The Numerical Proportions of Cell Types in Rat Liver during Carcinogenesis by 4-Dimethylaminoazobenzene (DAB) *

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The liver is an organ which is frequently utilized for biochemical investigations because of its large volume and its apparent homogeneity. Actually, the liver is not a homogeneous organ, since it contains, in addition to the hepatic cells, an appreciable number of other cells the presence of which must be taken into consideration when interpreting the biochemical data. Moreover, the histological architecture of this organ is known to be markedly altered in different pathological conditions. During carcinogenesis by 4-dimethylaminoazobenzene (DAB), for instance, important changes are observed in the relative proportions of the cell types as well as in the nature and the volume of the extracellular spaces (11, 15-17). The liver becomes highly heterogeneous in such a condition. In order to evaluate quantitatively the changes taking place in the cellular population of the liver during carcinogenesis, we have undertaken to determine the numerical proportions of the various cell types present in the liver of normal rats and rats fed DAB for different time intervals.

MATERIALS AND METHODS

Male, adult, albino rats (Wistar strain, 175-225 gm.) fed Purina Fox Chow and water ad libitum were separated into three groups. The animals of the first group were continued on Purina Fox Chow and water. Those in the second group were transferred to a semi-synthetic, low-protein diet (basal diet, Ref. 14, diet 8). Animals in the third group were transferred to a semi-synthetic diet containing the azo-dye DAB, for instance, important changes are observed in the relative nuclear sizes, since bigger nuclei have greater chances to be included in any section (1, 4).

To correct the results of the differential counts, the mean nuclear length of each cell type was calculated from measurements made by ocular micrometry on 200 parenchymal nuclei and 50 nuclei of each other type per animal. The measurements were made under oil immersion by a microscope which had been adjusted so that one division of the eye-piece micrometer corresponded to 1 micron on the stage micrometer. The objective was placed at random on a liver section, and the latter was displaced perpendicularly to the micrometer scale. The first 25 nuclei of each type, the centers of which fell within the length of the scale (50 μ), were measured. All measurements were determined in a second series of measurements.

For differential counts of cell types in the tissue sections, a reticle delimiting a square field was placed in one of the oculars of a binocular microscope. The field was placed at random on a liver section, and all nuclei included in the field were examined under oil immersion. Differential counts of the following cell types were made: parenchymal cells, littoral cells, bile duct cells, cells of connective tissue, and cells of the blood vessel walls. Once all the nuclei in a field had been examined and classified, the counts were pursued in similar fields taken at random on different parts of the liver sections until at least 6,000 nuclei had been examined per animal. The results of these counts represent the apparent distribution of the cell types. Such data must be corrected for the relative nuclear sizes, since bigger nuclei have greater chances to be included in any section (1, 4).

The animals were given injections subcutaneously with 0.1 ml/100 gm body weight of a 0.1 per cent solution of colchicine, 4 hours before sacrifice, for measuring the incidences of mitoses in the same sections as those used in the present study. Colchicine injection had no effect on the cytological composition of the liver, however, since differential counts of cell types in intact and colchicine-injected animals showed no appreciable difference between the two groups.

The term parenchymal cells instead of parenchymal nuclei is used throughout the present article, but these terms are not actually equivalent, since many parenchymal cells are binucleated (3, 5, 20, 24).

The term littoral cell used here after Abercrombie and Harkness (2) refers to the cells lining the blood sinusoids. They include the Kupffer cells and the so-called undifferentiated cells which cannot be easily distinguished from the other.

The measurements on parenchymal nuclei and those on nuclei of the other types were actually made in two different series. The mean diameters of the parenchymal nuclei were computed from a series of measurements on 200 nuclei per animal carried out for studying the distribution of nuclear sizes in liver parenchymas during DAB carcinogenesis (unpublished results). The nuclear lengths of the four other cell types, i.e., means of 50 nuclei of each type per animal, were determined in a second series of measurements.
made in the center of the field after the initial position of the nuclei on the micrometer had been noted. For each nucleus, the lengths of the long and short axes were determined (evaluated to 0.5 micron), and the nuclear length was taken as the mean of the two values. When 25 nuclei of each type had been measured, the slide was displaced at random, and the measurements were pursued in another part of the section. Hence, the measurements on parenchymal nuclei were made in eight different portions of the sections for a total of 200 nuclei per animal, while those on the nuclei of the other types were carried out in two different portions for a total of 50 nuclei of each type. After the mean nuclear length of each cell type had been calculated, the data on the apparent distribution of cell types were corrected according to Abercrombie (1), and the true distribution values were obtained. The results were finally expressed as the per cent distributions of cell types.

RESULTS

Normal rat liver.—The per cent distributions of cell types in normal rat livers are shown in Table 1. In 200-gm. rats (0 days), the parenchymal cells composed 61 per cent of the total number of cells and the littoral cells 30 per cent, the remaining 9 per cent being accounted for by the bile duct cells, the connective tissue cells, and the cells of the blood vessel walls. These proportions were observed to vary with age in the normal animals. The percentages of parenchymal cells were statistically higher in the rats sacrificed at later time intervals and the percentages of parenchymal cells and other cell types correspondingly lower. In the last group of normal rats investigated (180 days, 442 gm. body weight on the average), the parenchymal cells were found to constitute 61 per cent of the total number of liver cells, the littoral cells 30 per cent, and the other cell types 9 per cent.

It may be noted that, although the rats of the 120-day group had a higher mean body weight than those of the 60-day group, the mean liver weight in that group (for some unknown reason) was lower than in the latter group, and the percentage of littoral cells was accordingly lower.

The values obtained for these two groups were intermediate between those obtained at 0 and 180 days. This confirms that a quantitative change takes place in the cellular composition of the adult rat liver as the organ increases in weight.

Liver of rats fed the basal diet.—In rats fed the basal diet (Table 2), the body weights and liver weights were observed to decrease between 0 and 60 days and to increase afterward. These changes were accompanied by an initial (statistically significant) decrease in the percentage of littoral cells followed by a slight increase after the 90-day interval. The percentages of parenchymal cells and other cell types showed reciprocal variations. Feeding the basal diet thus altered the cytological composition of the liver in a direction opposite to that observed in animals fed the normal diet. At the 60-, 120-, and 180-day intervals, times at which both normal rats and rats fed the basal diet were investigated, the percentages of parenchymal cells were all statistically higher and the littoral cell values statistically lower in rats fed the basal diet than in the normal animals. By the end of the experiment (180 days), the values in rats fed the basal diet were close to those obtained at 0 day, but much different from the results obtained in the rats fed the normal diet for a corresponding time interval.

Liver of rats fed the carcinogenic diet.—The rats fed the DAB-containing diet (Table 3) showed a progressive decrease in body weight and liver weight up to 60 days, as was observed in rats fed the basal diet. These initial decreases were followed by progressive increases. After 180 days, the body weights of the DAB-fed animals were similar to those of animals fed the basal diet, but the liver weights were much higher in the former group than in the latter owing to the development of tumor masses, between 90 days and 180 days, in the liver of rats fed DAB.

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**TABLE 1**

NUMERICAL PROPORTIONS OF CELL TYPES IN NORMAL ADULT RAT LIVER

<table>
<thead>
<tr>
<th>TIME</th>
<th>BODY WEIGHT (gm. ± S.D.)</th>
<th>LIVER WEIGHT (gm. ± S.D.)</th>
<th>PER CENT DISTRIBUTION OF CELL TYPES (± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Parenchymal cells</td>
</tr>
<tr>
<td>(days)</td>
<td>(gm. ± S.D.)</td>
<td>(gm. ± S.D.)</td>
<td>60.7 ± 1.7</td>
</tr>
<tr>
<td>0*</td>
<td>200 ± 6</td>
<td>6.7 ± 0.7</td>
<td>56.1 ± 1.5</td>
</tr>
<tr>
<td>60</td>
<td>336 ± 14</td>
<td>11.4 ± 1.6</td>
<td>58.7 ± 2.2</td>
</tr>
<tr>
<td>120</td>
<td>300 ± 50</td>
<td>10.0 ± 1.4</td>
<td>55.7 ± 2.4</td>
</tr>
<tr>
<td>180</td>
<td>445 ± 52</td>
<td>12.7 ± 2.4</td>
<td>58.7 ± 2.2</td>
</tr>
</tbody>
</table>

* Time at which the animals were separated into three groups for studying the effect of (a) normal diet, (b) the basal diet, and (c) the carcinogenic diet.
The most striking histological change observed in the liver of DAB-fed animals is the formation of bands of bile duct and connective tissue cells as a result of an extensive proliferation of these tissue elements. The trabeculae surround nodules of parenchymal tissue (cirrhotic liver), and tumor masses develop at later stages from either the

from 3 per cent to 17 per cent during the same time interval. These changes were accompanied by striking decreases in the proportions of parenchymal and littoral cells. The livers of rats fed DAB for 90 days were composed, on the average, of 24 per cent of parenchymal cells, 15 per cent of littoral cells, 43 per cent of bile duct cells, 3 per cent to 17 per cent during the same time interval. These changes were accompanied by striking decreases in the proportions of parenchymal and littoral cells. The livers of rats fed DAB for 90 days were composed, on the average, of 24 per cent of parenchymal cells, 15 per cent of littoral cells, 43 per cent of bile duct cells, 3 per cent of connective tissue cells, and 1 per cent of cells in the blood vessel walls (Table 3). Such a composition is entirely different from those observed in rats fed normal or basal diets. The variations in the distribution of cell types were maximal after 90 days of DAB feeding, and the proportions observed in cirrhotic liver

<table>
<thead>
<tr>
<th>Time on diet (days)</th>
<th>BODY WEIGHT (gm.±S.D.)</th>
<th>LIVER WEIGHT (gm.±S.D.)</th>
<th>PARENCHYMAL CELLS (%)</th>
<th>LITTORAL CELLS (%)</th>
<th>CELLS OF BILE DUCTS (%)</th>
<th>CELLS OF CONNECTIVE TISSUE (%)</th>
<th>CELLS OF BLOOD VESSEL WALLS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>211±15</td>
<td>5.9±0.6</td>
<td>60.5±2.0</td>
<td>33.0±0.6</td>
<td>2.3±0.3</td>
<td>2.5±0.5</td>
<td>1.7±0.4</td>
</tr>
<tr>
<td>30</td>
<td>190±23</td>
<td>7.4±2.4</td>
<td>61.8±1.9</td>
<td>31.6±1.3</td>
<td>2.5±0.8</td>
<td>2.2±0.7</td>
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</tr>
<tr>
<td>60</td>
<td>155±22</td>
<td>5.2±0.8</td>
<td>62.4±2.2</td>
<td>30.3±2.4</td>
<td>2.8±0.8</td>
<td>2.1±0.4</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td>90*</td>
<td>177±22</td>
<td>7.2±1.7</td>
<td>62.6±2.3</td>
<td>28±2.2</td>
<td>3.8±1.3</td>
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<tr>
<td>120</td>
<td>201±36</td>
<td>7.0±1.3</td>
<td>64.2±0.6</td>
<td>29.6±1.7</td>
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<td>2.4±1.1</td>
<td>1.3±0.5</td>
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</tr>
<tr>
<td>180†</td>
<td>190±77</td>
<td>7.7±2.3</td>
<td>63.0±0.7</td>
<td>30.8±1.9</td>
<td>2.7±0.8</td>
<td>1.5±1.0</td>
<td>2.0±0.4</td>
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</tbody>
</table>

* One animal of that group showed an exceptionally high percentage of bile duct and connective tissue cells, and the results were excluded from the means. The per cent distribution of cell types in that liver was the following: parenchymal, 54.9; littoral, 29.8; bile duct, 7.0; connective tissue, 5.3; blood vessel walls, 3.0.

† One animal of that group showed an exceptionally high percentage of littoral cells, and the results were excluded from the means. The per cent distribution of cell types in that liver was the following: parenchymal, 50.8; littoral, 41.4; bile duct, 4.5; connective tissue, 1.9; blood vessel walls, 1.6.

**TABLE 3**

**NUMERICAL PROPORTIONS OF CELL TYPES IN THE LIVER OF RATS FED DAB**

<table>
<thead>
<tr>
<th>Time on diet (days)</th>
<th>BODY WEIGHT (gm.±S.D.)</th>
<th>LIVER WEIGHT (gm.±S.D.)</th>
<th>PARENCHYMAL CELLS (%)</th>
<th>LITTORAL CELLS (%)</th>
<th>CELLS OF BILE DUCTS (%)</th>
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* Means of counts on seven different hepatomas which developed in animals between 90 and 180 days.

† Means of counts on two cholangiomas which arose after 120 and 150 days of DAB feeding.
at later time intervals were about equivalent to those obtained at the 60-day period.

The tumor masses which developed from 90 days on, in the liver of the DAB-fed animals, presented particular distributions of cell types. In hepatomas, the parenchymal cells made up 76 per cent of the total number of cells, a value close to the normal one; the littoral cells 16 per cent, a value lower than normal; the bile duct cells 15 per cent and the connective tissue cells 10 per cent, values higher than normal; and the cells of the blood vessel walls 1 per cent. The cholangiomas, on the other hand, were composed almost exclusively of bile duct and connective tissue cells in proportions of 67 and 33 per cent, respectively.

**DISCUSSION**

The number of parenchymal cells in the liver of young adult rats accounted for only 61 per cent of the total number of cells (although they represent 85 per cent of the total volume [22]) and the nonparenchymal cells were present in a proportion of 39 per cent. Similar percentages of parenchymal cells in normal rat liver were reported by Abercrombie and Harkness, 59 per cent (2); Stowell, 60 per cent (21); Sibatani and Fukuda, 65 per cent (19); and Grant and Rees, 67 per cent (12). Figures of the same order were also reported for mouse liver, 56 per cent (25), and beef liver, 70 per cent (18). The results obtained in different laboratories vary whether: (a) young or older animals are used, (b) the nuclear counts are carried out exclusively on the cells composing the liver lobules or on both the lobular and interlobular cells, (c) blood elements are included in the counts or excluded, and (d) the results of the crude counts on tissue sections are corrected or not for the relative nuclear sizes. Each of the first three factors usually affects the results to only a small extent, but correcting the data for relative nuclear sizes decreases appreciably the percentage of parenchymal cells. In the present work, for instance, the crude counts gave an apparent percentage of parenchymal cells of 69 per cent (7), in agreement with the high (uncorrected) figures reported by some authors, while a true percentage of 61 per cent was obtained after correction. In any case, the different investigations lead to the same conclusion, namely, that the normal liver contains a high number of nonparenchymal cells and that, for an organ which is often considered as homogeneous, the liver shows an appreciable degree of cellular heterogeneity. The biochemical data obtained with liver homogenates cannot, therefore, be interpreted as though the organ were composed exclusively of parenchymal cells. In fact, such data cannot be translated in terms of any structural unit unless it is determined by histochemical methods in which tissue element the analyzed substances are located (9, 10).

The numerical proportions of cell types in the liver were found to be greatly altered during carcinogenesis by DAB. The percentage of parenchymal cells in cirrhotic liver was as low as 24 per cent on the average, after 90 days of DAB feeding, and the nonparenchymal elements were present in a proportion of 76 per cent. The liver tumors, for their part, showed particular cell distributions differing from those observed in both the normal and the cirrhotic livers. Grant and Rees (12) have recently confirmed the preliminary results obtained in the present study (7) and found that the changes taking place in the cell population of the liver during carcinogenesis by thioacetamide were of the same order as those described for DAB-fed animals. From a quantitative point of view, therefore, the normal, the cirrhotic, and the neoplastic livers show entirely different cytological compositions and cannot be considered as "homologous" tissues. Since cells of different types usually show quantitative differences in their chemical composition, changes in the proportions of cell types probably alter the average chemical composition of a tissue. It follows that variations in the composition of the "average liver cell" during DAB carcinogenesis may be due, in part at least, to changes in the cellular composition of the tissue and that they do not necessarily reflect actual variations in the cells.

The present analyses have dealt exclusively with the cell types which are permanent structural units of liver tissue. The blood cells were not included in the counts, since the percentage of these cells varies depending on whether the animals are exsanguinated or the livers perfused before carrying on analyses. Other cell types which have been excluded from the counts are the necrotic cells and the inflammatory cells which may be found in the necrotic areas of cirrhotic and neoplastic livers. The number of these cells was judged to be negligible in cirrhotic liver but appreciable in some tumors. Necrotic and inflammatory cells are probably included in varying numbers in the samples of neoplastic livers taken for biochemical assays, since it is impossible to dissect liver tumors so as to exclude all the necrotic regions.

In addition to the important variations taking place in the cell population of the liver during carcinogenesis, changes are also observed in the amount of fibrous elements as well as in the nature and the relative volume of extracellular fluids.
homogenates are generally hazardous and can be for investigating the chemical changes taking place of the cells, due to changes in concentration of extracellular fluid. Thus, methods other than analyses of tissue homogenates or a combination of biochemical and other methods must be used for investigating the chemical changes taking place in the structural units of tissues in the course of carcinogenesis or other pathological processes (6, 8–10, 21).

SUMMARY
The numerical proportions of cell types were determined by quantitative histological methods in liver sections of rats fed (a) a normal diet, (b) a low-protein, basal diet, or (c) a diet containing the carcinogen 4-dimethylaminoazobenzene (DAB).

1. The liver of young adult (200 gm.) normal rats contained 61 per cent parenchymal cells, 30 per cent littoral cells, and 9 per cent of other cell types. The relative number of littoral cells was observed to increase with age.

2. In rats fed the low-protein, basal diet, the losses of body weights and liver weights were accompanied by slight increases in the proportion of parenchymal cells and decreases in the percentage of littoral cells.

3. The cytological composition of the liver showed striking changes in rats fed DAB, due mainly to an extensive proliferation of the bile ducts and connective tissue elements. In cirrhotic liver, after 90 days of DAB feeding, the parenchymal cells accounted for only 24 per cent of the total number of cells and the littoral cells for 15 per cent, while the other cell types were present in a proportion of 61 per cent. Liver tumors (hepatomas and cholangiomas) which arose between 90 and 180 days of DAB feeding showed cellular distributions different from those of both the normal and the cirrhotic livers.

The results were discussed in relation to the interpretation of the biochemical data derived from assays on homogenates of normal, cirrhotic, and neoplastic livers.

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