A CRYSTALLOID INCLUSION IN THE RABBIT BLASTOCYST

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INTRODUCTION

Intracellular crystals or crystalloids have been reported from a wide variety of cells and tissues over the past century, ever since Auerbach (1) in 1855 found crystals in the cytoplasm of an ameba. They represent a heterogeneous group formed by a wide variety of substances from calcium salts to complex viruses, in cell types ranging from bacteria (7) to vertebrate oocytes (3, 10). In many cases crystalloid structures are of sporadic or variable occurrence, as in the digestive glands of certain molluscs (14) or the interstitial cells of the human testis (9). They may also provide an indication of certain pathological conditions, for example hemosiderosis (12). In other cases they may form an integral part of certain biological systems, such as in the yolk platelets of certain snail oocytes (4).

Although intranuclear crystalloids have been described in mammalian liver and kidney (2, 16), in most cases they have been found in the cytoplasm. Electron micrograph studies have shown some crystalloids to be apparently unrelated to the membrane systems of the cell (13), but in other cases they are bounded by smooth membranes that suggest an affinity with the Golgi apparatus (14), and certain crystalloids have been found within the mitochondria (5, 11).

Some intracellular crystals are apparently formed from simple inorganic salts such as the calcium phosphate inclusions of *Paramecium* (8). Crystals of carbonyl diurea are commonly distributed within the cytoplasm of *Amoeba* (16). Most crystal-like inclusions, however, apparently contain proteins as a major component, and in a number of cases the protein may be conjugated with iron (5, 14).

This paper describes a crystalloid inclusion that is widely and abundantly distributed within the early embryonic cells of the domestic rabbit. Although light microscope observations have been made on crystalloids from the rabbit oocyte (3, 10) this is the first description known to us of such inclusions in the early mammalian embryo.

MATERIALS AND METHODS

Blastocysts were recovered from albino New Zealand rabbit does 6 days after mating. Following a brief rinse in saline to remove adhering mucus, the material was fixed for 30 minutes in cold 1 per cent OsO_4, dehydrated in a graded series of alcohols, and embedded in a 5:1 mixture of butyl and methyl methacrylate. Some specimens were fixed in acrolein, dehydrated in a graded series of acetones and were embedded in vestopal. Sections were cut on a Porter-Blum microtome and were observed in an RCA EMU-3C electron microscope. To increase contrast, sections were "stained" for 1 hour in a saturated aqueous solution of uranyl acetate (15).

OBSERVATIONS

Crystalloids were of common occurrence in the majority of cells in the blastocyst, including trophoblast, endoderm, and embryonic shield. Their general appearance, as seen in the light microscope, is shown in Fig. 1. They were spherical to elongate in shape, varying in width from 0.1 to 1 μ, and were up to 10 μ in length. Some elongate crystalloids were expanded centrally by amorphous "droplet" material, and similar droplets also occurred separate from the crystalloids. The material of both crystalloids and droplets stained with the periodic acid–Schiff reaction for polysaccharides, were faintly basophilic but contained no detectable RNA. In these respects the staining resembled that of the surrounding zona pellucida with which these cells are closely associated.

In the electron microscope the crystalloids were seen to have rounded ends. The smaller crystalloids were usually oval in profile (Figs. 2 and 4). The larger inclusions had pale centers, in which the

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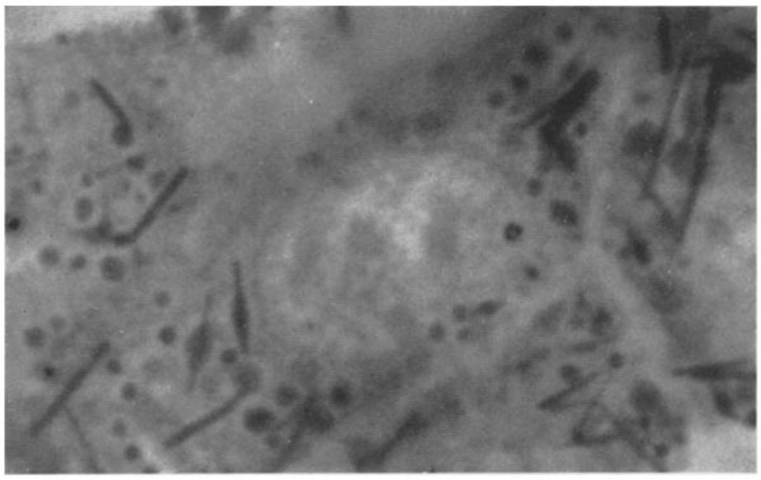

FIGURE 1

Cells from a portion of the trophoblast membrane, stained with the periodic acid–Schiff reaction for polysaccharides. The micrograph is of an entire cell, not a section. Note the PAS-positive crystalloids of various shapes, and also droplets of stainable material. Zenker fixation. × 2,700.

crystalloid structure was disrupted, probably through fixation or embedding artifact. Such pale centers were absent from material fixed in acrolein and embedded in vestopal. Crystalloids were bounded by a smooth membrane. This, however, was frequently incomplete, and in some cases was seen to be pulled away from the crystalloid material (Figs. 5 and 6).

Longitudinal or oblique sections through the crystalloids showed parallel rows of alternating dense and less dense lines. These varied in their spacing according to the plane of cut. In cross-section they appeared as a hexagonally packed array of light centers 70 A in diameter, delineated by dense material. The minimum distance from the mid-point of one center to the next was 100 A (Figs. 3 and 5).

Bundles of disoriented filamentous material were seen in the cytoplasm surrounding the crystalloids (Figs. 4 and 6), but bearing no obvious relation to them.

DISCUSSION

The crystalloid structures here described bear at least a superficial resemblance to the yolk platelets found by Wischnitzer (17) in the *Triturus* oocyte. It is possible that these structures are a form of yolk, persisting from the oocyte on into the early life of the embryo. Certain factors argue against this view. Similar structures have not been found during electron microscope observations on mature rabbit ova (Hadek, unpublished data). Yolk platelets were abundant in this material, but were quite different from the crystalloids in size, shape, and electron density, and no internal structure was evident. If the crystalloid structures represent yolk, the material must change markedly in appearance during early embryonic development. Also, the presence of a wide variety of sizes, from small bodies round or oval in section, up to large elongate structures, suggests that the process of crystalloid formation and growth is continuing in these cells. The crystalloids described by Cesa-Bianchi (3) were commonly needle-like, considerably longer and thinner than most of the inclusions seen in the blastocyst, and appear to be a differen type of structure.

Small round bodies, with density similar to the smaller crystalloids but without internal structure, occur in the region of the Golgi membranes (Fig. 4). Their relationship with either the Golgi material or the crystalloids is at present uncertain. Since the crystalloids are positive to the periodic acid–Schiff reaction, as is also the surrounding zona pellucida, it is possible that they bear some relation to the maintenance of that structure.

SUMMARY

Elongated crystalloids measuring 0.5 to 10 μ in length and 0.1 to 1 μ in width were found in the

cells of the 5½ day rabbit blastocyst. They were found to contain polysaccharides (PAS reaction) but no detectable RNA (azure B). Under the electron microscope they gave a striated appearance in longitudinal sections, and in cross-sections showed a hexagonal packing of units each about 100 A in diameter, with a light center 70 A across.

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REFERENCES

1. AUERBACH, L., Über die Einzelligkeit der Amoeben, *Z. wissensch., Zool.*, 1855. **7**, 365.
2. BERG, W., Zum mikroskopischen Nachweis des Stoffwechsels im Gewebe, *Z. mikr. anat. Forsch.*, 1929, **16**, 213.
3. CESA-BIANCHI, D., Über das Vorkommen besonderer Gebilde in den Eiern mancher Säugetiere, *Arch. mikr. Anat.*, 1906, **67**, 647.
4. ELBERS, P. F., Electronmicroscopy of protein crystals in ultrathin sections of egg of Limnea stagnalis, *Proc. K. Nederl. Akad. Wetentsch. Series C*, 1957, **60**, 96.
5. FAVARD, P., et CARASSO, N., Origine et ultrastructure des plaquettes vitellines de la Planorbe, *Arch. anat. micr. et morphol. exp.*, 1958, **47**, 211.
6. GRIFFIN, J. L., The isolation, characterization, and identification of the crystalline inclusions of the large free living amebae, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 227.
7. HANNAY, C. L., and FITZ-JAMES, P., The protein crystals of *Bacillus thuringiensis* Berliner, *Can. J. Microbiol.*, 1955, **1**, 694.
8. KUDO, R. R., Protozoology, Springfield, Illinois, Charles C. Thomas, 3rd edition, 1950.
9. LENHOSSEK, VON M., Beiträge zur Kenntniss der Zwischenzellen des Hodens, *Arch. Anat. u. Entwcklngsgesch.*, 1897, 65.
10. LIMON, M., Cristallides dans l'oeuf de "*Lepus cuniculus*," *Bibl. anat.*, 1903, **12**, 235.
11. NAPOLITANO, L., and FAWCETT, D., The fine structure of brown adipose tissue in the newborn mouse and rat, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 685.
12. RICHTER, G. W., The presence of hemosiderin. Presence of ferritin and occurrence of crystalline lattices in hemosiderin deposits, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 55.
13. RUDZINSKA, M. A., Further observations on the fine structure of macronucleus in *Tokophrya infusorium*, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 425.
14. STRUNK, S. W., The formation of intracellular crystals in midgut glands of *Limnoria lignorum*, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 385.
15. SWIFT, H., AND RASCH, E., Studies on electron microscope cytochemistry, *RCA Scient. Instruments News*, 1958, **3**, 1.
16. WEATHERFORD, H. L., A morphological and experimental study of the intranuclear crystals in the hepatic cells of the dog, *Anat. Rec.*, 1938, **71**, 413.
17. WISCHNITZER, S., The ultrastructure of yolk platelets of amphibian oocytes, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 1040.

FIGURE 2

Trophoblast cell, showing longitudinal and cross-sections of crystalloids. Note microvilli on outer cell surface. × 4,000.

FIGURE 3

Higher magnification of framed area in Fig. 4 below. × 170,000.

FIGURE 4

Trophoblast cell, showing crystalloids of different size and density. The micrograph shows three small crystalloids of uniform density in oblique section, one crystalloid of larger diameter (in frame) with a lighter center and showing internal structure, and one large crystalloid at top, with a large pale central area. Filamentous material surrounds the crystalloids (arrows). × 17,000.

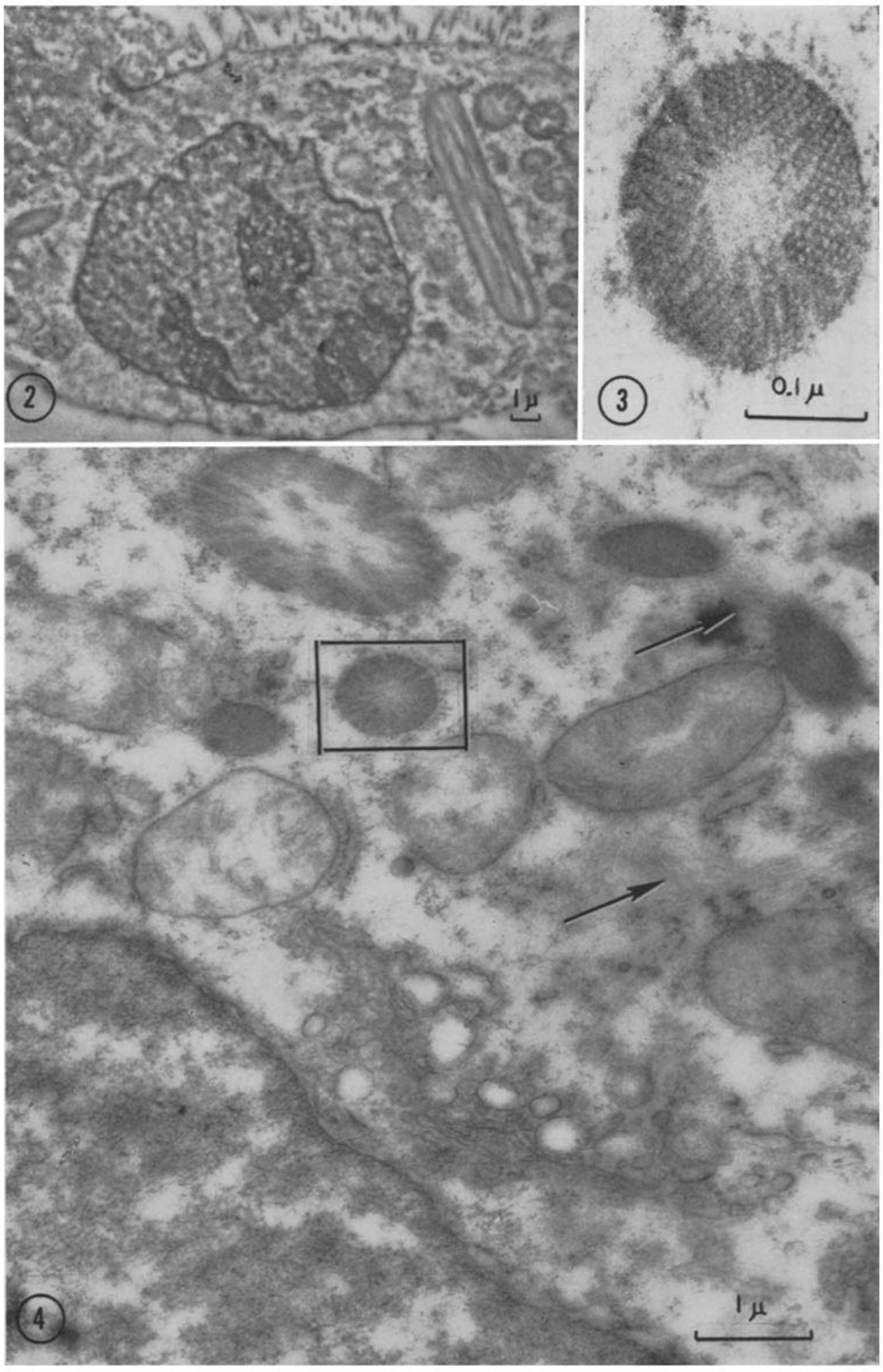

FIGURE 5

Transverse, longitudinal, and oblique sections through a cluster of crystalloids. × 144,000.

FIGURE 6

Filaments of electron dense material in cytoplasm surrounding crystalloids. × 68,000.