The Estimation of Nucleic Acids in Individual Isolated Nuclei of Ascites Tumors by Ultraviolet Microspectrophotometry and Its Comparison with the Chemical Analysis

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Previous biochemical work by one (12, 13) of us has shown that the average amount of desoxyribonucleic acid (DNA) per cell, as compared to that of normal tissues, varies in different tumors. In certain tumors it was falling within the range which different authors have established to be characteristic for normal mouse tissues with a diploid cell population, while in other tumors the average DNA value per cell was significantly higher than this value. This elevation was not correlated with the mitotic index, but showed a certain rough parallelism with average nuclear size. In this and in earlier work by other authors (2, 5, 6, 15, 21), the importance of expressing amounts of nucleic acids (NA) in terms of average amounts per cell, instead of with dry or wet weights as basis, has been emphasized. The analysis of the ascites tumors has been performed by carrying out determinations on suspensions containing known numbers of cells or of nuclei and by computing the nucleic acid content for a single cell or nucleus. The ascites tumors presented certain advantages with regard to the difficulties in isolating nuclei from solid tumors and with regard to the absence of stroma and large amounts of necrotic tissue (6–8, 12, 13).

The computed amounts of nucleic acids per cell in the different ascites tumors represent naturally only an average value and do not give any information about the variability from cell to cell in the tumor cell population. In the cases where DNA was found to be elevated, the high average value might result from a mixture of different cell types with varying DNA contents, or the value might be characteristic for most cells of the particular tumor. An elucidation of this point can obviously come only from cytochemical technics which allow the analysis of individual cells for their nucleic acid content. Such a method is the ultraviolet microspectrophotometry as described by Caspersson in 1986 (4). With this technic, which utilizes the natural absorption of ultraviolet light at 257 mµ by the nucleic acids of the cells, it is possible to analyze in microscopical sections single cells for their nucleic acids. Recently, it was found by C. Leuchtenberger et al. (15) that isolated nuclei are a much more suitable material than tissue sections for the ultraviolet microspectrophotometry of nucleic acids, especially if a quantitative estimation of the DNA in individual cells is attempted.

Among other advantages which isolated nuclei have over whole cells in tissue sections and which have been discussed in detail in the previous paper (15), are the more homogeneous distribution of the absorbing material and the marked decrease in light scattering, both being of importance for microspectrophotometry. A comparison between the data on the DNA content of isolated nuclei of normal tissues by the ultraviolet microspectrophotometry and by the biochemical analysis on the same material showed a close agreement (15). Furthermore, the ultraviolet microspectrophotometry on individual cells revealed variations in the amount of DNA in the nuclei of the same tissue, as, for instance, in the rat liver, while the biochemical analysis on a mass of nuclei gave of necessity only an average value, which may be misleading, since it need not represent the content of any one individual nucleus (15).

It seemed of interest, therefore, to analyze isolated nuclei of tumors for their nucleic acids by ultraviolet microspectrophotometry and to compare the results with the biochemical data on the same material.

MATERIALS AND METHODS

For these studies the Ehrlich ascites tumor and the DBA ascites lymphoma were chosen.

Detailed data on these ascites tumors are to be found in previous publications (9, 11–13). For the present experiments, 20 × 10⁶ Ehrlich ascites tumor cells were inoculated intraperi-
toneally into hybrid albino male mice weighing 20–25 gm. The resulting ascitic fluids were collected 5–8 days after inoculation. About 60 × 10⁶ lymphoma cells were inoculated into male DBA mice intraperitoneally; the resulting ascitic fluids being collected 9–14 days after inoculation. The cellular composition of the ascitic fluids was studied on fresh Papanicolaou smears; only those showing the typical picture of the well developed ascites tumor were used for the isolation of nuclei and subsequent chemical determinations of nucleic acids and proteins. For the studies of normal tissue, liver and sperm of freshly killed beef were used. Isolation of nuclei was carried out by Vendrely and Vendrely (84). The final suspension of the nuclei was made with m/18 citric acid. Chemical determinations on suspensions containing known numbers of isolated nuclei were carried out as previously described (9). Nitrogen was determined by a micro-Kjeldahl technic, and the amount of protein calculated according to the formula used by Davidson and Leslie (5).

TABLE 1
AMOUNTS OF NUCLEIC ACIDS AND PROTEINS IN ISOLATED NUCLEI OF TUMORS AND NORMAL TISSUES BY CYTOCHEMICAL AND CHEMICAL ANALYSES

<table>
<thead>
<tr>
<th>Material</th>
<th>No. of nuclei measured</th>
<th>NA* per nucleus in 10⁻⁹ mg.</th>
<th>DNA† per nucleus in 10⁻⁹ mg.</th>
<th>RNA† per nucleus in 10⁻⁹ mg.</th>
<th>DNA‡ per nucleus in 10⁻⁹ mg.</th>
<th>RNA‡ per nucleus in 10⁻⁹ mg.</th>
<th>Protein per nucleus mg/nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlich ascites</td>
<td>39</td>
<td>18.0 ± 1.7</td>
<td>14.0 ± 1.5</td>
<td>4.0 ± 0.4</td>
<td>17.3</td>
<td>12.9</td>
<td>4.4</td>
</tr>
<tr>
<td>DBA lymphoma</td>
<td>30</td>
<td>8.8 ± 1.9</td>
<td>6.8 ± 0.6</td>
<td>2.0 ± 0.2</td>
<td>8.2</td>
<td>6.6</td>
<td>1.6</td>
</tr>
<tr>
<td>ascites tumor</td>
<td>42</td>
<td>5.9 ± 0.11</td>
<td>5.9 ± 0.11</td>
<td>0.0 ± 0.0</td>
<td>6.7</td>
<td>6.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Beef liver</td>
<td>56</td>
<td>2.8 ± 0.08</td>
<td>2.8 ± 0.08</td>
<td>0.0 ± 0.0</td>
<td>3.4</td>
<td>3.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Bull sperm</td>
<td>56</td>
<td>2.8 ± 0.08</td>
<td>2.8 ± 0.08</td>
<td>0.0 ± 0.0</td>
<td>3.4</td>
<td>3.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

NA* = Total nucleic acid.
DNA† = Desoxyribonucleic acid.
RNA‡ = Ribonucleic acid.

The microspectrophotometric studies were carried out on nuclei of the same suspension of isolated nuclei used for chemical analysis. The isolated nuclei were immersed for 1 hour in glycerol, put on quartz slides, and analyzed for nucleic acids by the ultraviolet microspectrophotometric method as described previously (15). For the differentiation of DNA and RNA the nuclei were treated with Worthington crystalline ribonuclease (0.3 mg/1 cc distilled water) for 2 hours at 37° C. In order to free the enzyme from possible proteolytic activity, the ribonuclease was purified with saturated ammonium sulphate according to the procedure of M. McDonald (personal communication). From the difference in absorption between control nuclei (kept for 2 hours at 37° C, in distilled water) and ribonuclease-treated ones, the amounts of DNA and RNA were computed.

RESULTS
The results of the cytochemical and chemical analyses are presented in Table 1. The data show the rather close agreement between the values obtained by chemical analyses and the mean values of DNA and RNA obtained by ultraviolet microspectrophotometry.

It can be seen, furthermore, that, while the Ehrlich ascites tumor cell has twice the amount of DNA of that of the beef liver cell, the DNA content of the DBA lymphoma is approximately the same as that of the normal cell. This tetraploid DNA content of the Ehrlich ascites tumor and the approximately diploid one of the DBA lymphoma is in good agreement with the earlier chemical determinations (12, 13).

A comparison between the ribonucleic acid content in the nuclei of the tumor and that of the normal cells shows that both tumors contain considerably higher amounts of nuclear RNA than do the normal cells (20–25 per cent, as compared to 5–7 per cent of the total nuclear NA). The relatively high amount of RNA in the tumor nuclei is all the more striking, since during the isolation procedure some of the RNA is lost from the nuclei (24).

Since the cytochemical data presented in Table 1 are computed mean values and, therefore, do not reveal the variations occurring from nucleus to
DNA value is approximately 30 per cent less than the highest, while the remaining nuclei outside of this range vary up to 50 per cent.

A similar variation in the DNA content can be seen to occur in the nuclei of the ascites tumor. While the amount of DNA per nucleus is approximately twice that of the beef liver, 35 out of 39 tumor nuclei measured show a DNA content of from 10 to $14 \times 10^{-9}$ mg. — therefore, also, an approximate variation of 30 per cent.

**DISCUSSION**

The close agreement between the results of the microspectrophotometric and biochemical analyses on nucleic acid content of isolated tumor nuclei is in accordance with the earlier work on isolated nuclei of normal tissue (15). In both studies it was observed that, while the mean value of DNA per cell is approximately the same regardless of whether it is computed from the microspectrophotometric analysis of individual nuclei or from the biochemical analysis of a mass of nuclei, the microspectrophotometry reveals a variation in the content of DNA from nucleus to nucleus in normal as well as in tumor cells. Whether such differences in amounts of DNA from cell to cell within the same tissue are due to errors inherent in microspectrophotometry or are true biological variations cannot be decided with certainty at this time. However, the possibility exists, as discussed in detail in previous publications (16, 17), that these variations are, at least in part, biological ones. While such a suggestion is contrary to the opinion of some investigators (23, 18) who claim that the DNA content is the same for all cells of all somatic tissues of an organism and therefore independent of any metabolic function of the cells, recent findings by Leuchtenberger and Schrader (16) support the view that biological variations in DNA content might occur within a tissue. These workers observed that the nuclei of the salivary gland from the snail *Helix aspersa* Müller carried widely varying amounts of DNA in cells of the same gland (in an order of magnitude of 1:80) and that the amount of DNA in a nucleus was closely related to the production of secretory granules in the cytoplasm. Though the differences of DNA as found in the present study are of course much less marked than those in the salivary gland nuclei, they might nevertheless be indicative of changes in metabolic activities or of polyteny of cells within the same tissue.

Ever since Boveri (3) suggested that tumors are composed of cells with a "faulty chromosome complement," it has been generally assumed that all tumors show variations in their nucleic acid content. The results presented in this study are only partially in accordance with such a view, namely, in regard to the RNA, but not if one considers the DNA. While the Ehrlich ascites tumor contains twice the amount of DNA of that of a normal cell, the DBA lymphoma shows a normal DNA value. Furthermore, cells with twice the DNA content occur also in normal tissues, as shown by previous work on rat liver, and are thus not a characteristic deviation of tumor cells. The conclusion from these data that the DBA lymphoma is a tumor with predominantly diploid nuclei, while the Ehrlich ascites tumor carries predominantly tetraploid nuclei, is supported by the findings of Hauschka and Levan (10), who have carried out metaphase chromosome counts on the same tumors. These workers observed that the chromosome numbers occurring with maximum frequency in the Ehrlich ascites tumor were around tetraploid, namely between 75 and 84, while the chromosome number of the DBA lymphoma gave a unimodal peak at 40, which is the diploid number of the mouse. Earlier investigations on the DNA content of mouse tumors (14) and studies on human tumors, now in progress, are in accordance with the concept that tumors are not characterized by special deviations in their amounts of DNA as compared to normal cells.

The picture is different, however, if we consider the RNA in the two ascites tumors. Both tumors show (in spite of the normal DNA content of the lymphomas) a marked increase of RNA in their nuclei, as compared to the RNA of nuclei of normal cells as seen in Table 1, where the RNA/DNA ratios are listed. Arneson and co-workers (1) found a similar augmentation in the RNA content in leukemic as compared to normal spleen nuclei, though the DNA content of the leukemic spleen nuclei was normal. Petermann and Schneider (19) also observed a pronounced increase of RNA in the nuclei of a transplanted mouse leukemia over that of normal spleen nuclei; in this case the DNA content of the leukemic cells was 45 per cent higher than the DNA of the normal cells.

On the basis of these various results it seems that the RNA content of the tumor nuclei so far examined is higher than that of normal nuclei, regardless whether the DNA is increased or not. Whether all tumors show such a high value of RNA in their nuclei and whether this is characteristic for tumors only or holds also for other rapidly growing tissues must await further studies. But the apparently consistent higher values of RNA in the tumor nuclei thus far examined are rather remarkable if one considers the probability that a part of the RNA is lost from the nuclei during the isolation procedure.

On the basis of the relatively high RNA/DNA
ratios in tumor nuclei, one might expect a similar increase in the protein/DNA ratio, but, as can be seen from the last column of Table 1, the protein/DNA ratio in tumor nuclei is rather close to the protein/DNA ratio of normal nuclei. These findings are in accordance with the results of other workers (19). Since it has been demonstrated that considerable amounts of proteins are extracted from the nuclei during the isolation procedures (21), the rather constant protein/DNA ratios in isolated nuclei of various tissues is of interest. One might speculate that the protein left in the nuclei after isolation is very stable because of its linkage with the DNA which is not affected at all by the isolation procedure. Perhaps we deal here with a protein which might be a type of "chromosomal protein." Studies on Arvelius by Schrader and Leuchtenberger (22) clearly indicate two different types of proteins in nuclei: the so-called "chromosomal proteins" which are linked to the DNA and the "extrachromosomal proteins," the synthesis of which seems independent of the DNA and which is possibly concerned with the metabolic processes of the cells.

SUMMARY

1. The results of the microspectrophotometric analysis on nucleic acids in isolated nuclei of tumors and normal tissues were in good agreement with those of the biochemical analysis on the same material.

2. The DNA content/cell in the Ehrlich ascites tumor is approximately twice that found in normal diploid nuclei (corresponding to tetraploid nuclei), while the relative deviations from the mean value do not differ significantly from that found in normal cells. The DBA ascites lymphoma contains amounts of DNA similar to those of normal diploid cells. These results are in agreement with the chromosome counts of Hauschka and Levan on the same material.

3. The average RNA content per nucleus was markedly increased (20–25 per cent of the total nucleic acid) in the ascites tumors over that of the nuclei of normal tissue (3–7 per cent of the total nucleic acid).

REFERENCES


13. ———. Comparative Studies of Mouse Tumors with Respect to Their Capacity for Growth as "Ascites Tumors" and Their Average Nucleic Acid Content per Cell. Exp. Cell Research, 2:518–73, 1951.


