Quantitative Analysis of Ultrastructural Components of Nucleoli of the Walker Tumor and Liver

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Summary

Nucleolar components were defined as fibrillar, granular or light areas on the basis of ultrastructural appearance. Quantitative measurements were made of these areas with the aid of a planimeter and the percentage of total nucleolar area composed of the various areas was determined. The highest content of fibrillar component was found in the nucleoli of the livers of actinomycin D-treated animals and the lowest was found in the Walker tumor. The Walker tumor nucleoli contained a number of regions that could be classified as fibrillogranular regions, but very few pure fibrillar regions were found. The finding that the percentage of the total nucleolar area composed of fibrillar regions was related in part to the adenylic acid + uridylic acid/guanylic acid + cytidylic acid ratio of nucleolar RNA supports previous suggestions that the fibrillar regions contain an RNA fraction richer in adenylic and guanylic acid.

Introduction

Recent studies have suggested that nucleoli may be biochemically and morphologically differentiated during various stages of growth, maturation and function of cells (5, 6, 23, 28, 30). This differentiation is related to the amounts and distribution of the subcomponents of the nucleoli which include granular and fibrillar ribonucleoprotein structures as well as the light spaces of varying size that contain DNP fibrils and possibly an amorphous "nucleoloplasm." Compact nucleoli (23) have a rather uniform distribution of the ribonucleoprotein structures and a relatively small amount of the light spaces. These nucleoli are found in young or growing cells such as tumor cells (3, 5), immature leukocytes (23), and cells of pupal stages of Drosophila melanogaster (30). Ring-shaped nucleoli are found in mature cells such as lymphocytes (23), plasmocytes (23), cells of mature pupae of D. melanogaster (30), and mature oocytes (7), or in cells about to undergo division such as Ehrlich ascites cells in prophase (31). In addition, they were also found in HeLa cells and cells of livers of actinomycin D-treated animals (8, 23, 26). These nucleoli have ribonucleoprotein structures predominantly in their periphery.

Studies of Marinozzi and Bernhard (12, 13) have suggested that the fibrillar component of the nucleolar ribonucleoproteins may represent messenger RNA and the granular component may represent the precursor of the ribosomal elements in the cytoplasm. In view of the marked differences in the base ratios of the nuclear and nucleolar nucleic acids found in tumors and other tissues in recent studies in this laboratory (4, 14–16, 18, 25), it seemed possible to correlate quantitative studies on the relative size of areas containing ribonucleoprotein components with the composition of the nucleolar nucleic acids. To make a quantitative analysis of the ultrastructural nucleolar components, maps of nucleoli were prepared from which areas of fibrillar and granular components were determined with the aid of a planimeter. These studies showed that of the tissues examined, the Walker tumor had the lowest relative areas of fibrillar components, a finding which agrees substantially with the high content of GC-rich RNA found in previous studies (14).

Materials and Methods

Animals

Male albino rats from the Holtzman Rat Company, weighing 180–250 gm, were used in this study. Partial hepatectomy was performed as described previously (15) and the animals were sacrificed 15 hr later. Thioacetamide-treated animals received i.p. injections of a 2% solution of thioacetamide in 0.15 M NaCl in a dose of 50 mg/kg body weight daily or at specified times prior to death (1). Rats were killed 9 days after the initial injection of thioacetamide. Animals treated with actinomycin D received 1 i.v. injection of 150 μg of the drug/kg body weight 60 min before they were sacrificed. The Walker 256 carcinosarcoma used in these studies was excised 6–8 days after transplantation.

Electron Microscopy

Thin slices of Walker tumor and rat liver from experimental and control animals were fixed in 1% osmium tetroxide buffered with a-collidine at pH 7.4 (2) for 1 hr at 4°C. Specimens embedded in Epon 812 (11) were cut with an LKB ultramicrotome and post-stained with uranyl acetate (29) and lead hydroxide (9). Ultrathin sections were examined with a Philips Norelco 200 electron microscope.

Planimetry

Whole nucleolar areas and areas containing fibrillar and granular components were measured on electronmicrographs magni-
fied 2-6 times with a planimeter (OTT, Burrel Corporation, Pennsylvania), as shown in Figs. 1 and 2. The proportions of the total nucleolar areas containing the fibrillar and granular components were calculated as well as the areas occupied by the light spaces of the nucleolus.

By definition, a “fibrillar area” is one of greater density than the light spaces but does not contain defined granular components up to 30,000 magnifications directly in the electron microscope. By definition, at the same magnification, a granular area contains “granules” predominantly.

Results

Studies on Normal Liver Nucleoli

Fig. 1 shows the ultrastructure of normal liver nucleoli. Fig. 2 shows the method for preparation of the nucleolar map which differentiates the fibrillar areas from the total nucleolar area.

On the basis of planimetric analysis, approximately 19% of the total area of nucleoli of normal liver was accounted for by the fibrillar component. For the purposes of statistical analysis, a number of electron micrographs of normal liver nucleoli were analyzed in the same way. The values in Table 1 show the percentages of the fibrillar and granular areas in nucleoli from various tissues.

Serial Sections

To determine the reliability of planimetric studies on nucleoli, a series of serial sections was prepared from 2 different liver nucleoli. Consecutive sections, 800-1000 Å in thickness, were cut and mounted on separate grids. Out of each group of 20 sections, 1 was selected for planimetric analysis. The selection was made in such a manner that no 2 sections were closer than 1 μ to one another in situ. The results of the planimetric analyses are shown in Table 2 for 2 nucleoli of normal liver. Only minor changes were found in the values for the areas containing the fibrillar and granular nucleolar components.

**Regenerating Liver and Liver of Thioacetamide-treated Rats**

Similar analyses were carried out on regenerating liver 15 hr after hepatectomy, as shown in Figs. 3 and 4, at this same point. The fibrillar component accounted for 12% of the total nucleolar area. Although the fibrillar component was somewhat decreased in the nucleoli in cells of regenerating liver, planimetric analysis of the percentage of fibrillar component in the nucleoli of livers of rats treated with thioacetamide for 9 days (Table 1, Figs. 5, 6) revealed a much lower content of fibrillar components, i.e., 4.5%.

**Livers of Rats Treated with Actinomycin D**

By contrast with regenerating liver and the livers of rats treated with thioacetamide in which the percentage of nucleolar area accounted for by the fibrillar component was decreased, there was a marked increase in the fibrillar component following administration of actinomycin D. Although a series of changes occurred within 60 min after the i.v. injection of actinomycin D, the nucleolar pattern was relatively stabilized in treated livers at 60 min (Figs. 7, 8); at this time, the fibrillar component comprised 48% of the total nucleolar area. There was variability in the appearance of nucleoli of liver cells of actinomycin D-treated animals; some nucleoli were ring-shaped and some were compact with segregation of granular and fibrillar components (8, 17, 20, 26, 27).

**Walker Tumor**

Figs. 9 and 10 present the planimetric maps for the Walker tumor. Unlike the liver nucleoli of all types studied in which fibrillar areas were virtually completely devoid of granules, granular components in the Walker tumor were found in almost all areas. The appearance of the nucleoli in electron micrographs

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**TABLE 1**
**Percentage Composition of Total Nucleolar Areas**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Areas containing fibrillar components</th>
<th>Areas containing granular components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver (10)</td>
<td>19 ± 4</td>
<td>77 ± 5</td>
</tr>
<tr>
<td>Regenerating liver, 15 hr after partial hepatectomy (6)</td>
<td>12 ± 3</td>
<td>84 ± 4</td>
</tr>
<tr>
<td>Liver 9 days after thioacetamide administration (6)</td>
<td>4.5 ± 0.3</td>
<td>91 ± 2</td>
</tr>
<tr>
<td>Liver 1 hr after actinomycin administration (29)</td>
<td>48 ± 23</td>
<td>50 ± 22</td>
</tr>
<tr>
<td>Walker tumor (15)</td>
<td>3 ± 2</td>
<td>93 ± 3</td>
</tr>
</tbody>
</table>

* Areas containing fibrillar and granular components were measured with a planimeter on micrographs of ultrathin sections and were expressed as % of the total nucleolar areas measured in the same sections. The means and standard deviations of values obtained are presented. The numbers of different nucleoli studied are shown in parentheses. A representative section from each nucleolus was analyzed. The numbers of animals used for studies on the normal liver, regenerating liver, livers of thioacetamide-treated rats, actinomycin D-treated livers and Walker tumors were 6, 2, 6, 2, and 4, respectively.

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**TABLE 2**
**Percentage Composition of Total Nucleolar Areas in Serial Sections of Normal Liver**

<table>
<thead>
<tr>
<th>Serial section</th>
<th>Areas containing fibrillar components</th>
<th>Areas containing granular components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleolus 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14.8</td>
<td>83.6</td>
</tr>
<tr>
<td>2</td>
<td>14.2</td>
<td>81.8</td>
</tr>
<tr>
<td>3</td>
<td>15.5</td>
<td>83.0</td>
</tr>
<tr>
<td>4</td>
<td>14.2</td>
<td>83.5</td>
</tr>
<tr>
<td>Nucleolus 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19.6</td>
<td>77.5</td>
</tr>
<tr>
<td>2</td>
<td>18.7</td>
<td>80.0</td>
</tr>
<tr>
<td>3</td>
<td>20.8</td>
<td>78.8</td>
</tr>
<tr>
<td>4</td>
<td>17.0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

* Serial sections of a given nucleolus were prepared by mounting 10 consecutive sections, 800-1000 Å in thickness, on separate grids. The 10 consecutive sections were placed on a grid and the next 10 sections were discarded. This process was repeated several times. One micrograph was prepared for each of the 10 sections; thus the micrographs taken represented sections of the nucleolus spaced 2-μ thickness apart.
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suggests that 2 predominant types of areas exist in the Walker tumor nucleolus, i.e., granular areas and fibrillar granular areas. By comparison with the liver in which structures in some nucleolomata were virtually completely fibrillar, there were few, if any, such nucleolomata in the Walker tumor. As a result, the percentage of total nucleolar area in the Walker tumor composed of fibrillar components was extremely low, i.e., 2.8%.

Granular Component

Table 1 also presents the content of the granular components of the nucleolus which ranged in area from 52% to 93% of the total nucleolar area. Inasmuch as the fibrillar component is such a large part of the total nucleolar area in nucleoli treated with actinomycin D, the granular component only accounted for about 0.5 the total nucleolar area. As shown in Figs. 7 and 8, the line of demarcation of the granular and fibrillar component is frequently very sharp in nucleoli of livers of actinomycin D-treated animals. Although the granular component was largest in area in nucleoli of the Walker tumor, the differences in the percentages of the total nucleolar areas accounted for by the granular component were not significantly different from the nucleoli of thioacetamide-treated livers. In addition, the nucleoli from regenerating livers had a higher content of granular component than those of normal liver. On the other hand, the percentages of areas occupied by the nucleolar light spaces were not markedly different in the various nucleoli analyzed.

Fibrillar Granular Areas

Frequently in nucleoli of the Walker tumor and occasionally in the nucleoli of thioacetamide-treated rats (Figs. 11, 12), nucleolominal areas were composed of masses which appeared to contain a mixture of fibrillar component and granular component. In the Walker tumor, only minute areas of "pure" fibrillar component were found as contrasted to the nucleoli of any of the liver samples.3

Discussion

Previous studies on the ultrastructural components of the nucleolus have been directed at analysis of the types of components present (3, 12, 15, 20, 27, 28). In the present study, an effort has been made to determine the approximate quantitative composition of the nucleolus in terms of the areas occupied by components visible at magnifications providing optimal resolution of these various components. Some agreement with biochemical analysis has been obtained in that, on the basis of calculations of the base composition, there is an agreement between the areas composed of fibrillar components and the comparative A + U/G + C content of the nucleolar RNA (5, 14, 15, 18, 25). A strict comparison cannot be made because, in the biochemical analyses, tens of billions of nucleoli are analyzed for individual components as compared to the few nucleoli analyzed with the planimetric procedures. In addition, the analysis of the nucleoli by biochemical methods does not take into consideration the marked variations from the perportal to the central vein in liver which are important in the effects of various drugs, as was pointed out by Khoulish and Kleinfield (10).

The greatest changes found in the compositions of the nucleoli of liver cells on the basis of planimetry were those in the thioacetamide-treated rats as compared to the livers of rats treated with actinomycin D.4 These quantitative results compare favorably with the previous over-all impressions from observations of the electron micrographs (6, 8, 17, 20, 24, 26, 27) and also with the changes in base composition found for isolated nucleoli and nuclei of rats treated with these 2 agents (4, 14, 18, 25). However, only minor changes were found in the base composition of nucleoli of regenerating livers (15), and these results correspond to the relatively small changes in the amounts of the areas occupied by the granular and fibrillar ribonucleoprotein components.

As noted previously by a number of workers (21, 22), there were apparently no specific morphologically differentiated nucleolar structures characteristic of the cells of the Walker tumor with the possible exception of dense granules 200–400 A in size (19). However, it would appear that the nucleolomata of the Walker tumor nucleoli contain very few areas that are pure fibrillar areas. In many areas, the fibrillar components contain many granules in the nucleolomata of the Walker tumor. Since similar areas were noted in the nucleolomata of the nucleoli of livers of thioacetamide-treated rats, it is not clear whether these "fibrillar granular" areas are representative of changes in neoplastic tissues or preneoplastic tissues or simply represent an excessive production of the granular nucleolar component.

Acknowledgments

The authors are extremely appreciative to Dr. William J. Steele for supplying samples of animals treated with actinomycin D and thioacetamide, to Dr. Tae S. Ro for supplying samples of livers of animals treated with actinomycin D, to Dr. Masami Muramatsu for supplying samples of regenerating livers, and to Mr. Charles Taylor for supplying samples of Walker tumor. The authors are also appreciative to Dr. Steele for his many helpful suggestions and comments.

References

4. Busch, H., and Adams, H. A. Effects of Thioacetamide on

4 When representative sections containing all the nucleolar elements were analyzed the means of the values for the areas containing fibrillar and granular components were very similar to the means obtained when all sections were included. However, the range of the deviations were actually lower when many of the representative sections were analyzed from livers of actinomycin D-treated animals.
Nucleolar Components of Walker Tumor and Liver


The measured lines in Figs. 1-12 each represent 1 \mu.

Fig. 1.—Nucleolus of a liver cell from a control rat.  \times 48,000.
FIG. 2.—Map of the nucleolus of Fig. 1. The whole nucleolar area and the areas containing the fibrillar components are outlined. × 48,000.
FIG. 3.—Nucleolus of a liver cell from regenerating liver, 15 hr after partial hepatectomy.  $\times 37,000$.

FIG. 4.—Map of the nucleolus of Fig. 3. The whole nucleolar area and the areas containing the fibrillar components are outlined.  $\times 37,000$.  

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FIG. 5.—Nucleolus of a liver cell after administration of thioacetamide in a dose of 50 mg/kg body weight for 9 days. × 10,000.

FIG. 6.—Map of the nucleolus in Fig. 5. The whole nucleolar area and the areas containing the fibrillar components are outlined. × 16,000.
Fig. 7.—Nucleolus of a liver cell 1 hr after treatment of the rat with a dose of 150 μg of actinomycin D per kg body weight. × 45,500.

Fig. 8.—Nucleolar map of the nucleolus in Fig. 7. The nucleolar areas and the areas with the fibrillar components are outlined. × 45,500.
**Fig. 9.**—Nucleolus of a Walker tumor cell.  $\times 29,700$.

**Fig. 10.**—Map of the nucleolus of Fig. 9. The nucleolar area and the areas containing the fibrillar components are outlined.  $\times 29,700$. 
FIG. 11.—Portion of a nucleolus in a liver cell after administration of thioacetamide showing areas in nucleolonemata containing both fibrillar and granular components. X 50,000.

FIG. 12.—Portion of a nucleolus in a Walker tumor cell with areas composed of both fibrillar and granular components. X 59,000.
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Cancer Res 1966;26:786-796.

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