CYTOCHEMICAL STUDIES ON TUMOR CELLS, V.
MEASUREMENT OF DESOXYRIBONUCLEIC ACID (DNA) BY
FEULGEN-MICROSPECTROPHOTOMETRY IN SOME HUMAN
UTERINE TUMORS*
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It is well accepted that desoxyribonucleic acid (DNA) is one of the important constitu-}
ents for the maintenance of cellular phenomena in the living body. It has been
shown that DNA is an essential ingredient of the chromosomes, and plays an impor-
tant role in the process of cell division. Recently, quantitative aspects of DNA in indi-
vidual cell nuclei have been made clear through biochemical studies, particularly on
the basis of cytophotometrical measurements of its amount with Feulgen stained
materials. Further, many investigators have offered evidence indicating that there
is an intimate correlation between DNA amount and chromosome number. McLeish
(1959) summarized the data presented by several authors as follows: (1) under normal
conditions the amount of DNA per cell is directly proportional to the chromosome
number (Swift 1950a, b, Leuchtenberger et al. 1954), (2) there occurs a quantitative
constancy in some cells (Boivin et al. 1958, Vendrely and Vendrely 1948, Ris and Mirsky
1949), (3) the amount of DNA can vary within predicated limits according to ploidy
and to the stage of chromosome replication or division (Swift 1950a, Walker & Yates
1952, Patau & Swift 1953, Franzer & Davidson 1953), (4) some variations occur accord-
ing to the metabolic activity of the cell concerned (Leuchtenberger & Schrader 1952,

It would be reasonable to expect that the amount of DNA could be influenced by
physiological or pathological disturbances which strongly affect the entire cells. Most
studies in that field indicate that in some cases the amount of DNA is the same in
cancer and normal tissues (Mark & Ris 1949, Cunningham et al. 1950a, b, Price
& Laird 1950, Price et al. 1950, Metais & Mandel 1950, Davidson et al. 1951a, b,
Barder 1959). On the other hand, in some others, DNA amount per nucleus is higher
in cancer tissues than in normal ones (McIndoe & Davidson 1952, Klein and Klein
1951, Leuchtenberger et al. 1954).

Results of the recent chromosomal studies of human tumors by Makino et al.
(1959), Ishihara (1959), Tonomura (1959) and some others have shown that the number
of chromosomes is distributed in near-diploid and hyper-diploid ranges in many

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uterine carcinomas. In the present study a comparison was made on the amount of DNA between malignant cells based on some uterine carcinoma and normal human tissue cells.

Cordial thanks are offered to Dr. Akira Tonomura for supplying unpublished chromosomal data with important advices. Gratitude is extended to Professors G. Ogawa, Y. Okuda and J. Mikami, Hokkaido University Hospital, through whose generosity the greater part of the material herein employed was supplied. The authors are also obliged to Dr. H. Tsukada, Sapporo Medical College, for histopathological examinations of the materials used.

**Material and Methods:** In this investigation, eight human uterine tumors and one specimen of normal tissue from the liver of an eight months embryo as a control were used as material. Some clinical and pathologic data of tumors here under study are listed in Table 1. They were obtained directly from the patient immediately after removal of the tumors by operation.

Table 1. List of eight human uterine tumors under study, with a summary of clinical and pathological data.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Age</th>
<th>Clinical diagnosis</th>
<th>Pathological diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>46</td>
<td>Uterine carcinoma</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>No. 2</td>
<td>50</td>
<td>Carcinoma portionis 2nd</td>
<td></td>
</tr>
<tr>
<td>No. 3</td>
<td>47</td>
<td>Uterine carcinoma</td>
<td>Basal cell carcinoma</td>
</tr>
<tr>
<td>No. 4</td>
<td>56</td>
<td>Carcinoma portionis</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>No. 5</td>
<td>56</td>
<td>Corpus carcinoma</td>
<td>Cylindrical cell carcinoma</td>
</tr>
<tr>
<td>No. 6</td>
<td>61</td>
<td>Carcinoma cervicoportionis 2nd</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>No. 7</td>
<td>48</td>
<td>Carcinoma cervicoportionis 2nd</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>No. 8</td>
<td>40</td>
<td>Carcinoma portionis 2nd</td>
<td></td>
</tr>
</tbody>
</table>

The tissues were fixed with Carnoy's acetic alcohol solution for 60 minutes. Sections were made eight to ten micra in thickness suitable for microspectrophotometry; the slides were subjected to the Feulgen reaction according to the standard procedure after Stowell. The methods of measurement of absorption were essentially similar to those of Pollister and Ris (1947), Pollister and Moses (1949) and Leuchtenberger (1950). For histological studies, tissues were fixed with 10 per cent formalin. The sections were prepared according to the usual paraffin method and colored with Delafield's hematoxylin followed by counter staining with eosin.

**RESULTS**

The results of DNA measurements in individual nuclei of the primary human uterine carcinoma as well as of the normal embryonic liver here studied are as shown in Figure 1. In Table 2 are presented the variation of DNA values in relation to chromosome numbers.

It is evident from Figure 1 that there is a remarkable constancy of DNA content in somatic cells from the embryonic liver here examined, since the average value is approximately 33.0 to 33.5 in arbitrary units. A major mode is represented at approximately 50 per cent of the essential value. There is a small scatter of values
around the mode: it is probably due to metabolic and mitotic activity of cells. The average value here obtained is tentatively regarded to be a 2n-value and is taken as control.

It can be seen from the histograms presented in Figure 1 that the DNA contents of uterine carcinomas show a wide variation in contrast to those of normal embryonic tissue. With respect of DNA values, the tumor are grouped into three categories as follows:

The first group (no. 6) has a DNA value which is slightly higher than the 2n-value of the normal tissue and lies in a near-diploid range (36.93 ± 10.39). The modal peak of DNA value in this tumor shows over 50 per cent with a slightly narrow deviation (± 10.4).

The second group (no. 1, no. 2, no. 3, no. 4 and no. 5) is characterized by DNA values approximately 30 per cent higher than the 2n-value. The values lie generally in hyper-diploid region. The modal peaks are fairly remarkable in a hyper-diploid range (over 30%); the deviations show a larger scatter than the first group.

In the third group (nos. 7-8) the value of DNA shows a wide spread in the standard deviation (± 20): some of the cells have DNA contents which are significantly higher than the 2n-value lying in a hypertetraploid region.

A direct relationship between the chromosome number and the DNA contents of tumor cells is shown in table 2. From this table the two specimens, Nos. 7 and 8, were evidently estimated to have DNA values which differ very slightly from each other and show nearly similar chromosome variations. Also it is noted that no. 6 has a DNA value lying at a near-diploid range, while the chromosome ranges show a hypo-triploidy. As a whole it is apparent that all the primary uterine carcinomas here considered show cells carrying DNA contents which vary markedly. But it may be suggested that the frequency distribution of DNA contents in tumor cells of

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Mean am't of DNA</th>
<th>Major range of chrom. no. variation</th>
<th>Modal number of chroms.</th>
<th>Ploidy range from chroms.</th>
<th>Ploidy range from DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>42.99 ± 14.84</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Hyper-2n</td>
</tr>
<tr>
<td>No. 2</td>
<td>42.32 ± 6.99</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>No. 3</td>
<td>42.48 ± 4.91</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>No. 4</td>
<td>45.04 ± 12.15</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Hypo-3n</td>
</tr>
<tr>
<td>No. 5</td>
<td>41.50 ± 12.37</td>
<td>48-66</td>
<td>50</td>
<td>Hyper-2n</td>
<td>Hyper-2n</td>
</tr>
<tr>
<td>No. 6</td>
<td>36.93 ± 10.39</td>
<td>58-87</td>
<td>66 &amp; 75</td>
<td>Hypo-3n</td>
<td>Near-2n</td>
</tr>
<tr>
<td>No. 7</td>
<td>78.33 ± 19.55</td>
<td>54-100</td>
<td>83</td>
<td>Hypo-4n</td>
<td>Hyper-4n</td>
</tr>
<tr>
<td>No. 8</td>
<td>77.41 ± 20.45</td>
<td>50-100</td>
<td>62</td>
<td>Hypo-3n</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33.21 ± 9.80</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>prob. 2n</td>
</tr>
</tbody>
</table>

Table 2. List of eight human uterine tumors under study, with a summary of DNA data obtained. Chromosomal data were supplied by Dr. A. Tonomura.
the uterine carcinomas here concerned tends to correspond in value to the chromosome-number frequency irrespective of the pathological diagnosis of the tumors. It is remarkable that four out of the eight tumors here studied showed DNA value at a hyper-diploid range.

Fig. 1. Nine histograms indicating relative amounts of DNA in one specimen of the normal embryonic liver and eight uterine carcinomas.
DISCUSSION

Cytophotometric data have recently shown that there is a constancy of DNA-amount per nucleus in parallel to that in the number of chromosomes. Working with DNA measurements of rat tissues, Swift (1950a) found that the amount of DNA per nucleus was approximately the same in the liver, pancreas, lymphocytes and some other parts. To verify the above view, supplementary data have been accumulated by many authors (Ris & Mirsky 1949, Leuchtenberger et al. 1951, Franzer & Davidson 1952, Swift & Kleinfeld 1953, Makino et al. 1958). But, some other authors have presented data which suggest a variation of the amount of DNA in the nucleus under the influence of physiological and pathological disturbances. For instance, Leuchtenberger et al. (1954) made a comparative study of DNA contents between the normal and malignant tissues in various types of tumors. They found that the DNA content showed a certain but limited degree of variation from cell to cell in normal tissues, while in malignant tissues it showed a wider scatter from cell to cell than do normal cells. They also showed that in malignant tissues the amount of DNA per cell was much larger than in normal cells. Atkin and Richard (1956), and Barder (1959) found that in normal tissues the DNA content was almost constant, while in malignant tissues the DNA value showed a considerable variation and was higher than in the control.

Data presented from the present study have shown that in non-malignant cells measured DNA values vary rather slightly and show a basic mean value which lies between 30 and 40 in arbitrary units. In uterine tumors so far studied in this investigation, however, the amount of DNA showed a slight increase over the basic value. Further, it has been found that four out of eight tumors under study showed a DNA value lying in a hyper-diploid region, one in a near-diploid range, and the remaining three in a high ploidy range. The evidence presented is interesting in reference to the recent reports that in many uterine carcinomas the stemline numbers are distributed in near-diploid and hyper-diploid ranges (Makino et al. 1959, Tonomura 1959). Further, the data obtained here from DNA-measurements significantly supplement the hypothesis of stemline cells as progenitors of the growth of tumors (Makino 1957). There was no correlation between the DNA-value and the pathological property of the tumor.

SUMMARY

A microspectrophotometric study of the DNA content was made in one specimen of normal tissue and in eight human uterine carcinoma tissues. The results are shown in Table 2.

The normal liver tissue contained cells with a basic mean DNA content at 33.1 to
33.5 arbitrary units, showing a certain but limited degree of variation from cell to cell. Malignant tissues showed a much larger scatter of DNA-value from cell to cell than occurred in normal tissues. Four out of eight uterine carcinomas showed a DNA value lying in a hyperdiploid range. Generally, the DNA value of uterine carcinomas here considered tends to correspond to the stemline chromosome-number in each.

REFERENCES


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