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Histochemical Differences Between So-Called Megalocytosis and Neoplastic or Preneoplastic Liver Lesions Induced by *N*-Nitrosomorpholine*

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Abstract—*Differences in histochemical pattern were found between the so-called megalocytes and preneoplastic and neoplastic hepatocytes after short-term administration of sublethal dose of* N*-nitrosomorpholine in rats. Megalocytes were poor in glycogen and intensely pyroninophilic. The activity of alkaline and acid nucleases was normal or increased. On the other hand the clear and acidophilic hepatocytes considered to be preneoplastic showed excessive storage of glycogen and had usually decreased or absent activity of nucleases. Basophilic cells of neoplastic nodules and hepatocellular carcinomas were practically free from glycogen and nuclease activity. The majority of megalocytes which disturbed seriously the lobular architecture of liver during the NNM intoxication, disappeared soon after the withdrawal of carcinogen. The megalocytes are interpreted as the result of an interaction between toxic and regenerative events.*

INTRODUCTION

POLYPLOID hepatocytes with considerably enlarged nuclear and cytoplasmic volume have been described as megalocytes in different pathologic conditions. During mitosis many of the so-called megalocytes show an irregular distribution of the chromosomes. Megalocytosis was observed in the rat liver in senility [1], after partial hepatectomy of X-irradiated animals [2, 3] or after the administration of a great variety of toxic agents, including potent hepatocarcinogens, such as 3′-methyl-4-dimethylaminoazobenzene [4–6], 2-amino-2-azotoluene [7], aflatoxin B_1[8] or *N*-nitrosomorpholine [9–11].

Most of the above mentioned authors considered megalocytosis to be a reversible alteration. Some of them suggested that the megalocytosis which appears under the influence of liver poisons is due to an interaction of degenerative and regenerative effects. The death of cells induced by the poison calls for regeneration of other cells which during their mitosis are also injured by the poison [11, 12]. A disturbance of mitosis was also made responsible for the development of megalocytes after partial hepatectomy of X-irradiated rat livers [2, 3].

A pronounced increase in free and membrane bound ribosomes involving a strong cytoplasmic basophilia seems to be a cytoplasmic expression of the marked proliferative activity of megalocytes in liver parenchyma [11].

A clear-cut morphological distinction between basophilic megalocytes and basophilic cells which appear during late stages of hepatocarcinogenesis may be difficult or even impossible. Therefore, it seemed interesting to learn whether histochemical differences exist between the so-called megalocytes and the preneoplastic or neoplastic cells. The most convenient experimental model for such an investigation appeared to be rat liver carcinogenesis induced by short-term intoxication with a sublethal dose of *N*-nitrosomorpholine (NNM).

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MATERIALS AND METHODS

Male Sprague–Dawley rats weighing about 200 g at the beginning of the experiment were maintained in pathogene-free conditions and given a commercial diet (Altromin R pellets) and water. Over a period of 3 weeks the experimental animals received a maximum of 20 ml per day of a 50% mg aqueous solution of *N*-nitrosomorpholine* as drinking water. After that period they received pure water like control animals. Four to five experimental and three control rats were sacrificed by decapitation (1) immediately after the end of administration of the carcinogen; (2) 4 weeks; (3) 20 and (4) 50 weeks after the withdrawal of the carcinogen. Liver specimens were immediately frozen in the cryostat at −30°C and adjacent tissue fragments were fixed in Carnoy's solution for embedding in paraffin. The activity of acid DNAse and RNAse was detected histochemically by the lead nitrate method described by Vorbrodt [13]; for the alkaline nuclease the method described elsewhere was used [14]. Cryostat and paraffin sections were stained with hematoxylin–eosin, methylgreen–pyronin for nucleic acids, and the PAS reaction for glycogen.

RESULTS

Three weeks of continuous administration of NNM

Macroscopically, the liver of the experimental animals appeared shrunken and had a somewhat wrinkled surface. Microscopically the lobular architecture of the liver parenchyma was found to be greatly altered by hepatocellular necroses, by pronounced loss of parenchyma and by very intense reactive proliferation of bile ducts or mesenchymal cells. The bulk of the remaining hepatocytes revealed severe changes: the cytoplasm of these cells was enlarged and irregular in shape (Figs. 1–3). It was free of, or poor in, glycogen as demonstrated histochemically by the PAS-reaction (Fig. 4). Instead of the typical basophilic (or pyroninophilic) bodies a marked diffuse basophilia or pyroninophilia was found in most of the enlarged hepatocytes (Fig. 5). In addition megalocytic hepatocytes with a decrease or total loss of the basophilic material could be seen. The nuclei were enormous. Frequently, several nuclei were observed in one cell. As a rule, there were also multiple nucleoli. Sometimes up to 8 nucleoli were encountered in one plane of the section. Mitoses were frequent and often atypical. Many nuclei contained clear or acidophilic cytoplasmic inclusions. The condensed chromatin of the monstrous nuclei was usually distributed in a fine granular manner (Fig. 3 and inset).

Only a few hepatocytes appeared morphologically normal. They were usually situated in peripheral regions of the lobules. Their size was about normal and they contained glycogen as well as basophilic bodies. In some cells, however, the glycogen content was increased. The cytoplasma of these cells appeared clear in H.E. sections.

Alkaline DNAse and RNAse were active in the whole liver parenchyma. The activity of these enzymes was particularly intense on the surface of the enlarged hepatocytes or in the intercellular spaces corresponding to biliary canaliculi. A moderate activity was also found in the nuclei of the hepatocytes (Fig. 6 and inset). The nuclei of the proliferated ductular or mesenchymal cells showed a relatively strong activity of both enzymes.

Acid DNAse appeared to be more active in the whole liver parenchyma of the experimental animals than in the untreated rats. The histochemical reaction was intensely positive in all nuclei, including the monstrous ones, and in some cytoplasmic granules (lysosomes) of the enlarged hepatocytes (Fig. 7). Acid RNAse appeared to be somewhat less active than acid DNAse. No cells lacking in the activity of acid nucleases were seen.

Three weeks after the withdrawal of NNM administration

In almost all livers of the experimental animals little whitish spots could be seen macroscopically. Microscopically an almost normal lobular architecture of liver parenchyma reappeared (Fig. 2). However, many foci and rare neoplastic nodules containing altered liver cells (clear, eosinophilic or basophilic) were found. There was only a slight periportal fibrosis and bile duct proliferation. The so-called megalocytes were rare. As a rule, they were localized in the vicinity of periportal spaces. The cytoplasm of these enlarged hepatocytes no longer showed a diffuse basophilia, but was characterized by coarse basophilic bodies.

Glycogen was found in most hepatocytes and in many foci it was even stored in excess.

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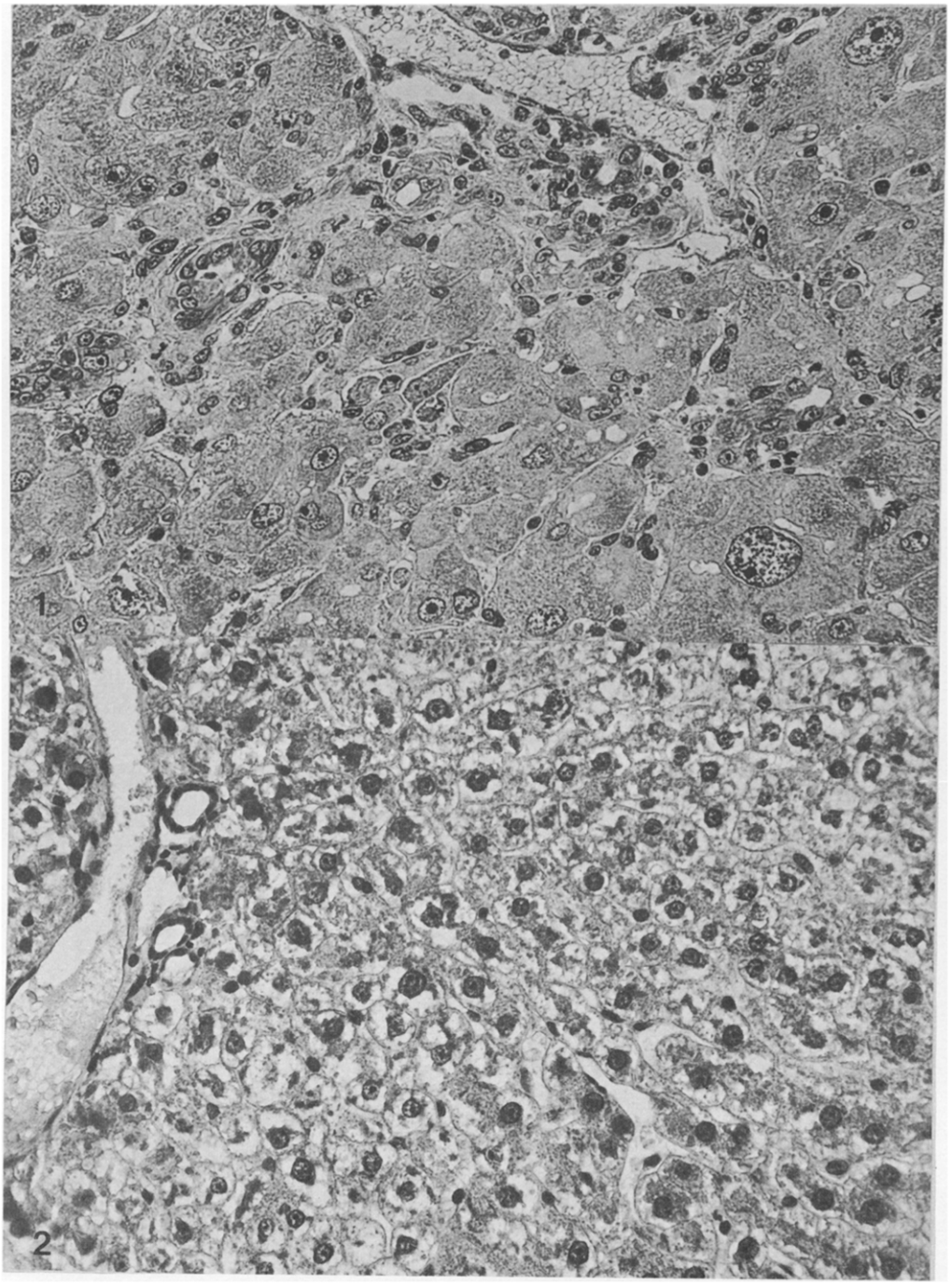


Fig. 1. *Almost completely disturbed liver architecture in a rat directly after the administration of sublethal dose of NNM. Megalocytes are very numerous. There are some necrotic cells and proliferates of bile ductules and of mesenchymal cells. H.E.* ×440.

Fig. 2. *Almost normal liver architecture in a rat* 3 *weeks after withdrawal of NNM. H.E.* ×440.

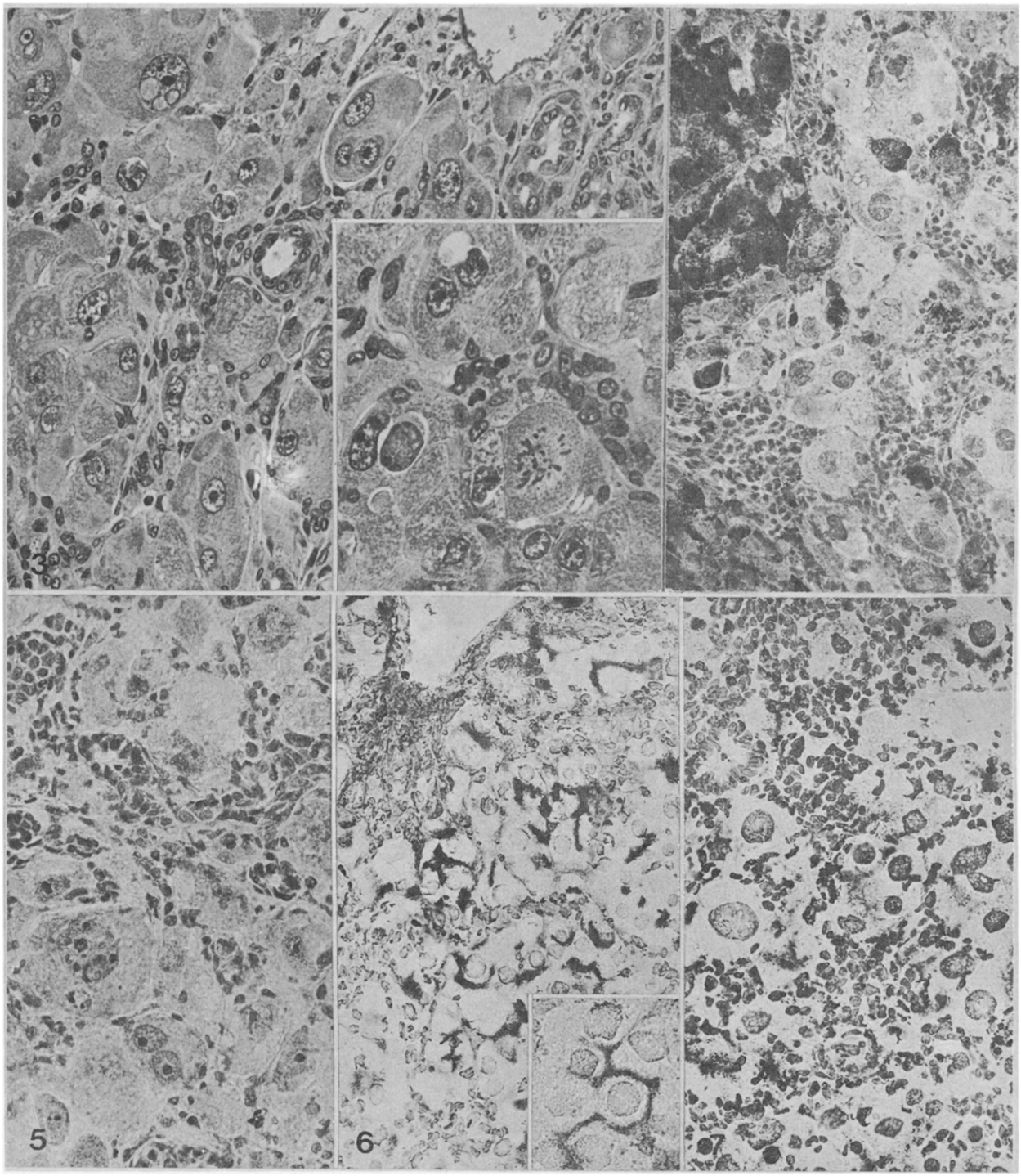


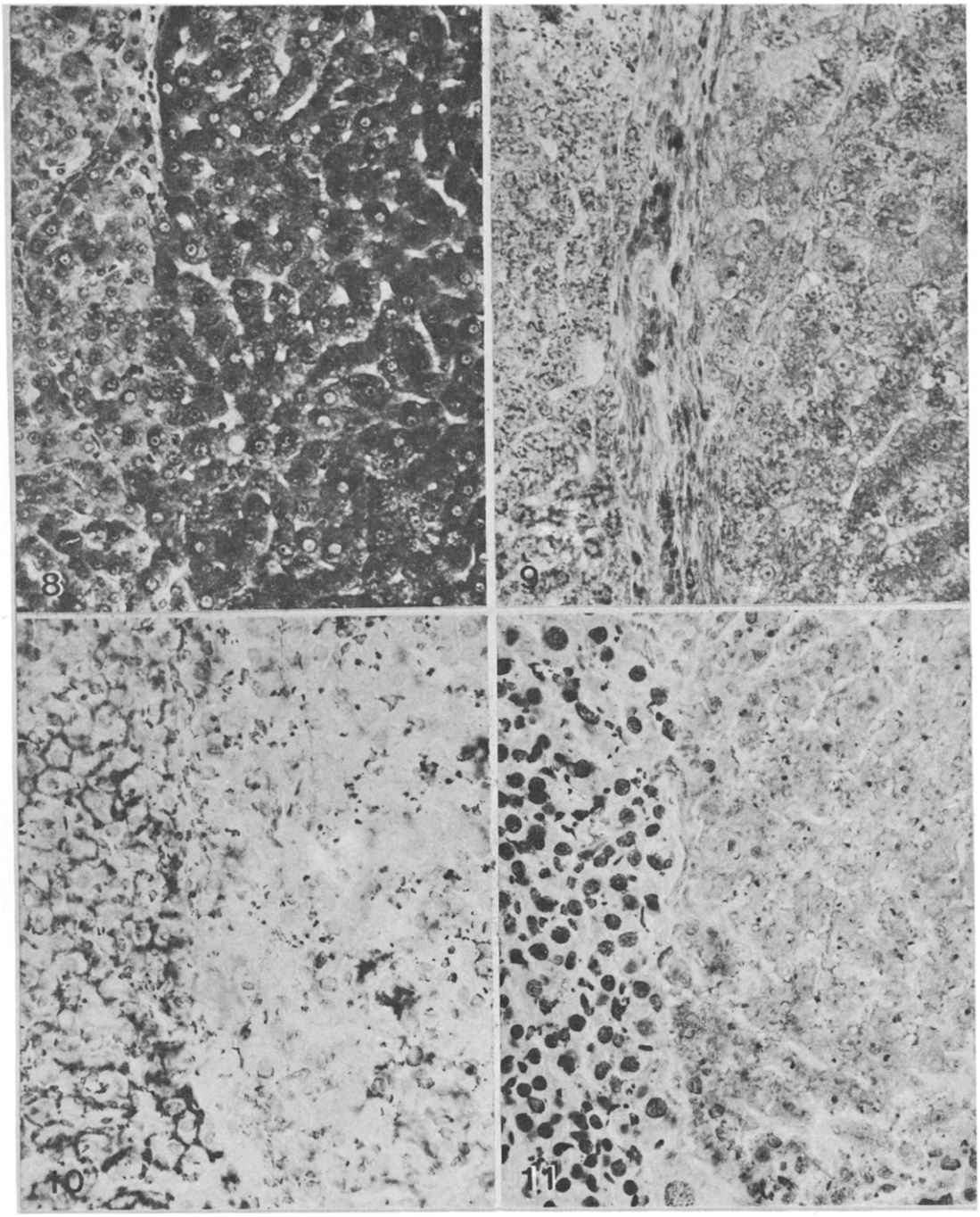
Fig. 3. Numerous megalocytes with monstrous nuclei containing multiple nucleoli and cytoplasmic inclusions. Some megalocytes are necrotic (unstained nuclei). Intense proliferation of biliary ducts and mesenchymal elements. H.E. × 250.
Inset: Atypical mitosis in a megalocyte. H.E. × 400.

Fig. 4. Most of the megalocyte are deprived of glycogen. Some smaller hepatocytes contain glycogen (upper left area). PAS × 180.

Fig. 5. Diffuse cytoplasmic pyroninophilia and very pyroninophilic nucleoli in the megalocytes. Intensely stained nuclei of proliferating biliary ducts and mesenchymal elements. Methylgreen–pyronin × 280.

Fig. 6. Very intense activity of alkaline RNAse in the intercellular spaces between the megalocytes and moderate nuclear activity × 180.
Inset: Alkaline DNAse is intensely active in the intercellular spaces and moderately positive in the nuclei of the megalocytes. × 280.

Fig. 7. Intense activity of acid DNAse in all nuclei (also in the monstrous ones). Some granular cytoplasmic activity in the megalocytes. × 180.



Figs. 8–11. *Neoplastic nodules in rat liver* 20 *weeks after withdrawal of NNM.*

Fig. 8. *Almost all hepatocytes in the neoplastic nodule (to the right) are excessively storing glycogen. Surrounding liver parenchyma (to the left) contain glycogen as in normal liver. PAS* × 280.

Fig. 9. *Irregular methylgreen–pyronin staining in the neoplastic nodule (to the right). Some cells are clear, others with diffuse cytoplasmic pyroninophilia. In the surrounding liver parenchyma (to the left) the pyroninophilia is granular. Methylgreen–pyronin* × 280.

Fig. 10. *Decrease of alkaline RNAse activity in the neoplastic nodule (to the right) with some completely negative cells.* × 280.

Fig. 11. *Distinct decrease of acid DNAse activity in the neoplastic nodule (to the right) with areas containing completely negative cells.* × 280.

These foci consisted of large clear or acidophilic cells. Numerous glycogen storage cells were also found in some of the neoplastic nodules (Fig. 8). In addition in some foci and in all neoplastic nodules different amounts of diffusely basophilic or pyrominophilic cells poor in glycogen were observed (Fig. 9).

The activity of alkaline DNAse and RNAse was decreased in almost all cells of the preneoplastic foci and nodules. In some cells enzyme activity was lacking (Fig. 10). The activity of acid DNAse was reduced to a greater extent than that of acid RNAse. Numerous cells showed a completely negative enzyme reaction (Fig. 11). The rare megalocytes which were localized outside the focal or nodular lesions preserved an intense nuclease activity.

Twenty weeks after the withdrawal of NNM administration

At this stage generally the histological and histochemical pattern in all livers of experimental animals was similar to that described in previous stage with the exception that more frequent neoplastic nodules have been observed.

Fifty weeks after the withdrawal of NNM administration

In three of the 4 animals included in this experimental group hepato-cellular carcinomas have been found. These tumors had the histological and histochemical pattern similar to that described previously [15]. Distinct basophilia, complete disappearance of glycogen and almost complete absence of alkaline and acid nuclease activity have been found in the cells of these hepato-cellular carcinomas.

In the livers of all control animals there was observed normal histological pattern, uniform and moderate distribution of glycogen and pyroninophilia as well as uniformly high activity of nucleases in all hepatocytes.

DISCUSSION

From the above described histochemical findings it appears obvious that essential differences exist between megalocytes and preneoplastic or neoplastic cells. The megalocytes which are very numerous at the phase following directly the administration of hepatocarcinogen have a cytoplasm poor in glycogen and rich in pryroninophilic material which according to previous observations [11] is due to an increase in ribosomes. The activity of all types of nucleases investigated histochemically is normal or even increased. An increased activity of acid DNAse in rat liver one and three days after administration of diethylnitrosamine has also been detected biochemically [16].

The clear and acidophilic cells which are included in foci and nodules and which are considered to be preneoplastic [17] may reach a similar size as the so-called megalocytes. However, in contrast to megalocytes, these cells are usually characterized by an excessive storage of glycogen. In addition, most of these cells show a decreased or negative activity of nucleases. Another important difference between the megalocytes and the putative preneoplastic hepatocytes is that the majority of megalocytes disappears soon after withdrawal of the carcinogen [5, 10, 11, 18] whereas the clear and acidophilic cells persist for weeks and months [17].

The basophilic cells of neoplastic nodules or hepatocellular carcinomas are poor in or free of glycogen. In this respect the neoplastic cells are very similar to megalocytes. However, unlike megalocytes most neoplastic cells exhibit a considerable reduction or even a total absence of the activity of nucleases [15, 19]. As pointed out elsewhere, the decrease in the activity of many enzymes in preneoplastic foci and neoplastic nodules could be a sign of a fundamental metabolic change taking place during the final step of the malignant transformation [15].

Finally, it should be emphasized that the development and significance of enlarged liver cells may be very different. Such alterations as disturbed regeneration, preneoplasia and neoplasia are only some of the pathologic conditions under which a considerable cellular enlargement may occur.

There is no doubt that several other pathologic alterations such as diverse storage diseases, may also produce an enlargement of liver cells. Therefore, the term "megalocyte" should always be used with some reserve. Histochemistry seems to be a valuable tool for discriminating among different types of enlarged hepatocytes.

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