INTRANUCLEAR CRYSTAL WITHIN THE PHAGOCYTES OF THE OVARY OF *ARBACIA PUNCTULATA*

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INTRODUCTION

The occurrence of intranuclear protein crystalloids in "amoebocytes" of sea urchins, was described in the last century by List (reference 13; *cf.* references 4 and 11 cited by List). During the course of an electron microscope study of the developmental processes of *Arbacia punctulata* oocytes, the author's attention was drawn to the presence of crystalloid bodies within the nuclei of the same type of cells in the ovarian tissues. The present paper describes the crystalline structure and the cytochemical properties of these bodies.

MATERIAL AND METHODS

Material for these observations was collected from mature *Arbacia punctulata* females at the Marine Biological Laboratory, Woods Hole, Massachusetts during the 1964 summer season. Small pieces of the superficial portion of fully grown ovaries were excised. The samples were fixed in 6 per cent glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2) (16) for 1 hour at 3°C, washed in the buffer for 1 hour, and postfixed in 2 per cent osmium tetroxide buffered with 0.1 M sodium cacodylate (pH 7.2) for 1 hour. They were dehydrated in a series of ethanols and embedded in Epon 812 (14). Some similar samples were fixed only in the glutaraldehyde for 20 minutes. After rinsing with the buffer solution, they were dehydrated in a series of glycol methacyrlate within 1 hour, transferred into a 7:3 mixture of glycol methacrylate and butyl/methyl methacrylate (GMA) (10), and quickly polymerized under a dark light lamp (115 volts, 0.38 amps) of 350 mμ wave length. All the procedures were performed in the cold room (3°C).

Ultrathin sections (0.03 to 0.05 μ) for electron microscopy and thick sections (0.5 to 2 μ) for light microscopy were cut with a diamond knife on a Porter-Blum microtome. Thick sections from GMA-embedded material were examined with the light microscope after various cytochemical tests (1). Some thin sections from GMA-embedded materials were examined after treatments with 10 per cent perchloric acid. Thin sections were stained with 2 per cent uranyl acetate. An RCA model EMU-3E microscope at 50 kv using a single condenser and a 25-μ objective aperture was used for electron microscopy.

OBSERVATIONS AND DISCUSSION

The wall of the *Arbacia* ovary is composed of five separate and distinct layers: an outer ciliated epithelium, collagenous connective tissue, smooth muscle tissue, a second layer of connective tissue containing scattered nerve elements, and finally a germinal layer. The germinal layer contains oogonia and oocytes. The inner lumen is filled

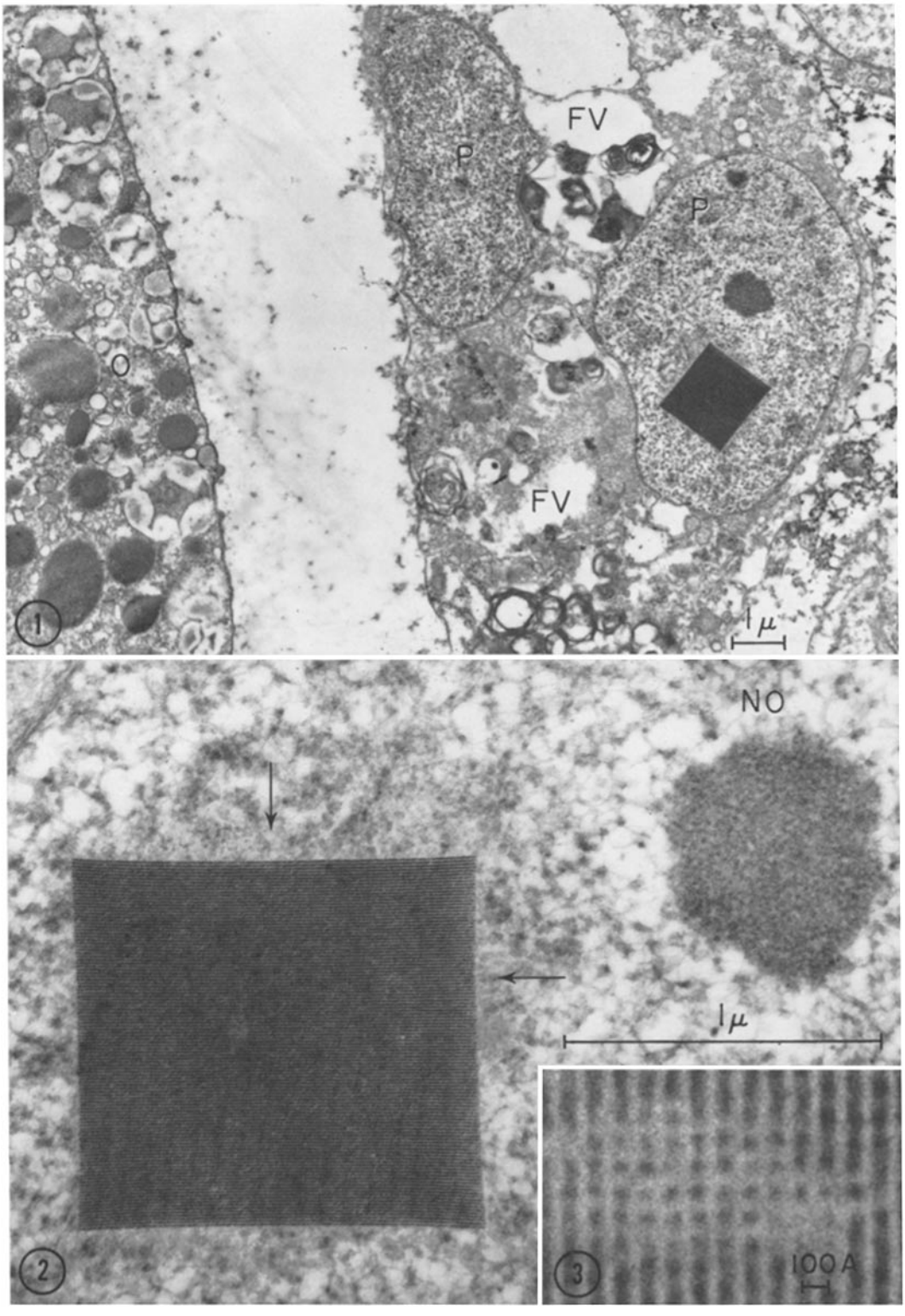
All electron micrographs are from thin sections of ovarian tissues of *Arbacia punctulata*.

FIGURES 1 to 3 Tissue was fixed in glutaraldehyde, postfixed in OsO_4, embedded in Epon, and stained with uranium.

FIGURE 1 Two phagocytes (*P*) and part of fully grown oocyte (*O*). Crystal is localized in the nucleus of a phagocyte. Phagocytes contain trephocytic materials in the food vacuoles (*FV*). × 8,000.

FIGURE 2 Part of the nucleus of a phagocyte in Fig. 1. Intranuclear crystal and nucleolus (*NO*). The crystal has no limiting membrane, but shows a zone of moderate dense material of unknown nature at the periphery (arrows). × 50,000.

FIGURE 3 High magnification of a portion of the crystal in Fig. 2, showing square array of dense dots with both spacings of about 100 A. × 400,000.



with full-grown loose oocytes (*cf.* 12, 18). Various types of leucocytes (12) are scattered throughout all these tissues. Of the leucocytes, only the so called amoeboid phagocyte contains crystalloids, and these bodies are always restricted to the nucleus of the cell. This form of phagocyte seems to correspond to the "amoebocyte" of earlier authors (13). Generally, the amoeboid phagocyte has a dark-staining nucleus with a large nucleolus, and it frequently contains almost intact trephocytes or trephocytic material in its food vacuoles. According to Liebman (12), during their growth periods the oocytes of *Arbacia* take up and assimilate the trephocytes or their fragments, as their normal process of nutrition. Also, he has mentioned that the phagocytes often accumulate in the lumen of the ovary and engulf the excess trephocytic materials.

In the present study, phagocytes containing such intranuclear crystalloids were frequently observed near fully grown oocytes (Fig. 1). From counts of 400 ameboid phagocyte nuclei in thick sections, the frequency of those containing crystalloids varied between 3 and 20 per cent among four animals.

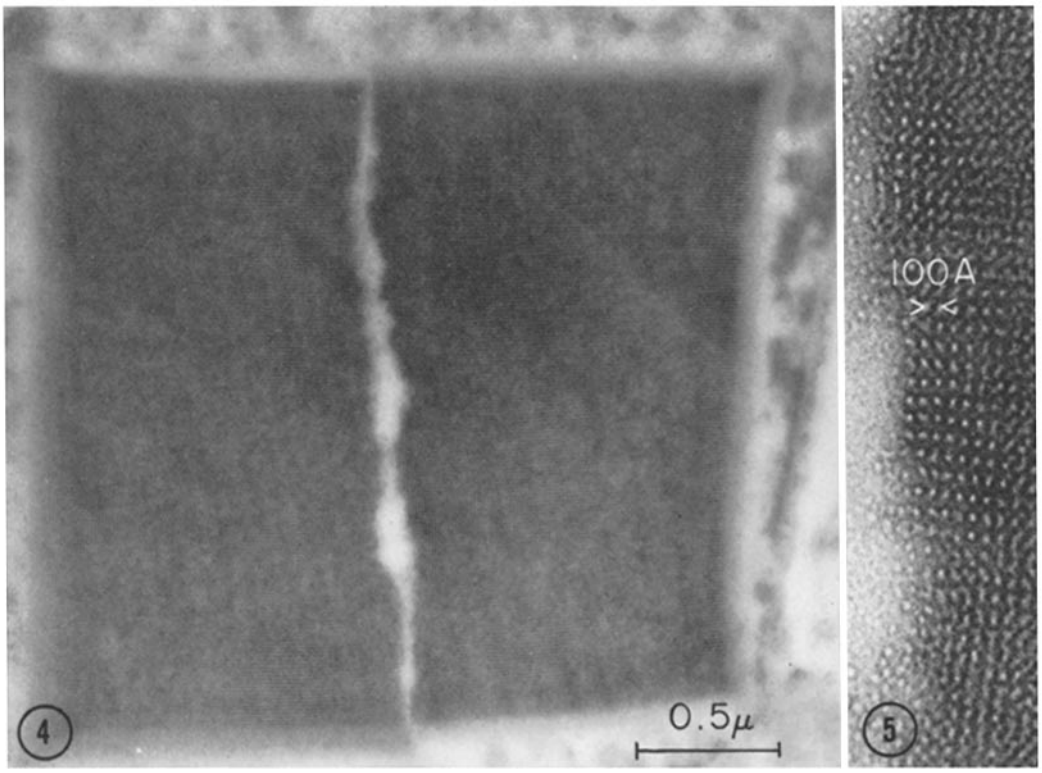
The intranuclear crystalloids are very basophilic and can be shown by serial sections to exist as hexahedrons, tetrahedrons, and occasionally as other types of polyhedrons. The large crystalloids range up to 5 μ in length, and smaller ones down to 0.1 μ. They are faint yellow in unstained sections of the glutaraldehyde-fixed material. Some cytochemical tests carried out on them and the results obtained are summarized in Table I. When the sections are stained without pretreatment for ferric iron by the Prussion blue reaction of Perl (1), only the intranuclear crystalloids show deep blue color. This fact indicates that considerable amounts of ferric iron are present in the crystalloids. The reaction is not significant in any other portion of the phagocytes. Oocytes or trephocytes are also negative to the test. An intense reaction to the mercuric bromphenol blue test (1) indicates that the crystalloids also contain protein. After treatment with 10 per cent perchloric acid at 60°C for 1 hour, which usually removes both RNA and DNA (1), little or no effect on the basophilia of the crystalloids is detected. Similarly, the staining of thin sections with uranyl acetate for electron microscopy is not altered after the same treatment.

TABLE I

Cytochemical Tests Conducted on Thick Sections of Ovarian Tissues Fixed in Glutaraldehyde and Embedded in Glycol Methacrylate

Cytochemical tests (*cf.* ref. 1)	Staining of amoeboid phagocytes	
	Intranuclear crystal	Nucleolus
Perl's Prussian blue	++ (deep blue)	−
Toluidine blue pH 5	+ (blue)	+ (blue)
pH 11	++ (deep blue)	+ (purple)
Mercuric bromphenol blue	++ (deep blue)	+ (blue)
Pyronin	+ (pink)	++ (pink)
Azure B	+ (green blue)	+ (blue)
Basic fuchsin	++ (red)	++ (red)
Eosin	±	+ (pink)
PAS reaction	−	−
Feulgen reaction	−	−

In the electron microscope, thin sections embedded either in Epon or GMA reveal a highly ordered periodicity in each intranuclear crystal (Figs. 2 to 8). Although the crystals have a high intrinsic density in unstained sections of aldehyde-fixed materials (Figs. 4 and 5), the density is generally increased significantly by uranium staining. Sections of the crystals reveal dot or band patterns. Their regularity usually extends over the whole crystalloid. The dot pattern packed in a square array is found in square or rectangular profiles of crystal sections (Figs. 2, 4, 7, 8). Less frequently, an almost hexagonal array of dots is found in triangular profiles (Fig. 6). The acute angles between the crossed bands vary between



FIGS. 4 to 8, phagocytes fixed in glutaraldehyde and embedded in GMA.

FIGURE 4 A large cracked intranuclear crystal with a square array of dots in square profiles. Unstained. × 40,000.

FIGURE 5 High magnification of a portion of the crystal in Fig. 4, showing square array of dots with spacings of about 100 A. Note the contrast reversal of crystal lattice pattern. This phase reversal effect can be observed in slightly out-of-focus images of the periodic objects. The same phenomenon was described by Farrant and Hodge (7) for ferritin crystals. × 200,000.

30 and 90 degrees. In 15 examples of a square pattern with angles of 90 ± 5 degrees, the mean of the center-to-center distances between the rows of dots or bands is 101 A (s = 3.6A). In four examples of hexagonal nets with angles of 60 ± 5 degrees, the average distance between the dots is 102 A (s = 4.3A). These facts suggest that the crystalline components are arranged in a face-centered cubic lattice. This structure may also be expected from the geometrical shapes of the crystal bodies themselves and from their isotropic nature (13) under polarized light. The basic structural components in all the crystals appear to have the same characteristics. When these are closely packed in a lattice, they appear to be spheroidal and have a diameter of about 100 A.

Similar periodic patterns have been reported by Farrant and Hodge (7) in thin sections of the ferritin crystal. According to x-ray diffraction analysis of the ferritin crystal by Fankuchen (6), the ferritin molecules are arranged in a face-centered cubic form with a lattice parameter of 186 A. The separation of molecules in projections on the (0 1 0) plane should, therefore, be 93 A for the ferritin crystal. The spacing found here for the intranuclear crystal is about 100 A and is quite similar to the x-ray data of Fankuchen (6). In consideration of the available data from the cytochemical studies, there is thus a possibility that the intranuclear crystalline material may be a variety of ferritin. However, at high magnification, as in Figs. 3 and 5, this material appears as

homogeneous particles about 60 A in diameter, which, at this resolution, show no substructure such as the ferric hydroxide micelles observed by Bessis and Breton-Gorius (3) and Richter (15). This crystalline pattern bears also a striking resemblance to that of some insect virus inclusions which were determined by Bergold to be crystals of face-centered cubic lattice form consisting of spherical molecules (2). The type of periodic pattern is different from that usually found in amphibian yolk platelets, which I consider to be crystals of simple hexagonal lattice form (9).

In the electron microscope, iron-containing protein crystals have been reported in yolk granules of the oocyte of *Limnea stagnalis* (5), in yolk granules of *Planorbis corneus* oocytes (8), and in midgut glands of *Limnoria lignorum* (17). Although these are formed in the cytoplasm, their morphological characteristics resemble those of the intranuclear crystals of *Arbacia*.

Recently Richter (15) has suggested the possibility of nuclear synthesis of iron-containing protein, based on the observation of intranuclear formation of ferritin aggregates which are produced in mouse liver after injection of iron-dextran. The large nucleolus of amoeboid phagocytes is deeply stained by pyronin (Table I) and consists mainly of granular components 150 A in diameter (Fig. 2), suggesting a high level of nuclear metabolism. The nucleoplasm of the same cells is fairly uniform in appearance, also indicating an active condition. Dense particles with a diameter of about 60 A and similar in size and shape to the visible unit of the crystal are frequently found scattered throughout the nucleoplasm (Figs. 2, 7 to 9), but are not encountered in the cytoplasm. Although the function and origin of the intranuclear crystals are unknown, one might speculate that a synthetic mechanism comparable to that proposed for ferritin aggregates (15) is operating in the formation of specific iron-containing protein within the amoeboid phagocyte.

SUMMARY

The nucleus of some amoeboid phagocytes in the ovary of *Arbacia punctulata* contains a large polyhedral body which according to cytochemical tests consists of protein and iron. Electron micrographs of thin sections reveal a crystalline periodicity in this body. The basic units of the crystal probably are spheroidal, each with a diameter of about 100 A, and appear to be closely packed in a face-centered cubic lattice.

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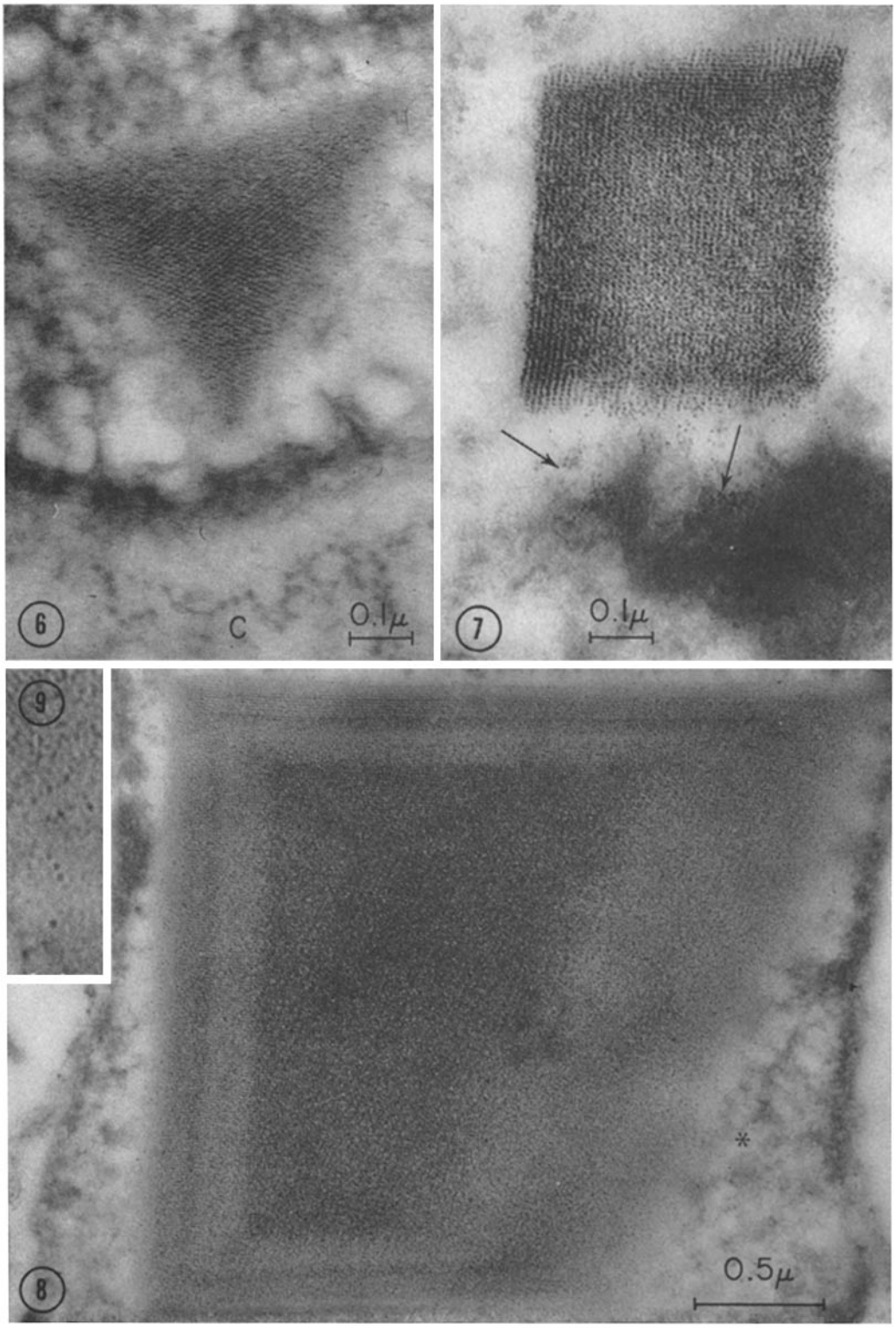
FIGURE 6 A small intranuclear crystal showing hexagonal array of dots. Cytoplasm (*C*). Uranium-stained. × 100,000.

FIGURE 7 A small intranuclear crystal showing square array of dots. Dense particles (arrows) are observed in chromatin. Uranium-stained. × 100,000.

FIGURE 8 A large intranuclear crystal showing a band pattern. The spacing is about 100 A. Note many dense particles in the nucleoplasm. Uranium-stained. × 40,000.

FIGURE 9 Enlargement of a portion of the nucleoplasm (*) in Fig. 8, showing dense particles in the chromatin material. Dense particles are about 60 A in diameter and are surrounded by a clear zone 20 to 30 A thick. × 170,000.



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