Virchows Arch [Pathol Anat] (1981) 390:339–345

Virchows Archiv A

Morphology of Foci of Altered Hepatocytes and Naturally-Occurring Hepatocellular Tumors in F344 Rats

J.M. Ward

Tumor Pathology Branch, Carcinogenesis Testing Program, National Cancer Institute, National Toxicology Program, National Institutes of Health, Bethesda, Maryland 20205, USA

Summary. The morphology of liver tumors of F344 rats used as controls in carcinogenesis bioassays were studied. Foci of cellular alteration composed of hepatocytes with basophilic cytoplasm were found commonly in F344 rats, 2 years of age. Eosinophilic and vacuolated foci were considerably less common. The morphology of 67 nodular hepatic lesions indicated that 54 were neoplastic nodules and 13 hepatocellular carcinomas. The majority of these tumors were composed of basophilic hepatocytes. Some of the carcinomas appeared to arise within neoplastic nodules. No tumors metastasized.

Key words: Liver neoplasms – Hepatocellular carcinoma – F344 rat.

Introduction

Naturally occurring liver tumors in most strains of rats are unusual occurrences. A few reports note high incidences of these tumors in select rat colonies (Adler et al. 1976). In contrast, liver tumors are among the most common natural tumors of mice. Genetics, diet and environmental factors may affect the incidence of mouse liver tumors. The causes of naturally occurring liver tumors in rats are unknown, although it has been suggested that dietary carcinogens in rat strains with a high incidence of these tumors may play a role (Pollard and Luckert 1979).

Many chemicals have been shown to induce hepatic neoplasms in rats. In general, hepatic foci of cellular alteration progress to neoplastic nodules which in turn may evolve into hepatocellular carcinomas (Bannasch 1978; Hirota and Williams 1979; Pitot 1977; Squire and Levitt 1975). Liver tumors occur in 1–3% of control F344 rats (Goodman et al. 1979). However, foci of cellular alteration have been seen in 20–90% of control F344 rats, 2 years of age. Because of the common occurrence of these foci, proliferative hepatocellular lesions of F344 rats were studied.

Offprint requests to: Dr. J. Ward, Del Ray 402, NIH, Bethesda, MD 20205, USA

0340-1227/81/0390/0339/$01.40

Material and Methods

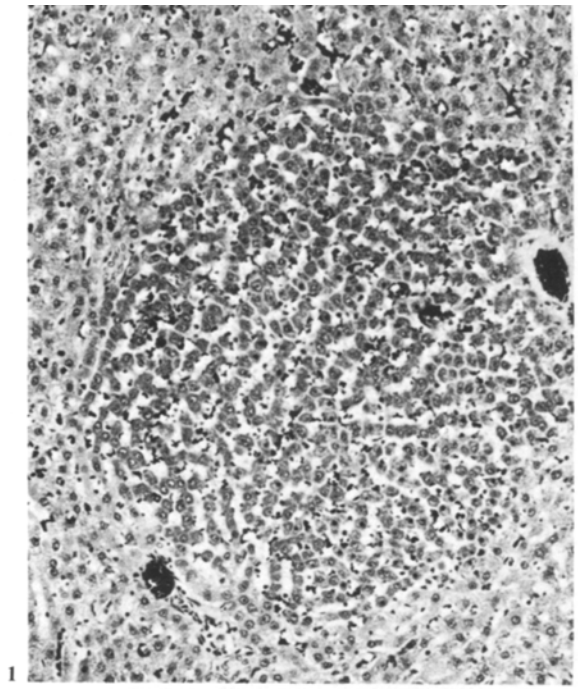
Liver lesions were reviewed from male and female F344 rats used as untreated controls in carcinogenesis tests (Goodman et al. 1979). Each test involved a chemical given in one of two doses to groups of 50 rats. Control groups varied in size from 20–50 rats. Liver tumors found in 39 carcinogenesis bioassays involving 2700 control male and female rats were reviewed. F344 rats were obtained from commercial suppliers (Charles River Breeding Labs., A.R. Schmidt or Frederick Cancer Research Center) generally at four weeks of age. At six weeks of age, they were put on test. Rats used as controls were generally placed 3–5 per polycarbonate cage and fed Wayne meal (Allied Mills, Chicago, IL) and water ad libidum. After 104–110 weeks on test, surviving rats were sacrificed. A complete necropsy was performed on each rat found dead or sacrificed. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin. Selected lesions were stained by the following methods: periodic acid-Schiff, toluidine blue and methyl green-pyronine.

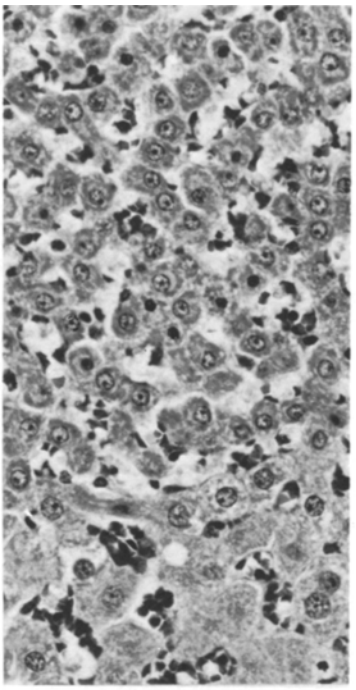
Liver lesions were classified according to a classification of rat liver lesions suggested by a workshop (Squire and Levitt 1975), a review (Inst. Lab. An. Resources 1980) and additional morphologic features found in this study. Other neoplastic and nonneoplastic lesions in these F344 rats have been described (Goodman et al. 1979).

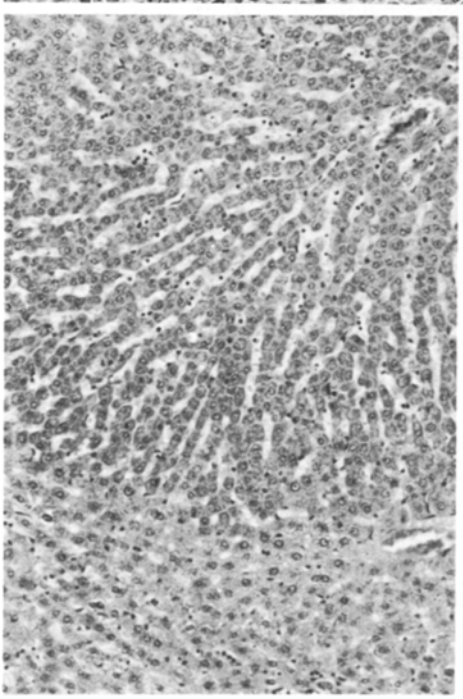
Results

Foci of cellular alteration were very common in F344 rats, two years of age. They were usually multiple in the liver and were found in 10–90% of the rats. The incidence may have been higher if additional hepatic sections were reviewed. Female rats had incidences approaching 100%. Histologically, the foci were found in various portions of the hepatic lobule, most frequently midzonal. The majority of the foci were composed of hepatocytes smaller than normal and with basophilic cytoplasm and a uniformly round nucleus and single prominent nucleolus (Figs. 1, 2). Few mitotic figures were seen. Sinusoids were lined by sinusoidal-lining cells and were inconspicuous in most cases, but more prominent if the liver was congested. The cytoplasm of the basophilic hepatocytes was dark blue after staining with toluidine blue and red after staining with methyl green-pyronine. Reticulin stains revealed less fibers within these foci than in normal liver. Fewer foci of cellular alteration were seen which were composed of eosinophilic or vacuolated hepatocytes.

The foci appeared to enlarge and form nodular lesions. The nodular lesions (neoplastic nodules and hepatocellular carcinomas) were usually found at the termination of the bioassay (104–110 weeks). A few were found from 92–104 weeks. In the 1350 male and 1350 female rats reviewed in this paper, there were 35 tumors in 32 males (2.3%) and 32 tumors in 28 females (2.1%). The lesions were classified as in Table 1. The morphologic types were similar in males and females. Neoplastic nodules have been referred to as hyperplastic nodules, adenomas and other terms. The smallest nodules compressed adjacent parenchyma and were composed of hepatocytes smaller than normal hepatocytes and with basophilic cytoplasm and round nuclei with prominent nucleoli (Figs. 2, 3). These cells were identical to those in basophilic foci and formed single cell plates only. Eosinophilic nodules were composed primarily of hepatocytes larger than normal hepatocytes and with pale eosinophilic cytoplasm (Fig. 4). Basophilic and clear or vacuolated hepatocytes were also present. The majority of these eosinophilic nodules occurred in livers from F344 rats with mononuclear cell leukemia.







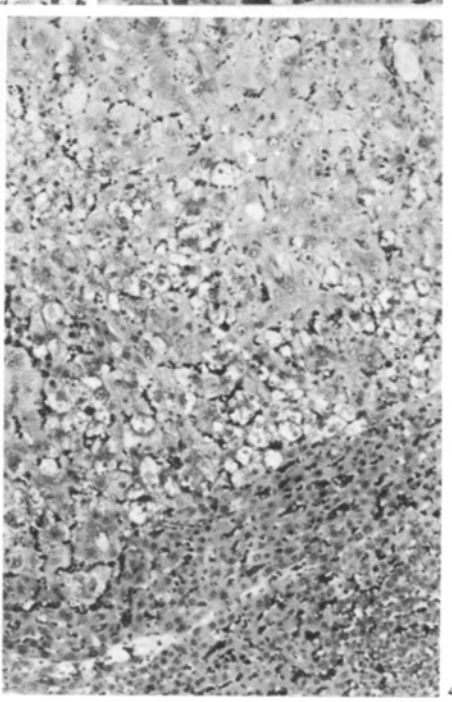


Fig. 1. Basophilic focus of altered hepatocytes. Note small size of cells. H and E, ×130

Fig. 2. Edge of basophilic focus with early compression of normal hepatocytes. Note small cell size and prominent nucleoli. H and E, ×330

Fig. 3. Neoplastic nodule composed of basophilic hepatocytes, smaller than normal, forming single cell cords. H and E, ×130

Fig. 4. Neoplastic nodule composed predominantly of large eosinophilic hepatocytes in leukemic liver. Some basophilic and clear cells are also seen in the nodule. H and E, ×80

Table 1. Histologic typing of sixty seven hepatocellular neoplasms of F344 rats

Tumor type	No. of neoplasms
Neoplastic nodule	
Solid	
Basophilic	22
Eosinophilic	8
Vacuolated	8
Mixed	2
Angiectatic	
Basophilic	11
Eosinophilic	3
Hepatocellular carcinoma	
Within neoplastic nodule	7
Trabecular	6

Other neoplastic nodules contained predominantly hepatocytes with vacuolated cytoplasm or vacuolated and basophilic hepatocytes. Some neoplastic nodules were composed of basophilic or eosinophilic hepatocytes which lined dilated sinusoids (angiectatic or peliosis) (Fig. 5).

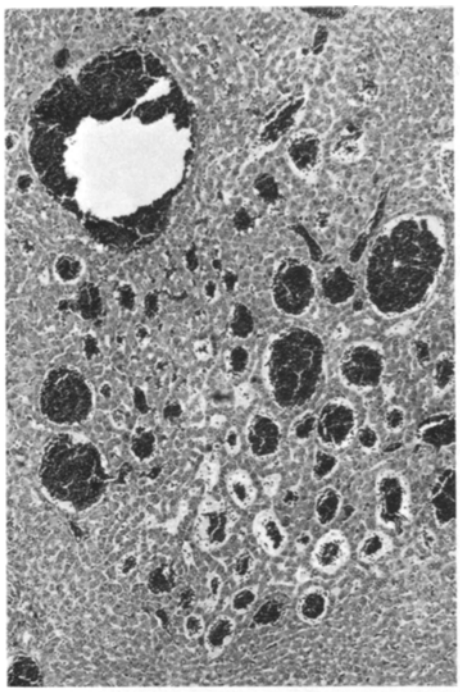
The largest neoplastic nodules frequently had foci or large areas of less differentiated hepatocytes or prominent trabecular formations (Figs. 6, 7). The hepatocytes were usually larger than normal hepatocytes and had large vesicular nuclei with prominent nucleoli. Trabecular areas were composed of large hepatocytes with basophilic cytoplasm, vesicular nuclei with prominent nucleoli and disorganized plates of hepatocytes lined by sinusoidal-lining cells.

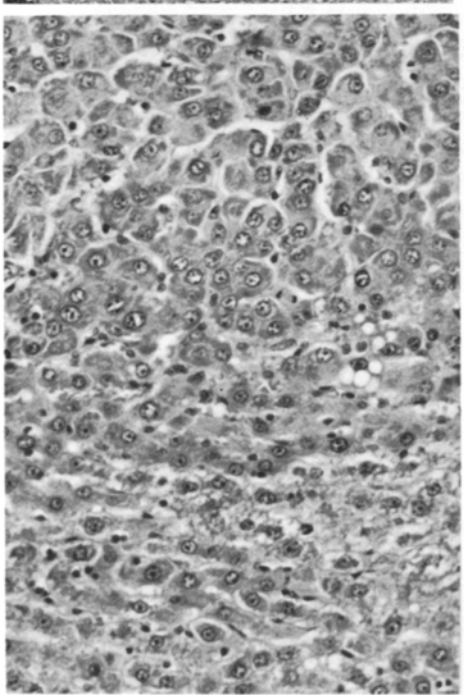
The largest liver nodules were trabecular carcinomas composed of masses of basophilic hepatocytes forming prominent trabeculae and with numerous mitotic figures. The tumor cells invaded adjacent parenchyma and blood vessels (Fig. 8). No distant metastases were found.

An unusual hepatic nodular lesion was occasionally seen in aging F344 rats. These lesions are probably very common but not well documented. The gross nodule is associated with a herniation of a small piece of liver through the diaphragm at the diaphragmatic hiatus. The nodule which results is merely a herniation of liver. Histologically, the hepatocytes in the nodule are in various stages of regeneration and hyperactivity. Nucleoli are frequently large and appear as inclusions. These lesions do not appear to be neoplastic or preneoplastic. Cholangiocellular neoplasms and hemangiosarcomas were not seen in this study and are rare in F344 rats.

Discussion

This morphologic study of liver lesions in aging F344 rats suggests that some basophilic foci of hepatic cellular alteration progress to form neoplastic nodules some of which in turn become hepatocellular carcinomas. This sequence of





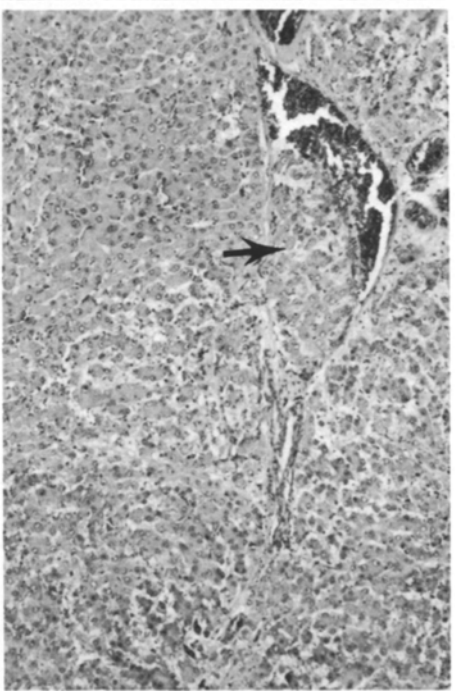


Fig. 5. Neoplastic nodule with prominent sinusoids. Hepatocytes in nodule are only slightly more basophilic than normal. H and E, × 55

Fig. 6. Neoplastic nodule composed of basophilic and vacuolated hepatocytes and an area of prominent trabecular formations (*arrow*). H and E, × 35

Fig. 7. Edge of hepatocellular carcinoma composed of hepatocytes forming irregular trabeculae. H and E, × 130

Fig. 8. Trabecular hepatocellular carcinoma with vascular invasion (*arrow*). H and E, × 80

development has been described for chemically-induced liver tumors in rats (Bannasch 1978; Hirota and Williams 1979; Institute of Laboratory Animals Resources 1980; Pitot 1977; Squire and Levitt 1975). The cause(s) of naturally occurring hepatocellular tumors of F344 rats is unknown. The high incidence of foci of cellular alteration and relatively low incidence of liver tumors might suggest that the foci were initiated by an unknown agent and the promoter, presumably dietary, occurred in low concentrations. Further work on exposure of F344 rats to promoters such as phenobarbitol may aid in our understanding of these lesions. Other hepatic lesions in aging F344 rats such as mild bile duct hyperplasia with fibrosis, were unrelated to these foci and tumors.

It has been proposed that specific hepatocarcinogens induce specific morphologic types of liver tumors in mice (Hoover et al. 1980; Reznik and Ward 1979; Ward et al. 1979). Similar findings have been observed for rats by the author and others. The induction of these unique tumors may depend in part on dose of carcinogen, duration of exposure and strain of rat. For example, nitrosamines induce predominantly clear cell foci and, in addition, a number of acidophilic cell foci which progress to hepatocellular carcinomas composed of basophilic hepatocytes (Bannasch 1978). Phenobarbitol and dioxins induce rat liver tumors composed of eosinophilic hepatocytes which develop from eosinophilic foci, according to the author's experience. The specific genotoxic or epigenetic mechanisms responsible for these different tumors may play a role in the type of tumors induced. Thus, carcinogens may directly or indirectly affect the morphology of tumor cells. The classification of naturally occurring rat liver tumors in different strains may allow us to classify induced tumors and attempt to compare the differences and similarities to the natural tumors. Subsequently, the significance of these differences or similarities will be evaluated. Further research of these induced tumors may aid us in understanding the carcinogenic process.

Acknowledgements. Supported in part by United States Public Health Service Contracts NO1-CP-43350 to Tracor-Jitco, Inc. and NO1-CP-43257 to EG&G Mason, Inc. The excellent technical assistance of Robert Nye, Jack Romine, Karen Brownell, and Dinah Lloyd is appreciated.

References

Adler JH, Roderig H, Ungar H (1976) Neoplastic liver nodules of unknown causes in a colony of Sand rats (*Psammomys obesus*). Israel J Med Science 12:1212–1215

Bannasch P (1978) Sequential cellular alterations during hepatocarcinogenesis. In: Butler W, Newberne PM (eds) Rat hepatic neoplasia. MIT Press, Cambridge p 58–93

Goodman DG, Ward JM, Squire RA, Chu KC, Linhart MS (1979) Neoplastic and nonneoplastic lesions in aging F344 rats. Toxicol Appl Pharmacol 48:237–248

Hirota N, Williams GM (1979) Persistence and growth of rat liver neoplastic nodules following cessation of carcinogen exposure. J Natl Cancer Inst 63:1257–1265

Hoover K, Ward JM, Stinson SF (1980) Histopathological differences between liver tumors in untreated mice and mice fed diets containing nitrofen. J Natl Cancer Inst, 65:937–948

Institute of Laboratory Animal Resources (1980) Histologic typing of liver tumors of the rat. J Natl Cancer Inst 64:177–206

Pitot HC (1977) The natural history of neoplasia. Am J Pathol 89:401–412

Pollard M, Luckert PH (1979) Spontaneous liver tumors in aged germ free Wistar rats. Lab An Science 29:74–77

Reznik G, Ward JM (1979) Carcinogenicity of the hair dye component 2-nitro-p-phenylenediamine (2-HPPN): induction of eosinophilic hepatocellular neoplasms in female B6C3F1 mice. Fd Cosmet Toxicol 17:493–500

Squire RA, Levitt MH (1975) Report of a workshop on classification of specific hepatocellular lesions in rats. Cancer Res 35:3214–3215

Ward JM (1980) Morphology of hepatocellular neoplasms in B6C3F1 mice. Cancer Lett 9:319–325

Ward JM, Bernal E, Buratto B, Goodman DG, Standberg JD, Schueler R (1979) Histopathology of neoplastic and nonneoplastic hepatic lesions in mice fed diets containing tetrachlorvinphos. J Natl Cancer Inst 63:111–118

Accepted October 17, 1980

Note Added in Proof.

Frozen liver sections from three 130-week-old F344 rats with basophilic foci in their livers, were stained for gamma-glutamyl transpeptidase activity (J Histochem Cytochem 17:517–526, 1969). No enzyme activity was found in the foci or normal hepatocytes, although bile duct cells stained red (had enzyme activity). Ultrastructural analysis of foci in portions of these three livers prepared for electron microscopy revealed that the cytoplasmic basophilia was due to aggregates of rough endoplasmic reticulum and polyribosomes and absence of smooth endoplasmic reticulum and glycogen.