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Sequential Morphogenesis of Liver Tumors in Mice Given Benzidine Dihydrochloride

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Abstract—*Benzidine dihydrochloride induced hepatic foci of cellular alteration, hepatocellular adenomas and hepatocellular carcinomas in mice. All three lesions occurred more frequently in the female, and all three lesions sometimes occurred in the same liver. The data suggest that the foci of cellular alteration would be precursors for the hepatocellular adenomas which are in turn precursors for the hepatocellular carcinomas.*

INTRODUCTION

A VARIETY of focal hepatic lesions are frequently seen in the liver of mice following the administration of carcinogens [1–4]. The relationship of these lesions to hepatocellular adenomas and hepatocellular carcinomas remains unsolved. The hypothesis that these early lesions are precursors for the hepatocellular carcinomas is known for the rat [5–10], but many investigators are reluctant to accept the same hypothesis for the mouse. This report will attempt to associate the development for hepatic foci of cellular alteration in the mouse with the development of benign and malignant liver tumors.

MATERIALS AND METHODS

The study included a total of 3456 F_1 (C57BL/6JfC3Hf/Nctr females × BALB/cStCrlfC3Hf/Nctr males) and F_2 (F_1 females × F_1 males) (Table 1). Weanling animals were housed, 4 per cage, in a room maintained at 22–24°C. The animals were fed Purina 5010C meal and dosed water *ad libitum* containing 0, 30, 60, 120, 200 and 400 parts per million (ppm) of benzidine dihydrochloride.‖ Groups of mice were killed at 40, 60, and 80 weeks.

Table 1. Experimental design

Dose level of benzidine (ppm)	Length of administration (weeks) 40	60	80	Total
	Number of Animals			
0	48	48	48	144
30	96	72	48	216
60	72	48	48	168
120	48	48	48	144
200	48	48	24	120
400	24	24	24	72
Total	336	288	240	864*

*Two crosses and two sexes = 2 × 2 × 864 = 3456 animals.

At death, each animal was given a carcass identification (CID) number. Detailed necropsies were performed and gross and microscopic findings were collected on approximately 45 tissues or organs as described by Frith *et al.* [11]. Sections of the median, left lateral and right lateral lobes of the liver were collected in addition to the other tissues. Additional sections were taken of grossly visible lesions not included with the above organs. The tissues were fixed in Bouin's solution for 18–24 hr. After fixation the tissues were routinely trimmed, placed in plastic cassettes for processing on an autotechnicon on a 4 hr cycle and embedded in paraffin blocks. Routine paraffin sections were cut at 5 μm and stained on an automatic stainer with

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‖Benzidine dihydrochloride was provide by Allied Chemical Co., Buffalo, N.Y., U.S.A.

hematoxylin and eosin (H & E) as described by Frith *et al.* [11].

RESULTS

Our results indicated a variety of histopathologic lesions including liver neoplasms which will be reported in detail in a separate paper. This report will concentrate on the relationship of hepatic foci of cellular alteration to the development of benign and malignant liver neoplasms. Animals were scheduled for sacrifice at 40, 60 and 80 weeks. The findings in an animal that died prior to its scheduled sacrifice were included in the scheduled sacrifice period closest to its death. Since no statistical differences were obvious between the two crosses, the F_1 and F_2 males and the F_1 and F_2 females have been combined. Animals that died prior to 30 weeks were excluded from analyses. These included 12 females and 22 males. None of these animals had any liver alterations.

Hepatic foci of cellular alteration were common histopathologic findings in this study and included acidophilic, basophilic and vacuolated cell foci of hepatocytes. The cells in the acidophilic foci were usually larger than adjacent normal hepatocytes. The cytoplasm of the cells was distinctly eosinophilic with a granular cytoplasm (Fig. 1). The basophilic cell foci consisted of cells usually smaller than adjacent normal hepatocytes with distinctly basophilic cytoplasm (Fig. 2). Vacuolated cell foci consisted of hepatocytes which contained distinct vacuoles of varying sizes. The nuclei of these vacuolated cells were either absent or flattened against the cytoplasmic membrane (Fig. 3). The cells of all three types of foci tended to interdigitate with adjacent hepatocytes and did not result in compression of adjacent hepatocytes. The foci were usually less than 1 lobule in size. Hepatic foci of cellular alteration were included in the tabulations irrespective of whether a hepatocellular neoplasm or any other alteration was or was not present.

Table 2 shows the occurrence of basophilic foci in all animals by sacrifice period. The females had considerably more basophilic foci than the males. The basophilic foci occurred at a relatively high incidence in the females at the 40-week sacrifice and were dose dependent. The highest incidence occurred at the highest dose (400 ppm) and decreased with lower dosages. At the 60 week sacrifice, the dose response shifted with basophilic foci occurring at a higher incidence at 200 ppm than at 400 ppm (Fig. 4). Somewhat similar findings were present at 80 weeks. The incidence of the basophilic foci also decreased with time in the female and the incidence was less in the 80-week females than the 60-week females (Fig. 4). The incidence of basophilic foci in the males was too low to provide meaningful comparisons, but the incidence with dose and time suggested that they were responding in a similar manner to the females.

Table 3 shows acidophilic foci of cellular alteration in all animals by sacrifice. Again females had considerably more acidophilic foci than males. The incidence of the acidophilic foci was lower at the 40 week sacrifice than the basophilic foci. A dose dependent relationship was evident at both the 60 and 80 week sacrifice. The incidence did not decrease at 60 and 80 weeks at the higher dosages as did the basophilic foci (Fig. 5). The incidence of acidophilic foci in the males was too low to provide meaningful comparisons.

Table 4 shows the occurrence of vacuolated cell foci in all animals by sacrifice periods. The incidence of vacuolated cell foci was low in both sexes and was dose dependent in the 80 week females, and was also dose dependent in the 60 week females at the higher dose levels.

Hepatocellular neoplasms were divided into hepatocellular adenomas and carcinomas based upon the current classification of liver neoplasms in use at NCTR [12]. The hepatocellular adenomas were small (usually <5 mm) and existed as district nodules which compressed adjacent parenchyma (Fig. 6) and sometimes bulged from the liver surface. Histologically they were composed of well differentiated hepatocytes resembling cells present in the foci of cellular alteration (Fig. 7). Most of the hepatocellular adenomas were composed of a single population of cells (basophilic, acidophilic or vacuolated), but some were composed of a mixed population of two or more cell types. The incidence of hepatocellular adenomas is presented in Table 5. Hepatocellular adenomas increased with dose at the lower dose levels and in the early sacrifice periods. At the higher dosages at the 60 and 80 week sacrifice period the incidence of hepatocellular adenomas decreased (Fig. 8).

The diagnosis of hepatocellular carcinoma was made on either a distinct trabecular pattern or cytologic features suggestive of malignancy. Figure 9 demonstrates the relationship between a hepatocellular carcinoma and adjacent hepatic parenchyma. Some hepato-

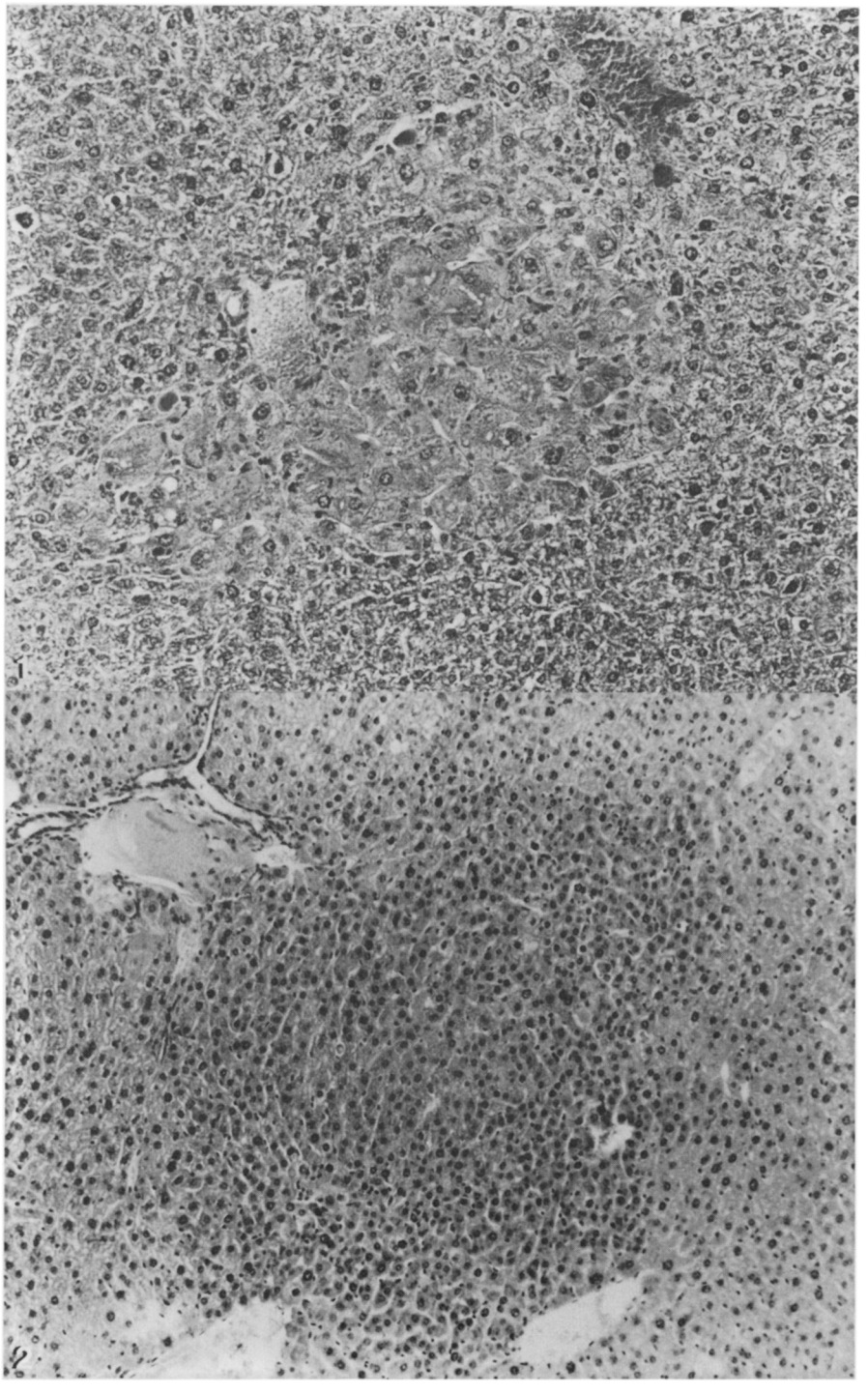

Fig. 1. *Photomicrograph of an acidophilic cell focus composed of large acidophilic cells with granular cytoplasm. H & E* ×208.

Fig. 2. *Photomicrograph of basophilic cell focus composed of small cells with basophilic cytoplasm. H & E* × 140.

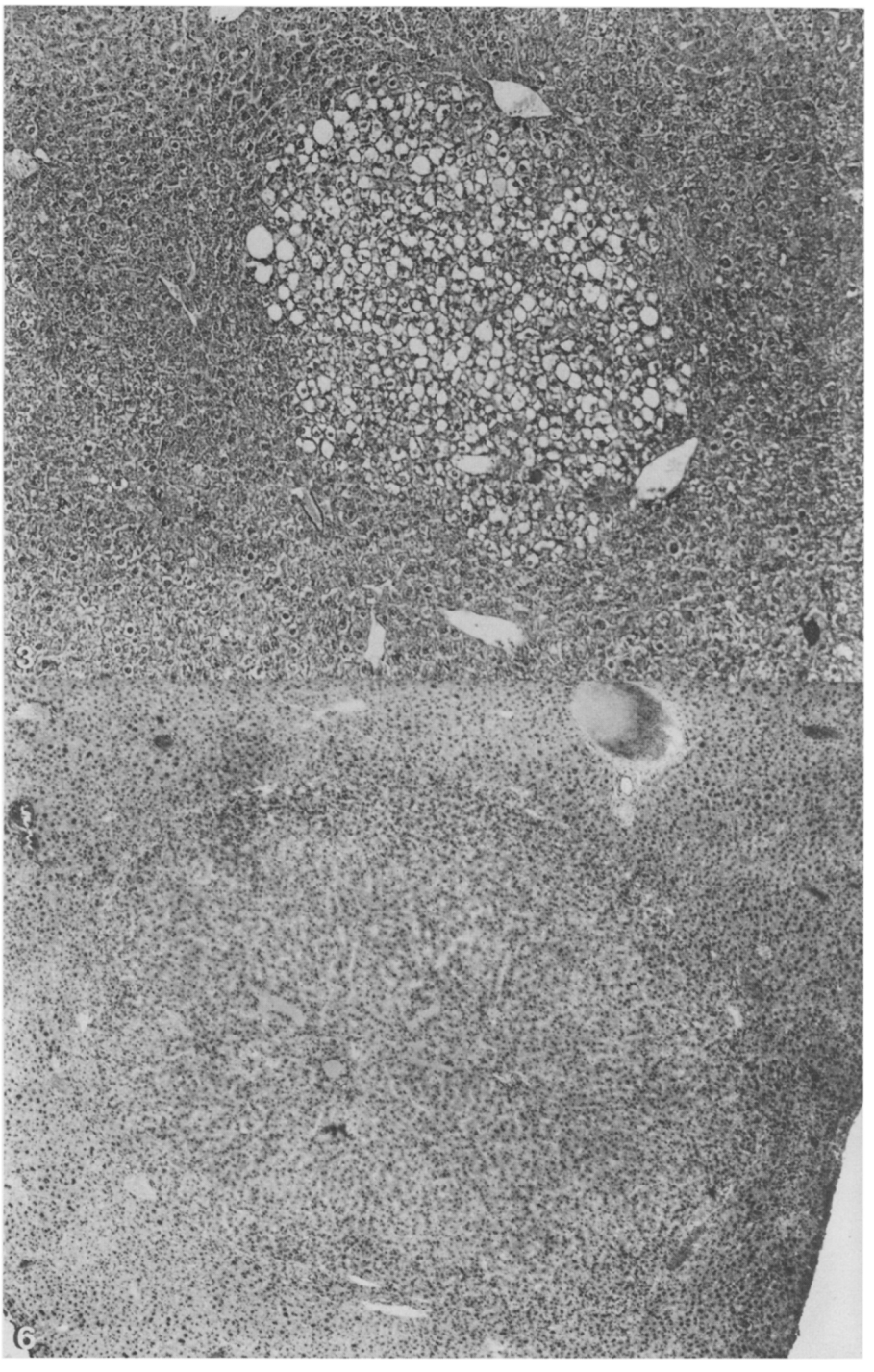

Fig. 3. *Photomicrograph of a vacuolated cell focus composed of hepatocytes with empty vacuoles.* H & $E \times 140$.

Fig. 6. *Low power photomicrograph of a small hepatocellular adenoma within a liver lobule.* H & $E \times 56$.

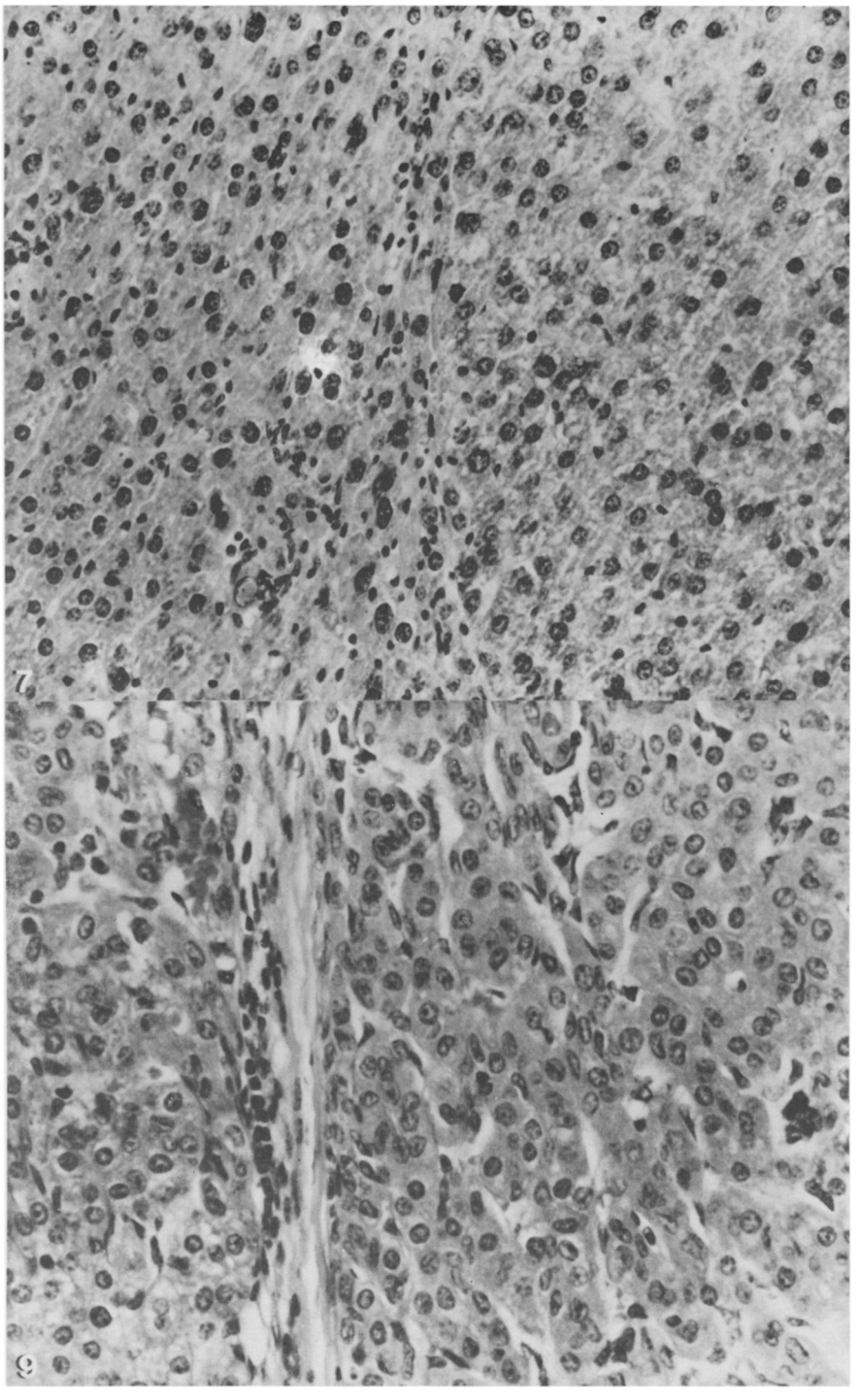

Fig. 7. Photomicrograph of junction of normal liver (left) and hepatocellular adenoma (right). Hepatocellular adenoma is clearly demarcated and is causing some compression. H & E × 350.

Fig. 9. Photomicrograph demonstrating junction of a hepatocellular carcinoma (right) with normal hepatocytes (left). H & E × 350.

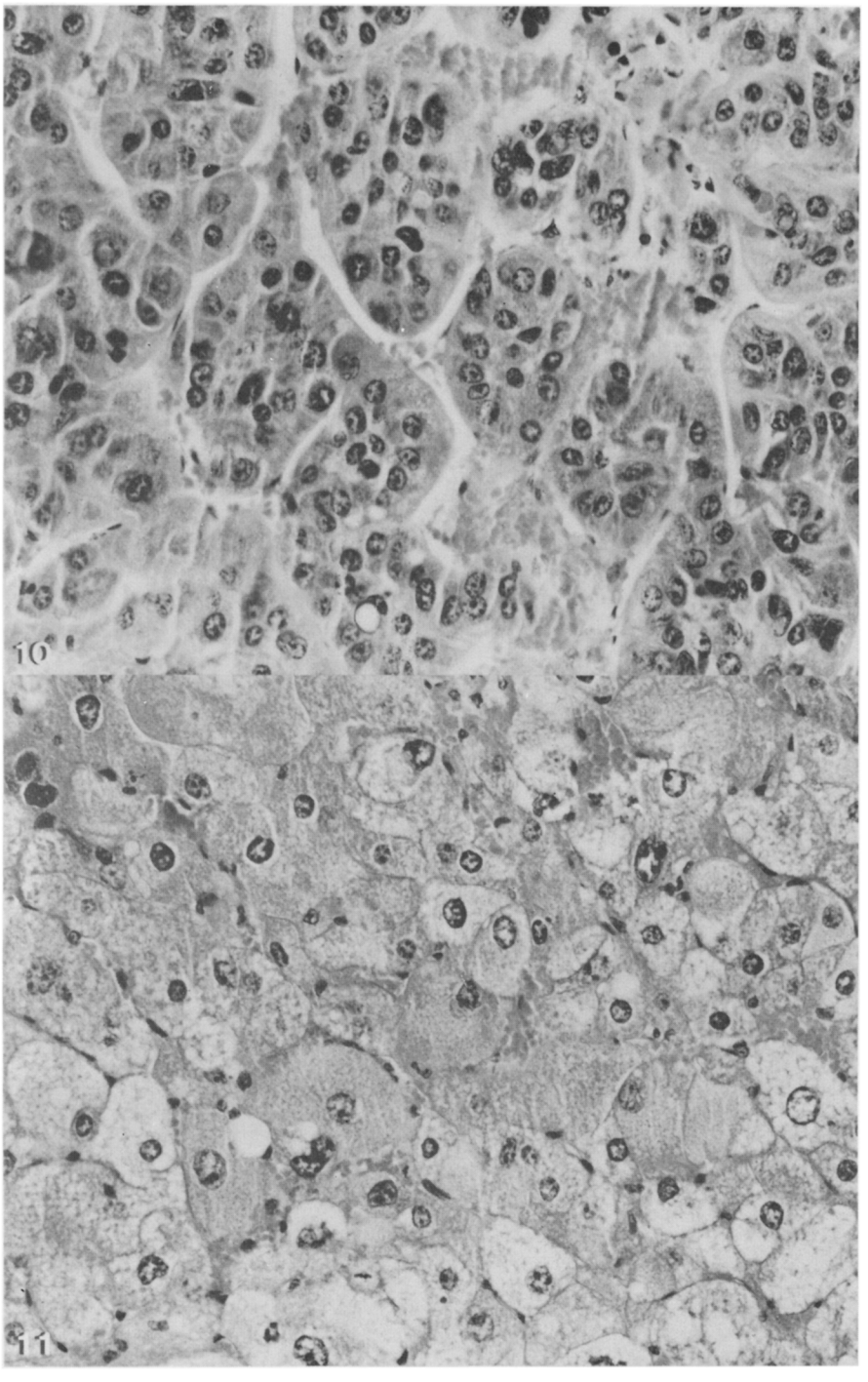

Fig. 10. *Photomicrograph of a basophilic trabecular hepatocellular carcinoma. H & E* × 350.

Fig. 11. *Photomicrograph of an acidophilic solid hepatocellular carcinoma. H & E* × 350.

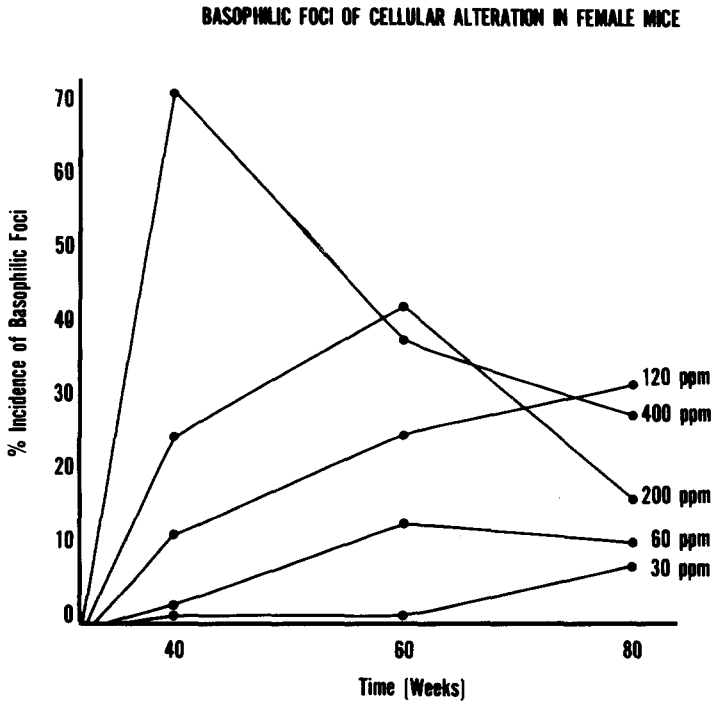

Fig. 4. Graph demonstrating that the basophilic cell foci appear early, but the incidence is lower at the higher dosages.

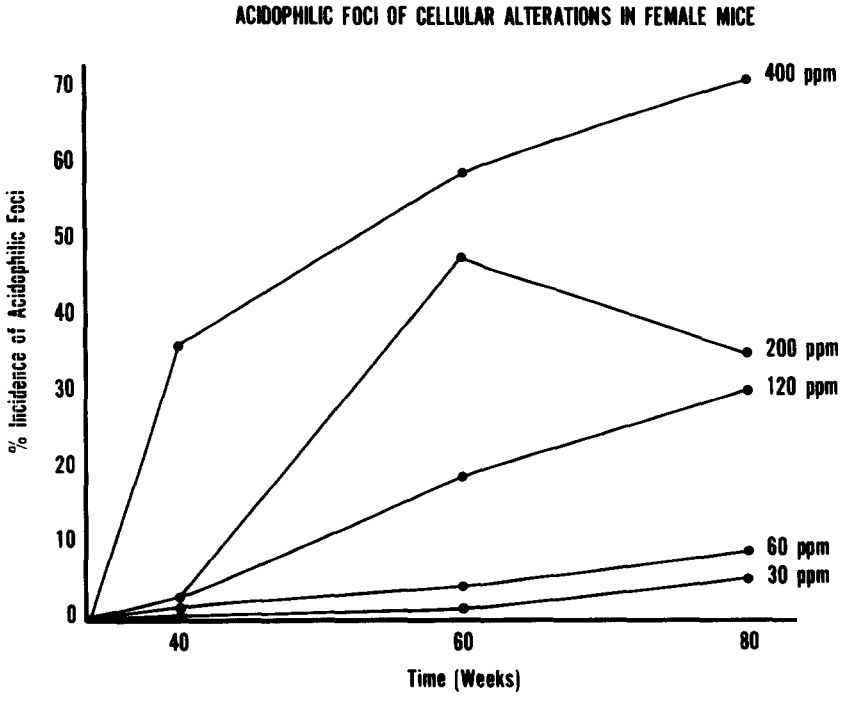

Fig. 5. Graph demonstrating a dose dependent relationship for the acidophilic cell foci.

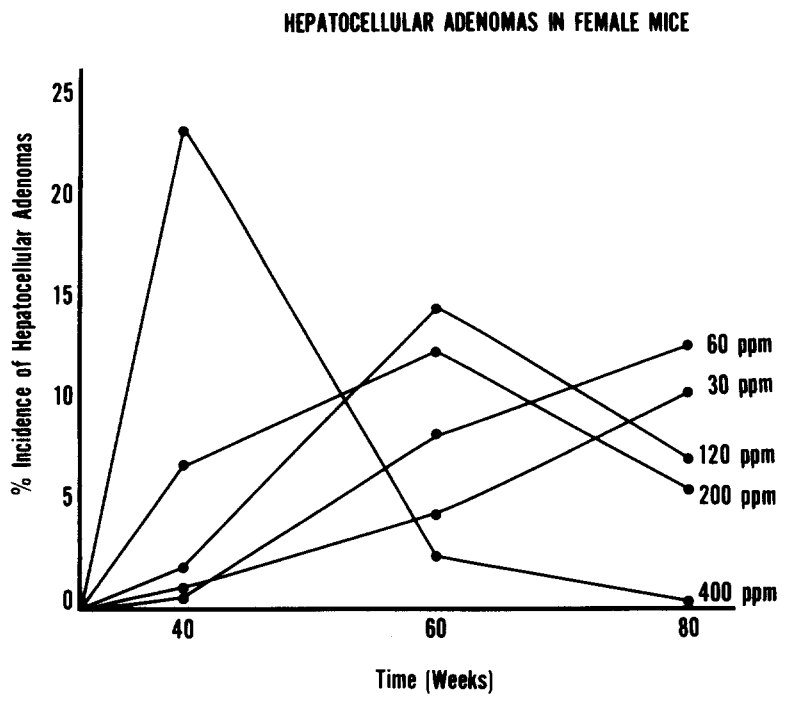

Fig. 8. *Graph demonstrating that the incidence of hepatocellular adenomas is lower at the higher dosages at both the* 60 *and* 80 week *sacrifice.*

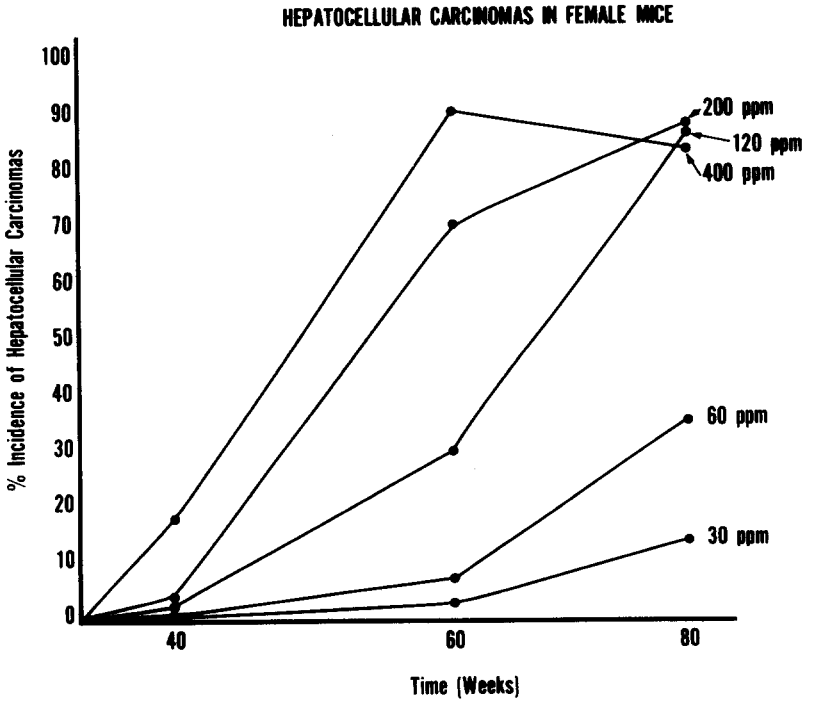

Fig. 12. *Graph demonstrating a dose dependent relationship for the hepatocellular carcinomas.*

Table 2. Basophilic foci of cellular alteration

Sacrifice period (weeks)	Sex	Total	Dose (ppm) 0	30	60	120	200	400
40	M	7/698 (1.0)*	0/99 (0)	2/199 (1.0)	1/143 (0.7)	1/100 (1.0)	2/102 (2.0)	1/55 (1.8)
	F	34/690 (12.2)	0/96 (0)	4/195 (2.1)	4/144 (2.8)	11/100 (11.0)	25/100 (25.0)	40/55 (72.7)
60	M	12/570 (2.1)	0/96 (0)	1/142 (0.7)	0/95 (0)	0/98 (0)	7/90 (7.8)	4/49 (8.2)
	F	129/662 (19.5)	0/96 (0)	3/146 (2.1)	15/106 (14.2)	29/114 (25.4)	52/121 (43.0)	30/79 (38.0)
80	M	11/432 (2.5)	0/91 (0)	0/85 (0)	0/90 (0)	4/89 (4.5)	2/40 (5.0)	5/37 (13.5)
	F	43/359 (12.0)	0/95 (0)	7/86 (8.1)	9/85 (10.6)	22/69 (31.9)	3/17 (17.6)	2/7 (28.6)

*Number in parentheses denotes incidence expressed in per cent.

Table 3. Acidophilic foci of cellular alteration

Sacrifice period (weeks)	Sex	Total	Dose (ppm) 0	30	60	120	200	400
40	M	0/698 (0)*	0/99 (0)	0/199 (0)	0/143 (0)	0/100 (0)	0/102 (0)	0/55 (0)
	F	25/690 (3.6)	0/96 (0)	0/195 (0)	1/144 (0.7)	2/100 (2.0)	2/100 (2.0)	20/55 (36.4)
60	M	3/570 (0.5)	0/96 (0)	0/142 (0)	0/95 (0)	0/98 (0)	2/90 (2.2)	1/49 (2.0)
	F	134/662 (20.2)	0/96 (0)	2/146 (1.4)	4/106 (3.8)	22/114 (19.3)	59/121 (48.8)	47/79 (59.5)
80	M	11/432 (2.5)	0/91 (0)	0/85 (0)	1/90 (1.1)	2/89 (2.2)	4/40 (10.0)	4/37 (10.8)
	F	44/359 (12.3)	0/95 (0)	4/86 (4.7)	8/85 (9.4)	21.69 (30.4)	6/17 (35.3)	5/7 (71.4)

*Number in parentheses denotes incidence expressed in per cent.

Table 4. Vacuolated foci of cellular alteration

Sacrifice period (weeks)	Sex	Total	Dose (ppm) 0	30	60	120	200	400
40	M	1/698 (0.1)*	0/99 (0)	0/199 (0)	1/143 (0.7)	0/100 (0)	0/102 (0)	0/55 (0)
	F	5/690 (0.7)	0/96 (0)	1/195 (0.5)	0/144 (0)	1/100 (1.0)	1/100 (1.0)	2/55 (3.6)
60	M	4/570 (0.7)	2/96 (2.1)	0/142 (0)	0/95 (0)	1/98 (1.0)	0/90 (0)	1/49 (2.0)
	F	33/662 (5.0)	0/96 (0)	0/146 (0)	0/106 (0)	5/114 (4.4)	13/121 (10.7)	15/79 (19.0)
80	M	3/432 (0.7)	0/91 (0)	0/85 (0)	0/90 (0)	1/89 (1.1)	2/40 (5.0)	0/37 (0)
	F	16/359 (4.5)	1/95 (1.1)	2/86 (2.3)	3/85 (3.5)	5/69 (7.2)	1/17 (5.9)	4/7 (57.1)

*Number in parentheses denotes incidence expressed in per cent.

Table 5. Hepatocellular adenomas

Sacrifice period (weeks)	Sex	Total	Dose (ppm) 0	30	60	120	200	400
40	M	4/698 (0.6)	0/99 (0)	0/199 (0)	0/143 (0)	1/100 (1.0)	2/102 (2.0)	1/55 (1.8)
	F	25/690 (3.6)	0/96 (0)	2/195 (1.0)	1/144 (0.7)	2/100 (2.0)	7/100 (7.0)	13/55 (23.6)
60	M	27/570 (4.7)	0/96 (0)	2/142 (1.4)	3/95 (3.2)	9/98 (9.2)	6/90 (6.7)	7/49 (14.3)
	F	51/662 (7.7)	1/96 (1.0)	7/146 (4.8)	9/106 (8.5)	17/114 (14.9)	15/121 (12.4)	2/79 (2.5)
80	M	30/432 (6.9)	2/91 (2.2)	2/85 (2.4)	6/90 (6.7)	11/89 (12.4)	5/40 (12.5)	4/37 (10.8)
	F	26/359 (7.2)	0/95 (0)	9/86 (10.5)	11/85 (12.9)	5/69 (7.2)	1/17 (5.9)	0/7 (0)

*Number in parentheses denotes incidence expressed in per cent.

Table 6. Hepatocellular carcinomas

Sacrifice period (weeks)	Sex	Total	Dose (ppm)					
			0	30	60	120	200	400
40	M	3/698 (0.4)*	0/99 (0)	1/199 (0.5)	0/143 (0)	0/100 (0)	1/102 (1.0)	1/55 (1.8)
	F	15/690 (2.2)	0/96 (0)	0/195 (0)	0/144 (0)	1/100 (1.0)	4/100 (4.0)	10/55 (18.2)
60	M	37/570 (6.5)	1/96 (1.0)	1/142 (0.7)	4/95 (4.2)	8/98 (8.2)	11/90 (12.2)	12/49 (24.5)
	F	202/662 (30.5)	1/96 (1.0)	3/146 (2.1)	7/106 (6.6)	33/114 (29.0)	86/121 (71.1)	72/79 (91.1)
80	M	61/432 (14.1)	0/91 (0)	5/85 (5.9)	7/90 (7.8)	16/89 (18.0)	10/40 (25.0)	23/37 (62.2)
	F	125/359 (34.8)	0/95 (0)	12/86 (14.0)	32/85 (37.7)	60/69 (87.0)	15/17 (88.2)	6/7 (85.7)

*Number in parentheses denotes incidence expressed in per cent.

cellular carcinomas were composed of smaller basophilic cells (Fig. 10) suggesting origin from a basophilic focus, and some were composed of larger eosinophilic cells suggesting origin from an acidophilic cell focus (Fig. 11). The incidence of hepatocellular carcinomas is presented in Table 6 and Fig. 12. (When benign and malignant liver tumors occured in the same liver, the lesion was considered to be malignant and tabulated with the hepatocellular carcinomas). The incidence of the carcinomas was low in both sexes at the 40 week sacrifice. The incidence was dose dependent in both sexes at the 60 and 80 week sacrifice periods. As with the cytologic alterations the incidence was much higher in females than males. The incidence in both sexes also increased with time. Hepatic foci of cellular alterations, and hepatocellular adenomas and carcinomas often occurred in the same liver.

Seven per cent of the hepatocellular carcinomas in the males and eight per cent in the females metastasized to the lungs. All tumors which metastasized contained areas of a prominent trabecular pattern.

DISCUSSION

A number of investigators have proven that benzidine is a carcinogen for the mouse [13–15]. This study confirms the carcinogenicity of benzidine in the mouse and also describes the incidence and relationship of hepatic foci of cellular alteration to hepatocellular adenomas and carcinomas.

The terminology used to describe liver lesions in the mouse has been quite varied and inconsistent. All hepatocellular nodules have been considered as carcinoma by some authors [16]. Other authors have classified these nodules simply as liver tumors [17] or hyperplastic nodules [18]. In an attempt to develop a more uniform classification for assessing hepatocellular lesions in mice, a workshop on "Hepatocellular Lesions in Mice" was held in Little Rock, Arkansas, in November, 1977, under the auspices of the National Center for Toxicological Research (NCTR) and the National Cancer Institute (NCI). The terms hepatocellular adenoma and carcinoma were chosen at this workshop as the preferred terminology for these lesions [12]. The adenomas are small, there is no invasion and they do not transplant or metastasize [19]. Our use of the term hepatocellular adenoma would be a synonym for the terms hyperplastic nodule or neoplastic nodule used by other investigators. The diagnosis of hepatocellular carcinoma is often made on a distinct trabecular pattern. Other investigators have shown that hepatocellular carcinomas with a trabecular pattern are more likely to metastasize [20, 21], and hepatocellular tumors in mice demonstrating a trabecular pattern are considered by most rodent researchers to be carcinomas.

Two crosses were utilized in the study to test the hypothesis that the F_2 mice would be more representative of the human population because of their genetic heterogeneity compared to the genetic homogeneity of the F_1 cross. The two crosses responded similarly and differences between sexes were generally more obvious than differences between the two crosses.

The foci of cellular alteration that occurred in this study were similar morphologically to those described for the rat [5] and for the mouse [2, 12]. All three types of foci and hepatocelluar adenomas and carcinomas were more common in the females than the males of both strains. It is not known exactly why the foci of cellular alteration assumed three distinct morphologic entities.

The basophilic foci occurred much earlier than the acidophilic foci. The shift from a high incidence of the basophilic foci at the higher dosage to a higher incidence at the intermediate dosage with time suggests that the basophilic foci are early appearing lesions, the appearance of which may decrease with the appearance of liver neoplasms. This observation supports the concept that the basophilic focus is a precursor to liver neoplasms.

In contrast to the basophilic foci, a marked decrease in acidophilic foci with an increase in liver neoplasms was not evident. Interpretation of these data suggests that acidophilic foci appear later than basophilic foci and that the progression to neoplasia may be quite rapid since the two lesions appear to be intimately associated with respect to time and dose. The occurrence of both acidophilic hepatocellular carcinomas and basophilic hepatocellular carcinomas in this study suggest that they may originate from acidophilic and basophilic cell foci, respectively.

The vacuolated cell foci responded in a similar manner to the acidophilic foci as far as dose level and length of administration of the carcinogen, but the incidence was much lower. The lesions occasionally progressed to vacuolated cell adenomas but did not appear to progress to hepatocellular carcinomas.

The fact that the hepatocellular adenomas occurred more frequently in the female than in male mice and that the hepatocellular adenomas were composed of cells morphologically similar to those which formed the foci of cellular alterations suggest that the alterations are precursors for the hepatocellular adenomas. The fact that the incidence of hepatocellular carcinomas was also higher in the females than in the males, and that the ratio of adenomas to carcinomas decreased with dose and time suggest that the hepatocellular adenomas are precursors for the carcinomas. The fact that foci of cellular alterations, hepatocellular adenomas and the hepatocellular carcinomas all occured in the same liver also suggests a relationship between the three. Investigators have shown that the foci of prominent trabecular formations may occasionally be seen within a solid pattern of a hepatocellular adenoma [2] suggesting that the adenomas are precursors to the carcinomas.

Although the mice in this study were administered the benzidine continuously, other studies at NCTR using 2-acetylaminofluorene (2-AAF) as the hepatocarcinogen [22, 23], have demonstrated that liver tumors of a comparable morphology to those seen in the present study continued to progress, grow and metastasize even if the 2-AAF was discontinued after 9 months of administration.

In conclusion, our study suggests a possible association between hepatic foci of cellular alteration, hepatocellular adenomas, and hepatocellular carcinomas in the mouse. We suggest that the foci of cellular alteration are preneoplastic lesions and give rise to hepatocellular adenomas and that the hepatocellular adenomas give rise to the hepatocellular carcinomas. We do not suggest, however, that *all* foci of cellular alterations develop into hepatocellular adenomas and that *all* hepatocellular adenomas develop into carcinomas. Since the response to various carcinogens differs, the possibility cannot be discounted that both hepatocellular adenomas and carcinomas may develop *de novo* without necessarily going through the changes described in this paper.

REFERENCES

1. J. E. Edwards and A. J. Dalton, Induction of cirrhosis of the liver and hepatomas in mice with carbon tetrachloride. *J. nat. Cancer Inst.* **3,** 19 (1942).
2. C. H. Frith and K. Dooley, Hepatic cytologic and neoplastic changes in mice given benzidine dihydrochloride. *J. nat. Cancer Inst.* **56,** 679 (1976).
3. R. D. Kimbrough and R. E. Linder, Induction of adenofibrosis and hepatomas of the liver in BALB/c mice by polychlorinated biphenyls (Aroclor 1254). *J. nat. Cancer Inst.* **53,** 547 (1974).
4. M. D. Reuber, Histogenesis of hyperplasia and carcinomas of the liver arising around central veins in mice ingesting chlorinated hydrocarbons. *Path. et. Microbiol. (Basel)* **43,** 287 (1975).
5. R. A. Squire R. A. and M. H. Levitt, Report of a Workshop on Classification of Specific Hepatocellular Lesions in Rats. *Cancer Res.* **35,** 3214 (1975).
6. E. Farber, Hyperplastic liver nodules. *Meth. Cancer Res.* **7,** 345 (1973).
7. E. Farber, Putative precursor lesions. Summary and some analytical considerations. *Cancer Res.* **36,** 2703 (1976).

8. E. Farber, Hyperplastic areas, hyperplastic nodules, and hyperbasophilic areas as putative precursor lesions. *Cancer Res.* **36,** 2532 (1976).
9. P. Bannasch, Cytology and cytogenesis of neoplastic (hyperplastic) hepatic nodules. *Cancer Res.* **36,** 2555 (1976).
10. H. C. Pitot, L. Barsness, T. Goldsworthy and T. Kitagawa, Biochemical characterization of stages of hepatocarcinogenesis after a single dose of diethylnitrosamine. *Nature (Lond.)* **271,** 456 (1978).
11. C. H. Frith, B. Highman and A. J. Konvicka, Advances in automation for experimental pathology. *Lab. Animal Sci.* **26,** 171 (1976).
12. C. H. Frith and J. M. Ward, A morphologic classification of proliferative and neoplastic hepatic lesions in mice. *J. Environ. Path. Tox.* **3,** 329 (1980).
13. G. M. Bonser, D. B. Clayson and J. W. Jull, The induction of tumors of the subcutaneous tissue, liver and intestine in mouse by certain dye stuffs and their intermediates. *Brit. J. Cancer* **10,** 653 (1956).
14. O. G. Prokofjeva, Induction of hepatic tumors in mice by benzidine. *Vop. Onkol.* **17,** 61 (1971).
15. S. D. Vesselinovitch, K. V. N. Rao and N. Mihailovich, Factors modulating benzidine carcinogenicity bioassay. *Cancer Res.* **35,** 2814 (1975).
16. H. L. Stewart, Comparative aspects of certain cancers. In *Cancer—A Comprehensive Treatise.* (Edited by F. F. Becker). p. 320 Plenum Press. New York (1975).
17. L. Tomatis, V. Turusov, R. T. Charles, M. Boiocchi and E. Gati, Liver tumors in CF-1 mice exposed for limited periods to technical DDT. *Z. Krebsforsch.* **32,** 25 (1972).
18. W. H. Butler and P. M. Newberne, *Mouse Hepatic Neoplasia*, p. 1. Elsevier, Amsterdam (1975).
19. J. B. M. Gellatly, The natural history of hepatic parenchymal nodule formation in a colony of C57BL mice with reference to the effect of diet. In *Mouse Hepatic Neoplasia* (Edited by W. H. Butler and P. M. Newberne) p. 77. Elsevier, Amsterdam (1975).
20. S. D. Vesselinovitch, N. Mihailovich and K. V. N. Rao, Metastatic rate of liver tumors induced by diethylnitrosamine in mice. *Cancer Res.* **32,** 2881 (1974).
21. J. M. Ward and G. Vlahakis, Evaluation of hepatocellular neoplasms in mice. *J. nat. Cancer Inst.* **61,** 807 (1978).
22. N. A. Littlefield, J. H. Farmer, D. W. Gaylor and W. G. Sheldon, Effects of dose and time in a long term, low-dose carcinogenic study. *J. Environ. Path. Tax.* **3,** 17 (1979).
23. C. H. Frith, R. L. Kodell and N. A. Littlefield, Biologic and morphologic characteristics of hepatocellular lesions in BALB/c female mice fed 2-acetylaminofluorene. *J. Environ. Path. Tox.* **3,** 121 (1979).