the nuclei in which the sites of nucleoli remain blank. Magnification is same as in Fig. 1.

synthesis of RNA in the hepatopancreas cells is accerelated by the participation of eyestalk hormones, and in the absence of these hormones synthesis may no longer occur.

DISCUSSION

According to Brachet (1957), it has been well established that the nucleolus plays a very important role in protein synthesis and that nucleolar RNA has very high metabolic activity. The results of the present work indicate that the incorporation of P^{32} is seen only in the nucleoli of the hepatopancreas cells of the normal crayfishes 20 hours after the $P^{32}O_4H_3$ injection. The incorporation of P^{32} in the nucleolus seems to show that it has been used as the precursor to nucleolar RNA. Therefore, in normal crayfishes, it is evident that nucleoli of the hepatopancreas cells are synthesizing RNA actively. On the other hand, in the destalked animals, there has been no incorporation of P^{32} into nucleolar RNA. It is not necessary to mention that in the destalked crayfishes the sinus gland-X organ complex has been removed. Since the sinus gland-X organ complex is the source of the so-called eyestalk hormones, the relationship between the eyestalk hormones and the RNA synthesis in the nucleoli of hepatopancreas cells must be taken into consideration. It is clear that the RNA synthesizing activity of the nucleolus is under the control of the eyestalk hormones. Synthesis of RNA occurs normally in the nucleoli of hepatopancreas cells under the influence of eyestalk hormones. The lack of the eyestalk hormones does not stimulate the RNA synthesizing activity of the hepatopancreas cell nucleoli; the precursor P^{32}, then, has not been incorporated.

Yamamoto (1960) has found that eyestalk removal causes degeneration of the hepatopancreas tissue, and the implantation of sinus glands into eyestalkless animals recovers from degeneration. Considering the results of Yamamoto (1960) and the present work together, it is probable that the removal of eyestalks (freeing from the control of eyestalk hormones) causes cessation of RNA synthesis in the hepatopancreas cells and thus the resultant lack of RNA suppresses protein synthesis in the hepatopancreas cells. The reduced activity of protein synthesis would then cause the degeneration of the tissue.

Among the cells constituting the hepatopancreas, Fe cells contain an extremely large amount of cytoplasmic RNA (Miyawaki *et al.*, 1961) as mentioned above. In the present work, however, the incorporation of P^{32} into cytoplasmic RNA has not been observed. This is probably due to the short term of the experiment. Higher turnover rate of the nucleolar RNA than that of the cytoplasmic one might be the reason of this. The incorporation of P^{32} into RNA takes place in the nucleolus at first and, at that time cytoplasmic RNA still remains unlabeled.

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