Cytological Aspects of Normal and Tumorous Liver*

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SUMMARY

Liver tumors were induced in male rats of the Wistar strain by feeding p-dimethylaminoazobenzene mixed into ground rat food. Surgical biopsies were taken before the animals were fed the azo dye, after 18 weeks of feeding, and when the animals were sacrificed. Tumorous liver was classified as cholangioma, trabecula hepatoma, and apparently normal areas. DNA content (by cytophotometry), mitotic activity, and nuclear volumes were obtained for the normal liver and different areas of the tumorous liver and were compared with earlier biopsies in each individual animal.

Normal parenchymal cells contained three DNA classes; the third class increased in frequency with age. Nuclear volumes were closely grouped around each DNA class. Enlarged nuclei belonging to DNA classes higher than the third class appeared after 18 weeks of feeding the azo dye. Mitotic indices in these tissue samples were within the normal range.

Apparently normal areas in the tumorous liver contained enlarged nuclei belonging to DNA classes higher than the third class, and again the mitotic index was not increased. A high mitotic index was observed in proliferating trabecula hepatoma areas, and consistent with this were many DNA values lying between the DNA classes. DNA measurements made with the two-wavelength method on interphase nuclei and on chromosome groups in dividing cells again revealed intermediate values. Intermediate values from interphase nuclei were thought to be due to either DNA synthesis or chromosome aberrations; however, intermediate values from chromosome groups were probably due to some type of chromosome aberration.

Normal bile duct cells contained a single DNA class, the diploid. Nuclear volumes were closely grouped around this class. Cholangioma nuclei were greatly enlarged but were still associated with a single diploid DNA class. The mitotic index in cholangioma was about the same as that in normal bile duct cells.

One of the nuclear parameters used to describe tumor cell formation has been changes in deoxyribonucleic acid (DNA) content. This material, relatively constant and stable in normal tissues, has been shown to increase in a number of tumors (3, 4, 12, 24). DNA content varies with chromosome number, and deviations from this relationship have been correlated with chromosome aberrations (13, 14, 22). Although some variation occurs in proliferating tissue, this has been attributed to DNA synthesis in preparation for mitosis (19). A constant relationship has also been found to occur in some tissues between DNA content and nuclear volume (2).

The present study was undertaken to investigate changes in DNA content, mitotic activity, and nuclear volume, with the hope that some new aspects of these parameters would be disclosed in pretumorous and tumorous liver. Samples of liver were taken from the same animals before they were fed carcinogen, during carcinogenesis, and when they were sacrificed.

MATERIALS AND METHODS

Nine normal, young adult, male rats of the Wistar strain were used in this study. Seven of the animals were fed, ad libitum, ground dog checkers which contained a 0.06 per cent concentration (by weight) of p-dimethylaminobenzene (26). The remaining two were fed ground checkers without the azo dye. Surgical biopsies of the liver were taken from all

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nine animals before treatment, 18 weeks after azo dye feeding, and when the animals were sacrificed. The sampling technic involved opening the abdomen under ether anesthesia and removing a piece of liver 3-8 × 2 mm. It was felt that a small number of animals would be adequate in this study because normal liver samples obtained at biopsy from each individual could be compared with liver samples taken from the same individual after carcinogen treatment.

Animals that developed large palpable masses in the abdomen were sacrificed after 29 weeks of feeding p-dimethylaminooazobenzene. The experiment was brought to a close at the end of 34 weeks by sacrificing the remaining animals.

Some tissues were fixed in an acetic acid-alcohol (1:3) mixture for 4-6 hours; others were fixed in a 10 per cent neutral formalin. All were dehydrated in isopropyl alcohol and were cut at appropriate thicknesses (10).

Sections of untreated normal liver samples, intermediate (after 18 weeks of azo dye feeding), and final liver samples from each animal were mounted on the same slide and subjected to identical procedures. In each animal that developed a tumorous liver, an effort was made to remove and fix samples from different areas of the liver. These areas were arbitrarily classified according to their histopathology as apparently normal, trabecula hepatoma (5), and cholangioma. DNA content measurements, mitotic activity, and nuclear volume calculations were made and plotted for each of these areas.

Cytophotometry.—Relative quantitative changes of DNA were studied in individual whole nuclei (24). DNA content in irregularly shaped nuclei was determined by the two-wavelength method (17, 18). A Bausch and Lomb 250-mm. grating monochromator was used to isolate the desired spectral region. The microscope was fitted with an oil immersion aplanatic-achromatic condenser and an apochromatic objective whose numerical apertures were both 1.50. The condenser diaphragm was closed to an appropriately small diameter. Compensating oculars were regularly used.

Mitotic activity determinations.—The mitotic index was determined on Feulgen-stained 18-μ sections. Every other section was used to avoid counting the same mitosis more than once and to provide more adequate sampling. Counts were made with an oil immersion lens at 970X. Fifteen to twenty fields per biopsy were scanned, permitting 2,500-5,000 nuclei to be counted. Normal biopsies contained few bile duct areas; thus fifteen to twenty of these fields yielded about 1,000 nuclei. Cholangioma nuclei were closely packed. Scanning fifteen to twenty of these fields produced 18,000-20,000 nuclear counts.

The mitotic index was multiplied by 100 in order to express results in terms of per cent. The following statistic for standard deviation was used, where \( Np \) equals the mean number of mitotic stages and \( q \) equals the total number of nuclei counted; \( \sigma \) equals the standard deviation of the number of mitotic stages.

\[
\sigma = \sqrt{Npq}
\]

Nuclear volume determination.—Calculations were based on measurements of major and minor axes of whole nuclei obtained at the time of DNA measurements. The following formula, in which \( a \) is equal to one-half the longer diameter and \( b \) is equal to one-half the shorter diameter, considers the nuclei as slightly elongated ellipsoids:

\[
V = \frac{4}{3} \pi ab^2.
\]

Measurements of nuclear diameters were made to ±0.25 μ. Volumes were plotted for those groups having approximately 50 nuclei in a given DNA class.

Stains and reactions.—General observations were made on hematoxylin and eosin-stained slides. Mitotic activity, nuclear volume, and cytophotometric determinations were made on Feulgen-reacted slides that were mounted in a Schillaberoil of proper refractive index (20); Carnoy's fixed sections were stained with azure-B at pH 4.0, and when used with specific nucleases DNA and RNA were identified (7). The periodic acid-Schiff (PAS) reaction combined with diastase digestion localized glycogen and nonglycogen PAS-positive material (10). Formalin-fixed frozen sections stained with Sudan Black-B were used to demonstrate fat.

RESULTS

Some cytological observations.—The RNA-basophilia in normal interphase parenchymal cells appeared characteristically clumped (Fig. 1). The cytoplasmic RNA-basophilia in tumorous liver cells differed from its normal predecessors and varied from one area to the next. RNA-basophilia in trabecula hepatoma was somewhat increased in density and not as clumped as that found in normal cells. Mitotic cells in this area contained large RNA-rich granules scattered around the spindle in a background of diffusely distributed basophilia (Figs. 2, 4). Nucleoli in the trabecula hepatoma cells were dense, diversely shaped, and often appeared to be in contact with the nuclear membrane (Fig. 5). After diastase digestion, spherical PAS-positive bodies were commonly found in these
parenchymal cells. Cells containing fat vacuoles had enlarged, dense, spherical nucleoli (Fig. 6). The cholangioma areas consisted of many groups of bile ducts surrounded by a connective tissue stroma (Fig. 8). These ducts often contained leukocytes and a material which was strongly PAS-positive after diastase digestion. The crowded cells bordering the duct contained large oval-shaped nuclei. Columns of cells similar to both bile duct cells and fibroblasts were observed extending from the duct region into fibrous stroma. The nucleoli in the nuclei of these cells were small and difficult to identify.

DNA.—The DNA content of parenchymal cell nuclei in the first control biopsies readily fell into classes, the first and second or diploid and tetraploid classes, respectively (Chart 1A). Samples of tissue from the same animal at the 18th and 34th weeks displayed further increases in the frequency of a third, octoploid class (Charts 1B, C). Standard deviations can be seen in Table 1. Data from azo dye-fed animals which did not develop tumors revealed DNA class distributions similar to that of the control normal animals.

Initial biopsies from animals that developed tumors contained a normal distribution of first and second DNA classes. Data from 18th-week biopsies in these animals differed from the normal pattern, showing DNA values belonging to a fourth DNA class (Chart 2D). Tumorous liver contained a number of deviations from the normal pattern. These varied with the histopathology of the sample. In regions of apparent normality found at the periphery of trabecula hepatoma, individual class peaks were displayed. There were, however, scattered DNA values above the octoploid class (Chart 2A). Data from trabecula hepatoma regions revealed many mitotic stages and continuous values between DNA classes (Chart 2C). Data from regions containing degenerating cells and cells with fat vacuoles revealed some intermediate DNA values and values greater than the octoploid class (Chart 2B). Parenchymal cell DNA measurements, made near proliferating bile ducts, revealed some values greater than the octoploid class; whereas measurements made on cells at a distance from the bile duct regions displayed an almost normal DNA distribution. Standard deviations were, in general, greater in tumors than in nontumorous liver (Table 1).

To determine whether intermediate DNA values, obtained from tumors, were associated only with DNA synthesis or whether abnormal chromosome numbers might be involved, measurements were made on chromosome groups as well as on interphase nuclei, using the two-wavelength method. For this method, parenchymal cell data were obtained from the following liver samples: prior to azo dye feeding, after 18 weeks of azo dye feeding, and from two areas of tumorous liver.

Charts 1A, B, C.—Frequency distribution of DNA (in arbitrary units) in normal liver parenchymal cells.

Charts 2A, B, C, D.—Frequency distribution of DNA (in arbitrary units) in azo dye-fed rat liver parenchymal cells. Chart 2D is a biopsy obtained after 18 weeks of azo dye feeding; 2A, B, C are tissue samples from different areas of the tumorous liver.
TABLE 1
STATISTICAL ANALYSIS OF REPRESENTATIVE DNA DATA IN ARBITRARY UNITS

<table>
<thead>
<tr>
<th>Week of</th>
<th>Liver samples from</th>
<th>Diploid (Mean ± S.D.)</th>
<th>Tetraploid (Mean ± S.D.)</th>
<th>Octoploid (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tissue</td>
<td>individual animal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Normal bile duct</td>
<td>4.4 ± 0.32</td>
<td>8.3 ± 0.78</td>
<td>16.3 ± 1.11</td>
</tr>
<tr>
<td></td>
<td>cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control par. cells</td>
<td>4.4 ± 0.46</td>
<td>8.2 ± 0.49</td>
<td>16.3 ± 1.14</td>
</tr>
<tr>
<td></td>
<td>Azo dye par. cells</td>
<td>4.4 ± 0.26</td>
<td>8.4 ± 0.62</td>
<td>16.3 ± 1.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4 ± 0.26</td>
<td>8.4 ± 0.62</td>
<td>16.3 ± 1.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4 ± 0.26</td>
<td>8.4 ± 0.62</td>
<td>16.3 ± 1.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.4 ± 0.54</td>
<td>8.3 ± 0.83</td>
<td>16.3 ± 1.08</td>
</tr>
<tr>
<td>18</td>
<td>Control par. cells</td>
<td>4.3 ± 0.33</td>
<td>8.2 ± 0.59</td>
<td>16.3 ± 1.11</td>
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<tr>
<td></td>
<td>Azo dye par. cells</td>
<td>8.2 ± 0.55</td>
<td>16.3 ± 1.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.8 ± 0.86</td>
<td>16.3 ± 1.08</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Azo dye*</td>
<td>4.6 ± 0.89</td>
<td>8.1 ± 0.78</td>
<td>16.3 ± 2.18</td>
</tr>
<tr>
<td></td>
<td>Azo dye†</td>
<td>4.4 ± 0.54</td>
<td>8.3 ± 1.22</td>
<td>15.6 ± 2.45</td>
</tr>
<tr>
<td></td>
<td>Azo dye‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Control par. cells</td>
<td>4.8 ± 0.89</td>
<td>8.2 ± 0.58</td>
<td>16.2 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>Control par. cells</td>
<td>4.5 ± 0.56</td>
<td>8.2 ± 0.60</td>
<td>16.3 ± 0.84</td>
</tr>
</tbody>
</table>

* Trabecula hepatoma.
† Apparently normal area of parenchymal cells in tumorous liver.
‡ Cholangioma area.
§ Fat-containing area in tumorous liver.

TABLE 2
COMPILED TWO-WAVELENGTH DATA, AZO DYE-FED ANIMAL, PARENCHYMAL CELLS

<table>
<thead>
<tr>
<th>0-WEEK BIOPST</th>
<th>18TH-WEEK BIOPST</th>
<th>29TH-WEEK BIOPST, REGION OF APPARENT NORMALITY</th>
<th>29TH-WEEK BIOPST, TRABECULA HEPATOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, 14.01* S.D., .50 Coef. var., 5.9%</td>
<td>Mean, 15.94* S.D., .59 Coef. var., 4.0%</td>
<td>Mean, 14.38* S.D., 1.14 Coef. var., 7.5%</td>
</tr>
<tr>
<td>12.93</td>
<td>13.21</td>
<td>13.44</td>
<td>14.01 Interphase</td>
</tr>
<tr>
<td>13.61</td>
<td>13.82</td>
<td>14.75</td>
<td>15.98 Interphase</td>
</tr>
<tr>
<td>13.68</td>
<td>14.12</td>
<td>13.91</td>
<td>16.70 Interphase</td>
</tr>
<tr>
<td>14.00</td>
<td>14.38</td>
<td>14.24</td>
<td>14.71 Interphase</td>
</tr>
<tr>
<td>14.14</td>
<td>14.26</td>
<td>13.54</td>
<td>21.53† Interphase</td>
</tr>
<tr>
<td>14.35</td>
<td>13.78</td>
<td>14.41</td>
<td>19.03† Anaphase</td>
</tr>
<tr>
<td>15.08</td>
<td>13.43</td>
<td>14.10</td>
<td>25.69 Metaphase</td>
</tr>
<tr>
<td>14.39</td>
<td>13.84</td>
<td>14.83</td>
<td>24.15 Metaphase</td>
</tr>
<tr>
<td>13.57</td>
<td>13.63</td>
<td>14.36</td>
<td>21.08† Anaphase</td>
</tr>
<tr>
<td>14.39</td>
<td>13.90</td>
<td>14.38</td>
<td>15.30</td>
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<tr>
<td></td>
<td></td>
<td>14.38</td>
<td>15.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.02</td>
<td></td>
</tr>
</tbody>
</table>

* Mean is for Class 2 interphase stages.
† Intermediate value.
A study of normal bile duct cells revealed only one DNA class, the diploid (Chart 3A). Measurements on cholangioma bile duct cells again yielded only a single DNA class. No second class or intermediate values were observed (Chart 3B).

**Mitotic activity.**—The per cent mitotic index (mitotic index \( \times 10^2 \)) in normal liver parenchymal cells differed from that of its tumorous counterpart. Observations on mitotic activity in initial biopsies from control animals revealed a range for the standard deviation at the 2-sigma level of from 0.2 to 0.5 per cent (Chart 4). The percentages of prophase and metaphase stages were about equal. When these animals were sacrificed 34 weeks later, the mitotic activity was in the same range.

Animals that were sacrificed after being fed the azo dye for 34 weeks, and that did not develop tumors, contained mitotic activity similar to that found in the control, nonazo dye-fed animals.

Areas of apparently normal parenchymal cells some distance from trabecula hepatoma disclosed some activity, but it was within normal range (Chart 5). Mitotic activity in trabecula hepatoma was high and at the 2-sigma level had a range of 0.9–1.9 per cent (Chart 5), and here the ratio of metaphase to prophase stages was high.

Zero week biopsies containing normal bile duct cells displayed a range of 0–0.3 per cent at the 2-sigma level. Final biopsies taken from the same normal animals at the close of the experiment, 34 weeks later, revealed a range of 0.0–0.5 per cent (Charts 6A, B). A range of 0.2–0.3 per cent activity was calculated for bile duct cells in cholangioma. The metaphase stage contributed more to this percentage than did the prophase stage.

**Nuclear volumes.**—An initial biopsy containing normal liver parenchymal cells revealed nuclear volume means of 121.0 cu. \( \mu \) for class 1 nuclei and 149.0 cu. \( \mu \) for class 2 nuclei. A biopsy from this same animal 34 weeks later yielded a class 1 mean of 148.0 cu. \( \mu \), a class 2 mean of 205.0 cu. \( \mu \), and the appearance of a class 3 mean of 382.0 cu. \( \mu \). The difference between the initial and final biopsy means was not considered significant at the 2-sigma level of confidence. When the volume measurements from normal biopsies were plotted against DNA content, the values fell readily into discrete classes (Charts 7A, B).

Zero time biopsies from animals that eventually developed tumors were similar to the above control values. After 18 weeks of azo dye feeding, classes 2 and 3 had means within normal limits at the 2-sigma level of confidence.

Areas of apparently normal parenchymal cells peripheral to the tumorous area displayed means of 145.0, 249.0, and 553.0 cu. \( \mu \) for the three DNA classes, respectively, after 29 weeks of azo dye feeding.
feeding (Chart 8A). Marked tumorous regions yielded 387.0 and 771.0 cu. μ for class 2 and class 3, respectively (Chart 8B). Increased scattering of the nuclear volume data in tumorous tissue obscured normal relationships with the DNA classes. Nuclear volumes were clearly increased in the tumorous parenchymal cells (Table 3).

Both normal and tumorous bile duct nuclei fell into the same DNA group, class 1 (Charts 9A, B).

DNA.—With the aid of cytospectrophotometry and biochemical analyses, it has been shown that DNA is a relatively constant and stable entity (11, 24). Geometric multiples of the diploid DNA class occur in some tissues. Normal adult liver contained three such classes; class 2, tetraploid, was the most common. An increase in the octoploid class normally occurs with age and has been observed in this and other studies (8, 9). Accompanying this increase in polyploid nuclei were a decreasing number of binucleate cells and no increase in mitotic activity, implicating nuclear fusion as the mechanism which produced the large nuclei. Early appearance of polyploid nuclei after azo dye feeding, as observed in this and other studies, again suggested that some form of chromatin doubling had occurred without breakdown of the nuclear membrane (20, 23, 28).

In tumors, intermediate values (those DNA values that occur between classes) and DNA classes higher than the octoploid were found. Data from this and other studies suggested that DNA histograms which revealed intermediate values and a scattering of higher values could be useful in the diagnosis of malignancy (3, 4, 10, 12). Although polyploid nuclei are implicated in the tumorous process, their significance is not fully understood.
TABLE 3

STATISTICAL ANALYSIS OF REPRESENTATIVE NUCLEAR VOLUME DATA, IN MICRONS

<table>
<thead>
<tr>
<th>Week of sample</th>
<th>Liver samples from individual animal</th>
<th>Diploid (Mean±S.D.)</th>
<th>Tetraploid (Mean±S.D.)</th>
<th>Octoploid (Mean±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal bile duct cells</td>
<td>80.1±16.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control par. cells</td>
<td>121.0±12.2</td>
<td>194.4±48.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azo dye par. cells</td>
<td>174.0±39.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Azo dye par. cells</td>
<td></td>
<td>198.5±36.5</td>
<td>468.3±199.3</td>
</tr>
<tr>
<td>29</td>
<td>Azo dye*</td>
<td>249.3±91.2</td>
<td>420.5±119.1</td>
<td>728.7±170.9</td>
</tr>
<tr>
<td></td>
<td>Azo dye†</td>
<td></td>
<td>248.6±88.0</td>
<td>558.0±222.7</td>
</tr>
<tr>
<td></td>
<td>Azo dye‡</td>
<td></td>
<td>387.9±128.6</td>
<td>771.9±410.3</td>
</tr>
<tr>
<td>34</td>
<td>Control par. cells</td>
<td>148.2±19.8</td>
<td>204.8±40.8</td>
<td>382.5±147.2</td>
</tr>
</tbody>
</table>

*Cholangioma area.
† Trabecula hepatoma.
‡ Apparently normal area of parenchymal cells in tumorous liver.
§ Fat-containing area: tumor.

It has been claimed that those cells containing polyploid nuclei may behave as centers for hepatoma formation (8) and that metastatic tumors tended to have a higher ploidy than the primary tumors from which they were derived (6). Since intermediate values may be due either to DNA synthesis, which occurs during the interphase stage in most tissues, or to irregularities in chromosome duplication, the two-wavelength method, a cytospectrophotometric technic which reduces errors due to irregular distribution of the Feulgen dye, was used to measure chromosome groups. Measurement of chromosome groups in metaphase and anaphase stages revealed intermediate values. Although there was a lack of measurements on chromosome groups in normal liver and only a small number of measurements were made on chromosome groups in tumorous liver, as a preliminary conclusion, these variations were interpreted as meaning that irregular chromosome numbers or some failure in separation of previously synthesized DNA materials was involved.

Data from normal and cholangiomatous bile ducts revealed a single DNA class; no second DNA class or intermediate values were found. The DNA data were consistent with the low mitotic index observed. It would seem that either these cells have a relatively low growth rate or some other factors were involved in the growth of cholangioma. The few quantitative studies that have

Charts 9A, B.—Scatter diagrams of nuclear volume and DNA class in normal bile duct cells obtained at the beginning of the experiment and in cholangioma cells obtained 29 weeks later.
been made on growth characteristics of bile duct epithelium are in agreement that bile duct cells renew themselves but at a slower rate than do parenchymal cells (1, 29, 30). An increase in fibroblast-like cells in cholangioma had been observed in this study. A close association between the outgrowth of fibroblasts and bile duct cells has been described (1, 30). An association of these two cell types may, in some manner, contribute to the increase in cholangioma.

**Mitotic activity.**—Mitotic activity varied in normal liver and was probably due to the animal strain (27). Difficulty in determining onset of prophase and end of telophase may have contributed some error to the counts; however, criteria for these stages were established and consistently followed.

Mitotic activity in 18th-week biopsies from azo dye-fed animals was within the normal range. Marked increases in mitotic activity appeared in areas containing trabecula hepatoma; the activity in apparently normal parenchymal cells near the trabecula areas was again within the normal range. A recent study, with tritiated thymidine, found no increase in labeled ‘preneoplastic’ cells, and its authors concluded that cellular proliferation was not important in the development of hepatic tumors (15). Other workers noted that mitoses and especially mitotic anomalies were significant in the formation of hepatomas (16). Mitotic anomalies and chromosome aberrations could manifest themselves as variations from a DNA class. DNA data, especially those with the two-wavelength method, as discussed above, have shown that discrete DNA classes were present in all tissue samples except trabecula hepatoma. In this study, mitotic and chromosomal aberrations, disclosed by variations in DNA content, represented activity in definitive tumor rather than a contribution toward tumor formation.

**Nuclear volumes.**—Some normal tissues maintain a close association between nuclear volume and DNA content, whereas others do not. A consistently close relationship has been observed between nuclear volume and total nuclear protein (2). The three nuclear volume class means, obtained in this study, were closely associated with their DNA classes, and the values compared favorably with those obtained by others (21). Apparently normal parenchymal cells at the periphery of trabecula hepatoma had somewhat increased nuclear volumes for all three of their DNA classes. Nuclear volumes in trabecula hepatoma displayed marked increases. The association between nuclear volume and DNA class which occurs in normal liver became less distinct in tumorous liver.

Normal bile duct cell nuclear volumes were closely grouped around a single diploid DNA class. In cholangioma nuclear volumes were markedly increased and widely scattered; however, their DNA content was still associated with a single diploid DNA class.

These observations revealed the presence of two kinds of nuclear volume increase. Nuclear volumes in parenchymal cells were associated with an increase in ploidy as well as an increase within each ploidy. Nuclear volumes increased in cholangioma cells, but their ploidy remained the same. Nuclear volume changes occur readily in physiological and pathological conditions. This may be because of one or several factors: aneuploidy, polyploidy, polyteny, increased nuclear proteins, or water retention. The situation in tumorous tissue remains to be clarified; undoubtedly more than one factor is involved.

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