Studies on the Ultrastructure of the Nucleoli of the Walker Tumor and Rat Liver*

KAREL SMETANAT AND HARRIS BUSCH

(Department of Pharmacology, Baylor University College of Medicine, Houston, Texas)

SUMMARY

The ultrastructure of nucleoli was studied with ultrathin sections of cells and isolated nucleoli of the Walker tumor and liver. Septa-like extensions apparently penetrate into nucleoli from the nucleolus-associated chromatin at low magnifications. The nucleolonemas appear to be composed of granules, fibers, and small vesicles. At higher magnifications these structures appear to be components of tubular structures.

Recent studies in this and other laboratories (4) have provided evidence for an important role of the nucleolus in nuclear metabolism, methods for isolation of nucleoli (12), and evidence of a system of communication between the nucleolus and the cytoplasm (18). To learn more about the composition of the nucleolus, particularly when isolated from the cell, electron microscopic studies seemed to be essential.

Electron microscopic studies in recent years have shown that the nucleolus is composed in part of a fibrous or trabecular structure referred to as a nucleolonema (4) and lighter areas between the fibers that have been described as amorphous nucleoloplasm or light spaces. If these light areas are large, they correspond to the pars amorpha and nucleolar vacuoles seen by light microscopy (4). Around the nucleolus there is a layer of nucleolus-associated chromatin. By fixation procedures with the use of osmium tetroxide alone, this chromatin structure was poorly stained; now it can be more satisfactorily studied by recent fixation technics with formaldehyde (2).

The present study was designed to determine: (a) are DNA-containing structures normal components of nucleoli of the Walker tumor and liver (4), and if so what relationship is there between the DNA-containing structures within the nucleolus and the nucleolus-associated chromatin; and (b) what are the substructures of nucleoli? A number of studies have been made on the ultrastructure of nucleoli in situ (2, 4, 5, 11, 20). In the present studies these questions have been investigated on nucleoli of the Walker tumor and liver isolated by the procedure employing sonications (12) and the newer compression and rapid decompression procedure developed in this laboratory (6).

MATERIALS AND METHODS

For studies on nucleoli in situ small fragments of rat liver and Walker 256 carcinosarcoma were used. These fragments and pellets containing nucleoli isolated by the procedure employing sonications (12) or the procedure employing compression and rapid decompression (6) were fixed and stained according to two different procedures. In procedure A the specimens were fixed for 2–3 hours in 10 per cent neutral formalin and were post-fixed with osmium tetroxide at pH 7.5. In procedure B the specimens were fixed for 2–3 hours in osmium tetroxide solutions, buffered according to Palade (14). The pH of the solutions containing osmium tetroxide varied from 5 to 9.5. The samples were post-fixed with “neutral formalin” prepared with CaCO₃ (10). The absolute ethanol used in the dehydration procedure was diluted with 1 per cent uranyl acetate to a final concentration of 0.5, 0.3, and 0.1 per cent of uranyl acetate for staining and prevention of damage by the embedding procedure.

After the dehydrated specimens were embedded in Maraglas® (7), sections were cut with a Porter-Blum ultramicrotome and were observed with an RCA 3F electron microscope. Studies of a series of high magnification pictures, both over- and under-focused, eliminated the possibility of observation of focusing artifacts (13). For controls by light microscopy of observations made with the electron microscope, smears of nucleoli in situ and isolated nucleoli were stained with toluidine blue (16) and by the Feulgen reaction. In some experiments isolated nucleoli were treated overnight at 4°C with 2 M NaCl or with DNase (100 μg/ml) in 0.25 M sucrose containing 0.0075 M MgCl₂ and 0.05 M Tris at pH 7.4.

RESULTS

Studies on chromatin in and around the nucleolus.—Although nucleoli in situ and isolated nucleoli stained intensively with toluidine blue (Figs. 1, 2) the remainder of

---

* These studies were supported in part by grants from the National Science Foundation, the Jane Coffin Childs Fund, the American Cancer Society, and the U. S. Public Health Service.

† Visiting Associate Professor of Pharmacology on leave of absence from the National Academy of Sciences of Prague, Czechoslovakia.

Received for publication July 8, 1963.
the nucleus was stained to only a very small degree. When the Feulgen technic was employed the nucleolus-associated chromatin stained intensively, as well as other chromatin throughout the nucleus (Figs. 3, 4). However, the core of the nucleolus was stained to a very minor degree.

When procedure A was employed to fix and stain nucleoli in situ, both the nucleolar structure and that of the nucleolus-associated chromatin were clearly seen in the Walker tumor at low magnifications (Fig. 5). The dense nucleolus-associated chromatin was relatively sharply demarcated from the remainder of the nucleolus. When procedure B was utilized, the nucleolonemas of the nucleoli were more dense than the nucleolus-associated chromatin (Fig. 6), and septa-like extensions of the nucleolus-associated chromatin appeared to penetrate the nucleoli.

When isolated nucleolar preparations were fixed in formalin and then treated with osmium tetroxide and uranyl acetate, a dense thin layer was noted on the surface of the nucleoli (Figs. 7, 8) that corresponded in position to the nucleolus-associated chromatin. In addition, dense septa-like structures appeared to penetrate the nucleoli from the surface layers. Following treatment of the nucleolar preparations with DNase there was a decrease in the amount and density of the dense layer on the surface as well as the septa-like structures within the nucleolus (Figs. 7, 9). Moreover, treatment of the nucleoli with 2 M NaCl resulted in loss of the dense surface layer as well as much of the dense material within the nucleolus (Fig. 10).

When the nucleolar preparations were initially fixed with osmium tetroxide (14) the density of the surface layer of the nucleolus was lower than that seen after procedure A (Fig. 11). The presence of fibers in this surface structure is illustrated in the micrographs with higher magnification (Fig. 12). Following treatment of the nucleolar preparations with DNase, these fibers disappeared (Figs. 13-15). The relatively thin, dense layer on the surface that remained after DNase treatment (Fig. 13) was most dense when fixation was carried out in osmium tetroxide buffered at pH 9.5 (Fig. 14), the isoelectric point of some histone fractions.

Substructure of nucleoli.—At low magnifications, the nucleolonemas seem to be composed of granules that vary in density (Figs. 5, 6). At higher magnifications, the nucleolonemas appear to be composed of vesicular and tubular structures that are embedded in a matrix of low electron density (Fig. 12). Some fibers that may be derived from the nucleolus-associated chromatin were also noted at high magnifications. What appear to be longitudinal segments of tubules were occasionally seen in nucleolonemas; these are two parallel fibers that are 40-50 A in thickness, with a variable length and with a constant distance of 40-50 A between them. Some of the paired parallel fibers were connected at their ends. The diameters of the walls and central light cores of the vesicles were approximately the same as those of the tubular structures.

DISCUSSION

The present studies on isolated nucleoli with the electron microscope provide some support for previous studies that reported the presence of DNA-containing structures within the nucleolus (2, 4, 17). Septa-like structures apparently penetrate the nucleolus from the nucleolus-associated chromatin, and chromatin-like fibers were found on the surfaces of the nucleoli (8). The apparent penetration by chromatin structures into nucleoli contrasts with the apparent negativity of the Feulgen reaction of the nucleolar core (4). The chromatin structures may be present in nucleoli in such small amounts that the DNA is too low a concentration to be detected by histochemical reactions; alternatively, the possibility exists that the structures penetrating the nucleoli are devoid of DNA.

The nucleolonemas are apparently composed of fibrous and granular structures as viewed at low magnifications. At higher magnifications, these structures appear to be sections of tubules. Since the diameters of the walls and the central light cores of the vesicular structures found in nucleolonemas were the same as the diameters of the walls and central light cores of tubules, some of the vesicles may be cross-sections of the tubules. The finding of tubular structures corresponds to the report of Marinozzi (11), who described RNA containing fibers about 50 A in thickness and 300 A in length in nucleolonemas. These diameters were similar to the diameters of the outer walls (40-50 A) and the length of the tubules. The dense peripheral layer and the central light core of the tubular and vesicular structures in the nucleolus are similar to the substructures of other RNA-containing structures, including Palade granules in the cytoplasm (15, 17) and dark particles in the nucleus (9, 17).

The loss of granularity of components of isolated nucleoli may be the result of extraction of undefined nucleolar elements in the aqueous media utilized.

Fig. 1.—A smear preparation of Walker tumor cells stained with toluidine blue at pH 5.5 to demonstrate ribonucleoproteins of the nucleus and the cytoplasm.
Fig. 2.—A smear preparation of isolated nucleoli (12) stained with toluidine blue at pH 5.5.
Fig. 3.—A smear preparation of Walker tumor cells stained by the Feulgen technic showing the nucleolus-associated chromatin and other chromatin structures.
Fig. 4.—Isolated nucleoli (12) stained with the Feulgen technic to show the nucleolus-associated chromatin.
Fig. 5.—Electron micrograph of a nucleolus of the Walker tumor in situ. The specimen was fixed and stained by procedure A—i.e., fixation with formalin, postfixation with osmium tetroxide, and staining with uranyl acetate. Except where otherwise indicated, the length of the measured line is 1 micron. Ch = chromatin. X 16,000.

Fig. 6.—Nucleoli from liver cells in situ. The sample was fixed and stained by procedure B—i.e., fixation with osmium tetroxide, postfixation with formalin, and staining with uranyl acetate. Arrows = chromatin. X 23,500.
Fig. 7a.—Electron micrograph of an isolated nucleolus (12) from rat liver. The sample was treated by procedure A. Ch = chromatin. X 60,000.

Fig. 7b.—Electron micrograph of an isolated nucleolus (12) from rat liver after treatment with deoxyribonuclease. The sample was treated by procedure A. Some fragments of the peripheral dense layer remained on the surface. X 80,000.
Fig. 8.—A nucleolus from the Walker tumor isolated by the procedure employing compression and rapid decompression (6). The sample was treated by procedure A. Ch = chromatin. × 78,000.
FIG. 9.—A nucleolus from the Walker tumor isolated by the compression and decompression procedure (6). After treatment of the sample with deoxyribonuclease, it was treated by procedure A. × 130,000.
Fig. 10.—A nucleolus of rat liver isolated by the procedure employing compression and rapid decompression (6). After extraction with 2 M NaCl, the sample was treated by procedure A. \( \times 45,000 \).

Fig. 11.—A nucleolus from the Walker tumor isolated by the procedure employing compression and rapid decompression (6). The sample was treated by procedure B; the pH of the osmium tetroxide was 7.5. Ch = chromatin. \( \times 47,500 \).
Fig. 12a.—A nucleolus isolated from rat liver (12) treated by
procedure B; the pH of the osmium tetroxide was 8.7. T = tubule,
Ch = chromatin-like fibers. × 145,000.

Fig. 12b.—Electron micrograph of a section of the nucleolus of
a Walker tumor cell fixed and stained as described for Fig. 12a.
Arrows = chromatin fibers, T = tubules, V = vesicles. × 123,000.
FIG. 13.—Isolated nucleolus of the Walker tumor (6) fixed and stained as described for Fig. 11. The sample was treated with deoxyribonuclease prior to fixation. A dense thin layer remained on the surface of the nucleolus. × 32,500.

FIG. 14.—The same as Fig. 13, with the exception that the pH of the osmium tetroxide was 9.5. × 50,400.
Fig. 15. The same as Fig. 12a, following treatment of the sample with deoxyribonuclease prior to fixation. × 139,000.
ACKNOWLEDGMENTS

The authors are greatly indebted to Drs. Raoul Desjardins and Masami Muramatsu for isolation of the nucleoli, to Mr. Charles W. Taylor for transplantation of the Walker tumor, and to Dr. Krishnamoorthy Sankaranarayan for helpful advice.

REFERENCES


Studies on the Ultrastructure of the Nucleoli of the Walker Tumor and Rat Liver

Karel Smetana and Harris Busch


Updated version  Access the most recent version of this article at:  
http://cancerres.aacrjournals.org/content/24/4_Part_1/537

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.