SUMMARY

The activity of alkaline and acid DNase and RNase was histochemically investigated in the rat liver during different stages of \(N\)-nitrosomorpholine-induced carcinogenesis.

The activity of acid and alkaline nucleases was considerably decreased in focal areas and later in the hyperplastic nodules, whereas the surrounding liver parenchyma demonstrated normal activity of these enzymes. Nuclease deficiency appeared at the 38th to 59th day of carcinogenesis. It preceded by approximately 56 to 75 days the morphological signs of cancer. In malignant hepatocellular tumors, nuclease activity was practically negative. In the necrobiotic cells of those malignant tumors, a reappearance of nuclease activity was observed, indicating that the deficient nuclease activity in cancer is not equivalent with the real diminution or disappearance of those enzymes but depends probably upon their binding with inhibitors.

It has been suggested that nucleases might be involved in the protection of genetic stability of normal cells against transforming nucleic acids.

INTRODUCTION

Deficient nuclease activity in malignant tumors has been found by histochemical (9, 23–25) and biochemical methods (3, 5, 7), as well as by an immunochemical technique (16). In relation to these findings, it was important to gather information about the variations of nuclease activity in different stages of carcinogenesis. Thus the histochemical investigations of Amano and Daoust (2) and Daoust and Cantero (10) based on a substrate film method demonstrated a decrease in (and even the complete disappearance of) the activity of nucleases in the liver parenchyma of rats during experimental carcinogenesis induced by 4-dimethylaminoazobenzene. The results obtained by another histochemical procedure based on Gomori's lead nitrate method (1, 19) and by biochemical methods (5, 11, 12) confirmed this deficiency of nucleases in carcinogenesis.

Reports on the activity of nucleases during liver carcinogenesis are scanty and concern only one or two types of these enzymes. Moreover, all of those observations are based on the same experimental model of liver carcinogenesis induced by 4-dimethylaminoazobenzene, a compound which, in addition, produced necrosis and cirrhosis of the liver parenchyma. These complications make difficult interpretations of the activity of nucleases in liver carcinogenesis.

Our report deals with the histochemical study of the activity of 4 basic types of nucleases (alkaline and acid DNase and RNase) investigated in the liver parenchyma of rats during different stages of \(N\)-nitrosomorpholine-induced carcinogenesis. This model of liver carcinogenesis excludes major necrosis and cirrhosis (6). The activity of nucleases was not, until now, investigated in this carcinogenesis. Additionally, we have attempted to determine whether the variations in the activity of nucleases precede or follow the malignant transformation.

MATERIALS AND METHODS

Fifty-eight young, male albino rats (Wistar R strain) weighing 100 to 120 g at the beginning of the experiment were fed a normal, semisynthetic diet, Protector, and water \textit{ad libitum}. Each animal received \(N\)-nitrosomorpholine, 8 mg/kg, in water solution by intragastric intubation 5 times weekly. Two experimental animals and 1 control, which did not receive the carcinogen, were sacrificed each week.

Acid nucleases were detected by the histochemical method of Aronson et al. (4) as modified by Vorbrodt (24). Alkaline nucleases were revealed by a personal procedure (20) based on Gomori's lead nitrate method modified for the detection of phosphatases at physiological pH by Wachstein and Meisel (26).

The sites of enzymatic activity were revealed by deposits of black lead sulfide. Control sections were treated by the same procedure but without substrates in the incubation media. They were all negative. The liver and other organs were also examined in each case by the regular histological technique (formalin fixation, paraffin embedding, and hematoxylin and eosin staining.)

The following reagents were used for the histochemical procedure: DNA sodium salt from calf thymus (The British Drug Houses, Ltd., Poole, England); RNA sodium salt from yeast (The British Drug Houses, Ltd.); acid phosphatase from wheat germ, type I (Sigma Chemical Co., St. Louis, Mo.); and alkaline phosphatase (ex-calf intestinal mucosa) salt (Koch-Light Laboratories Ltd., Colnbrook, Bucks, England).

RESULTS

Until the 5th week of carcinogenesis, the morphological and enzymatic pattern of the liver parenchyma did not reveal any visible alteration.
In animals sacrificed after 38 days of N-nitrosomorpholine introduction, the liver had a normal weight and macroscopic appearance. Histological preparations revealed only a diffuse cellular polymorphism. The nuclei of certain liver cells were considerably enlarged. Some groups of liver cells contained clear cytoplasmic vacuoles. In histochemical sections, the activity of acid nucleases was almost similar to that of normal liver cell. Both nuclei and cytoplasmic granules were intensely stained. The cytoplasmic granules (probably of lysosomal origin) were localized mainly on the periphery of the liver cells. However, in some liver cells the cytoplasmic granules disappeared, and the intensity of acid DNase activity in the nuclei was not uniform (Fig. 1). The alkaline nuclease had the same localization and intensity as in normal liver.

After 59 days of carcinogenesis in the histological preparations, large and indistinctly limited areas of liver parenchyma cells with clear cytoplasmic vacuoles could be noted. These vacuoles were more pronounced than in the previous stage. Acid nuclease activity was considerably decreased in some groups of liver cells, whereas the alkaline nuclease revealed only slightly reduced activity.

On the 78th and 85th days of carcinogenesis, small (approximately 1 mm in diameter), whitish nodules could be detected in the liver by macroscopic examination. Microscopically, these indistinctly limited nodules demonstrated a trabecular structure without any morphological signs of cancer. Histochemically, those nodules revealed distinctly reduced activity of both acid and alkaline nucleases.

After 93 days, the nodules became larger and distinctly limited. Their trabecular structure was lost. This histological pattern could be interpreted as a stage of adenomatous proliferation. These nodular structures demonstrated the same decreased nuclease activity as in previous stages.

Histological preparations on the 125th day of carcinogenesis revealed further enlargement of the hyperplastic nodules without cellular monstrosity or atypical mitoses. Histochemically, activity of acid nucleases was practically negative in the hyperplastic nodules, whereas in the surrounding liver parenchyma it was distinctly positive (Fig. 2). The inset of Fig. 2 demonstrates in high-power magnification the negative, nonnecrotic cells inside a hyperplastic nodule. These cells contrast with the intensely positive cells of the surrounding liver parenchyma. Alkaline DNase and RNase activity was also practically negative in the hyperplastic nodules, whereas the surrounding liver parenchyma continued to reveal a histochemical pattern similar to that of normal liver. However, inside the nodules some small fusiform or stellate cells, probably of endothelial or macrophagic origin, demonstrated an intensely positive reaction of alkaline nucleases (Fig. 3).

In the sections of the liver examined on the 134th day of carcinogenesis and in later stages, morphological signs of malignant transformation in the hyperplastic nodules were observed (invasiveness, cellular monstrosity, hyperchromatic nuclei, and numerous atypical mitoses). These malignant tumors (hepatocellular carcinomas) did not reveal any activity of acid or alkaline nucleases. However, intense nuclease activity was observed in the peripheral zone of necrotic foci. This zone demonstrated morphological signs of necrobiosis (karyorrhexis, karyolysis, and pyknosis). This enzymatic reactivation in the necrobiotic cells concerned both acid and alkaline nucleases (Figs. 4 to 6). Deficient nuclease activity in the premalignant and malignant nodules observed during N-nitrosomorpholine carcinogenesis concerned all 4 types of nucleases. Nevertheless, certain quantitative and topographic differences were observed between some types of nucleases. Moreover, these enzymatic variations appeared at different periods.

**DISCUSSION**

The activity of acid DNase and RNase decreased in some areas of the liver parenchyma at about the 38th and 59th days of N-nitrosomorpholine carcinogenesis and disappeared completely in the hyperplastic nodules and in malignant tumors at later stages.

The activity of alkaline nucleases decreased somewhat later and was also almost negative in the hyperplastic and malignant nodules.

This deficient nuclease activity was found approximately 56 to 75 days before the appearance of the morphological signs of malignant transformation. It was detected even before the formation of benign, hyperplastic nodules. Thus, this enzymatic deficiency preceded the malignant transformation and should therefore not be interpreted as a secondary phenomenon of cancer.

It could be also suggested that nuclease deficiency is not produced by a direct action of the carcinogen on the liver cells, since it appeared after a latent period of approximately 38 to 59 days and it did not concern the whole liver parenchyma.

Deficient nuclease activity during liver carcinogenesis is probably due to an inhibition and not to a real decrease of those enzymes. This interpretation could be based on the reappearance of nuclease activity in the necrobiotic cells of the malignant nodules. This suggestion is further supported by the fact that the inhibitors of nucleases were increased in tumors (3, 7, 18). This intense nuclease activity histochemically detected in the necrobiotic tumor cells and in the mesenchymal elements of the stroma could explain the positive reaction of nucleases biochemically detected in the homogenates of malignant tumors by some authors (8, 15, 17), as well as that seen in the late stages of 4-dimethylaminoazobenzene carcinogenesis by Deckers-Passau et al. (11).

The results of this experiment concerning the decreased nuclease activity that precedes malignant transformation during N-nitrosomorpholine carcinogenesis in the liver agree with the hypothesis discussed in previous reports (13, 14, 21, 22).

According to this hypothesis, primarily low or secondarily reduced nuclease activity might facilitate the incorporation or production of abnormal nucleic acids that are able to induce malignant transformation. Those findings, as well as the opinion of other authors (9, 27), suggest that nucleases constitute some kind of mechanism protecting the genetic stability of the cell against the transforming nucleic acids.
REFERENCES


Histochemical Activity of Alkaline and Acid Nucleases in the Rat Liver Parenchyma during $N$-Nitrosomorpholine Carcinogenesis

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