The Histogenesis of Hepatoma Occurring Spontaneously in a Strain of Sand Rats *(Psammomys obesus)*

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Spontaneous hepatomas and hepatic preneoplastic changes were observed in sand rats *(Psammomys obesus)* from two colonies. Both colonies originated from 10 sand rats captured in the Judean desert in 1969. At the age of 6 months, and increasing in multiplicity with advancing age, histologic examination revealed nodules containing hepatocytes characterized by hyperbasophilia, accumulation of glycogen, eosinophilic cytoplasm, or a mixture of these cells. In animals over 25 months old hepatocellular carcinoma was diagnosed. The histologic changes described here were reported to be characteristic of chemical hepatomagenesis in rats. No external chemical carcinogen could be demonstrated in our animal colonies, and a hereditary predisposition to tumor formation is presumed. Identity of hepatic carcinogenesis, irrespective of etiology in distantly related rodents, ie, the laboratory rat and the sand rat, which in reality is a gerbil, supports the assumption of the existence of a general law governing hepatic carcinogenesis. (Am J Pathol 90:399–410, 1978)

WE RECENTLY REPORTED on the spontaneous occurrence of neoplastic liver nodules in aging sand rats from a colony maintained at the Hebrew University–Hadassah Medical School. Single or multiple nodules could be observed macroscopically.[1] In an attempt to clarify the development and character of the nodules, a systematic histologic study was undertaken on the liver of sand rats of different ages.

Materials and Methods

The animals studied were derived from the original stock of 10 sand rats captured in the Judean desert in 1969 and reared in subsequent generations at the animal farm of our school.[2] A subcolony from this stock was established in 1972 at the Kimron Veterinary Institute in Beth Dagan, Israel, and continued in parallel with our colony to date.

In the present study, 164 animals were used, including an approximately equal number of either sex, originating from the two different colonies:

1. One hundred thirty-three sand rats from the Animal Farm of the Hebrew University–Hadassah Medical School included specimens from weaning to the apparent limits of the expected life-span, which was estimated to be approximately 36 months. The animals were maintained on a free-choice diet of pelleted laboratory chow (Amrod No. 935 "for rabbits and guinea pigs", Ambar Feed Mills, Hadera, Israel) and salt-bush (*Atriplex halimus*), which is part of their natural diet.
2. Seven sand rats aged 7 to 12 months and 24 sand rats aged 24 to 27 months, bred at

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Accepted for publication August 31, 1977.

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Table 1—Neoplastic Nodules in the Livers of Sand Rats: Distribution by Age and Sex

	Males		Females		Both sexes			
Age (months)	No. of animals	Nodules	No. of animals	Nodules	No. of animals		Nodules	
Weaning-6	7	—	10	1	17		1	
7-12								
A	4	1	8	1	12	19	2	4
B	5	—	2	2	7		2	
13-18	7	3	16	4	23		7	
19-24								
A	13	7	18	7	31	43	14	24
B	8	6	4	4	12		10	
≥25								
A	25	20	25	16	50	62	36	45
B	6	5	6	4	12		9	
Total	75	42	89	39	164		81	

A = animals from the Hebrew University colony; B = animals from Veterinary Institute, Bet-Dagan.

the Kimron Veterinary Institute, were used. In this colony the dietary regime differed. Only the breeding stock received the diet described above. After weaning, salt-bush was withdrawn and the offspring were maintained on commercial pellets (Amrod No. 931 "for rats and mice"). Tap water was freely offered to all animals.

From among the animals described in this article, the group of up to 6 months of age were selected at random from our colony. Most of the animals between 7 and 24 months of age were used for various metabolic studies prior to necropsy. In the course of such studies, the diets in the "Hebrew University series" were modified as follows: in 9 animals (2 males and 7 females, aged 9 to 16 months) salt-bush was withdrawn for the last 3 months of life; 6 animals, 19 to 24 months of age, were fed only salt-bush for 12 to 14 days before death. Fifteen animals between 18 and 30 months of age were killed after a fast of 18 to 22 hours.

Histologic Methods

Livers were sliced through all lobes, irrespective of the presence of macroscopic nodules, and fixed in Bouin's fluid and, in many instances, also in Carnoy's fluid. Paraffin sections were stained with hematoxylin and eosin and periodic acid–Schiff, directly and following diastase treatment. In a considerable number of cases, toluidine blue, Gomori's methenamine silver method, Masson's trichrome, and, in several instances, iron reaction for hemosiderin were applied.

Results

The histologic changes were identical in the animals of both colonies and were independent of sex (Table 1).

Group I included 17 animals from the age of weaning to 6 months. At the time of weaning and at 3 months of age, no pathologic changes were

found. At 5 and 6 months, necrosis of single hepatocytes was observed in irregular distribution in every animal examined. Accumulations of lymphoid cells and, more rarely, granulocytes were seen around the necrotic cells. One animal, 6 months of age, had large groups of hepatocytes with abundant eosinophilic cytoplasm of "ground-glass" appearance. In 2 animals, 6 months old, small foci composed of small hepatocytes with a distinctly basophilic cytoplasm were seen. Mitotic figures were present in the same area (Figure 1).

Group II included 19 animals from 7 to 12 months of age; 12 of these animals were from the Jerusalem Colony and the remainder from the Veterinary Institute. In 8 animals, histologic examination revealed various changes presumed to represent early stages of tumorigenesis. The livers contained wide areas of mixed-cell hyperplasia composed of hepatocytes with ground-glass appearance of the cytoplasm and small hepatocytes with hyperbasophilia of the cytoplasm. Also, in one 7-month old male and two 12-month old females, basophilic hepatocytes were arranged in sharply defined nodules (Figure 2). In a number of instances, nodules appeared adjacent to small branches of the hepatic veins. Not infrequently, mitotic figures were observed in the hyperbasophilic hepatocytes.

Only 1 female, 9 months old, had a macroscopic tumor. The surrounding parenchyma showed signs of compression and contained lipofuscin in contrast to the hepatocytes forming the nodule which were devoid of pigment. Near the periphery of the nodule they showed excessive basophilia, while the hepatocytes near the center of the nodule were abundantly filled with glycogen; other groups of cells had undergone fatty changes (Figure 3).

In 10 animals, no changes were found besides single-cell necrosis moderately distributed, as observed in animals of the younger age group. Such foci were also present in animals showing the abnormal cell population. There was, however, no topic relationship between the proliferative lesions and single-cell necrosis.

Group III included animals from 13 to 18 months of age. Only 7 of the 23 animals in this group had livers free of pathologic changes. Single-cell necrosis was scarce. In the majority of animals, wide areas of ground-glass hepatocytes were seen alternating with small hepatocytes. In several animals, considerable variations in nuclear size of the hepatocytes and mitotic figures were present (Figure 4).

Hyperbasophilic hepatocytes were seen in multiple foci which were not clearly defined (Figure 5). The majority of the nodules were formed by small hepatocytes with a clear cytoplasm containing abundant glycogen.

The hepatocytes within the nodules were always free of lipofuscin, which was abundant in the surrounding parenchyma cells in animals of this age group.

Six animals were fed only salt-bush for the last 10 to 12 days of life. During this period they lost considerable weight due to undernourishment. Their livers showed marked fatty change, which, however, did not affect the hepatocytes within the nodules which retained abundant glycogen, in contrast to the surrounding parenchyma.

Groups IV and V included 105 animals older than 19 months, 81 of which originated from the animal farm of the Hebrew University–Hadassah Medical School and the rest from the Veterinary Institute. Nodules were apparent in 69 animals, with a greater incidence (45 animals of 62) in Group V than in the younger group. However, when present, the nodules were spread throughout the liver, varying from minute foci to nodules 5 to 10 mm in diameter (Figure 6).

The histologic patterns were essentially similar to those observed in the younger age groups, the various abnormal cell populations occurring simultaneously in the same liver and, occasionally, also in an individual nodule. Many nodules were composed of cells containing abundant glycogen which was unaffected by withdrawal of food for approximately 20 hours (Figure 7). Hepatocytes in other nodules were hyperbasophilic (Figure 8). In contrast to findings in young animals, mixed-cell populations were more frequent and, occasionally, atypical growth patterns were present in the center of such nodules (Figure 9), while adjacent nodules displayed eosinophilic ground-glass cytoplasm (Figure 10).

The hepatocytes in the older age groups contained abundant lipofuscin and, rarely, hemosiderin adjacent to large nodules. As was shown in the younger animals, the cells composing the nodules were consistently free of pigment (Figure 11).

In 8 animals, 5 males and 3 females over 25 months of age, a malignant change was diagnosed in one or two of the nodules, because of excessive pleomorphism loss of the trabecular pattern (Figure 12) and, in some instances, penetration of tumor across the wall of hepatic veins (Figure 13). The center of such nodules showed irregular widening of sinusoids, creating areas of peliosis (Figure 14).

Discussion

In the livers of laboratory-bred sand rats, various stages of proliferative preneoplastic and neoplastic changes were observed with a notable frequency. They were identical in animals of both sexes and of the two colonies, each bred and maintained at different locations and under different dietary regime but originating from a common stock.

The changes reported in this article were found in livers free of cirrhosis and inflammatory lesions. Single-cell necrosis of hepatocytes was present in a number of animals, mainly of the younger age groups, but never exceeded the mild incidence expected by apoptosis.[3] A topographic relationship between single-cell necrosis and the proliferation of hepatocytes, suggesting a regenerative reaction, was consistently absent.

Diffuse hyperplasia of mixed abnormal hepatocyte populations were already observed at the age of 5 to 6 months. Beginning at 6 to 7 months of age, with increasing frequency with advancing age, abnormal cell populations formed multiple well-defined nodules throughout the liver, which were rarely of macroscopic size during the first 2 years of life. Macroscopic lesions were evident in more than one half of the animals between 19 and 24 months of age and in approximately two thirds of the animals more than 24 months of age (Table 1).

Early nodules were composed of small hepatocytes showing hyperbasophilia and, occasionally, considerable mitotic activity. Other nodules revealed accumulation of glycogen and, in certain cases, fatty changes, both unrelated to the nutritional state of the animals. In addition, at various periods of life, nodules composed of hepatocytes with acidophilic cytoplasm of ground-glass appearance were observed.

Malignant changes were found in a number of animals 25 to 38 months of age. These changes developed only from nodules and never from hyperplasia; in these instances, tumor invasion of hepatic veins was seen but no distant metastases were found.

The failure to produce and store lipofuscin served as a constant marker of preneoplastic and neoplastic hepatocytes. This may have a relationship to the observation that in siderotic liver occurring naturally as, for example, in human hemosiderosis or resulting from experimental procedures, hepatocytes in stages of carcinogenesis are unable to accumulate protein-bound excess iron.[4,5]

The described patterns of cellular and histologic changes have, until now, been reported only in the course of chemically induced carcinogenesis in rats,[6-10] with very little variation, depending on the type of carcinogen employed.[11]

However, in contrast to these published observations, no regular sequence in the type of abnormal hepatocyte populations was evident in our sand rats. On the contrary, the various presumed precursor lesions were often found side by side in livers carrying the final malignant phase. We have no explanation for this phenomenon. Our findings raised doubt regarding the concept of a systemic triggering of malignant transformation, as suggested by observations in hepatoma in humans.[12]

The possibility that a chemical environmental carcinogen was inadver-

tently introduced in the described animal colonies could not be established. Commercial pelleted laboratory chow free of anitbiotics was fed. There was no difference in frequency and character of preneoplastic and neoplastic lesions in the animals maintained on Amrod No. 931 diet without alfalfa and those on Amrod No. 935 with alfalfa as a partial protein source.

There was no indication that fresh salt-bush (*Atriplex halimus*), included in the diet of our colonies, might contain an unknown carcinogen. This plant is part of the natural diet of sand rats in the Judean desert, Sinai, and the Nile delta. Moreover, no toxic effects of salt-bush were reported in cattle and sheep grazing on experimental fields of this plant in semiarid areas of Israel.[13] Australian workers studying the value of salt-bush species as animal feed have not reported on carcinogenic activity.[14] Neither was an estrogenic effect demonstrated in salt-bush collected in the Judean desert and offered *ad libitum* to our animals.[15]

Sand rats have been bred for experimental purposes in the United States,[16,17] Germany,[18] and Israel.[2,19] Until now, no spontaneous tumors were reported from any colony, some of which included animals belonging to the subspecies *P terraesanctae Thomas*,[16,18] from which our colonies apparently were derived.[1]

The point has been stressed in the literature that in view of the high initial mortality following transfer of wild sand rats to laboratory conditions, the established colonies are derived from limited genetic material.[17] In the present case, it is possible that a predisposition to liver tumors was present in the genetic material of the colonies at the Hebrew University and the Veterinary Institute; both of these colonies originated from no more than 10 animals caught near the Dead Sea in 1969. This conjecture would be favored by the absence of relevant observation in the reports from the Department of Zoology at the Tel Aviv University on sand rats from various sources in Israel differing from ours.[19]

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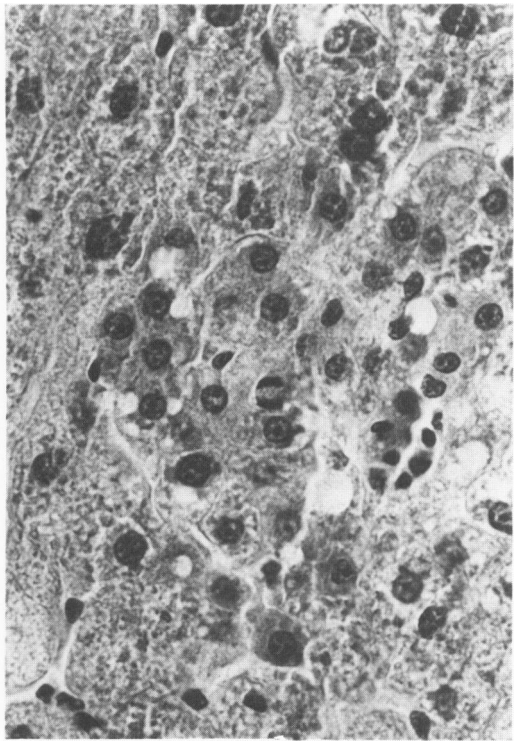
[*Illustrations follow*]



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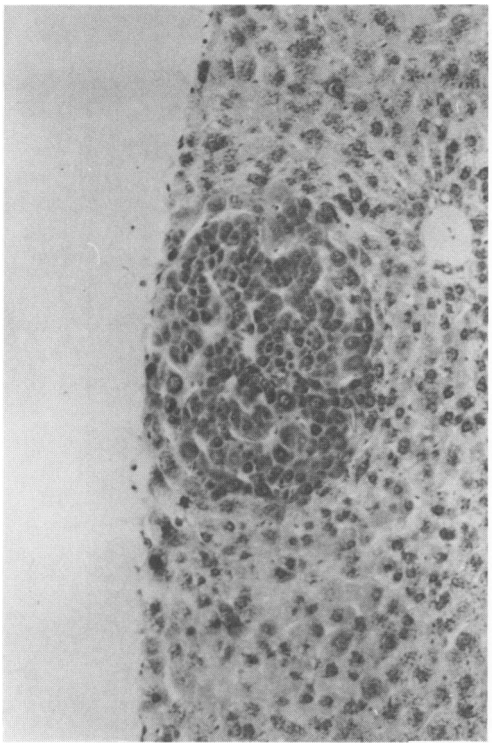

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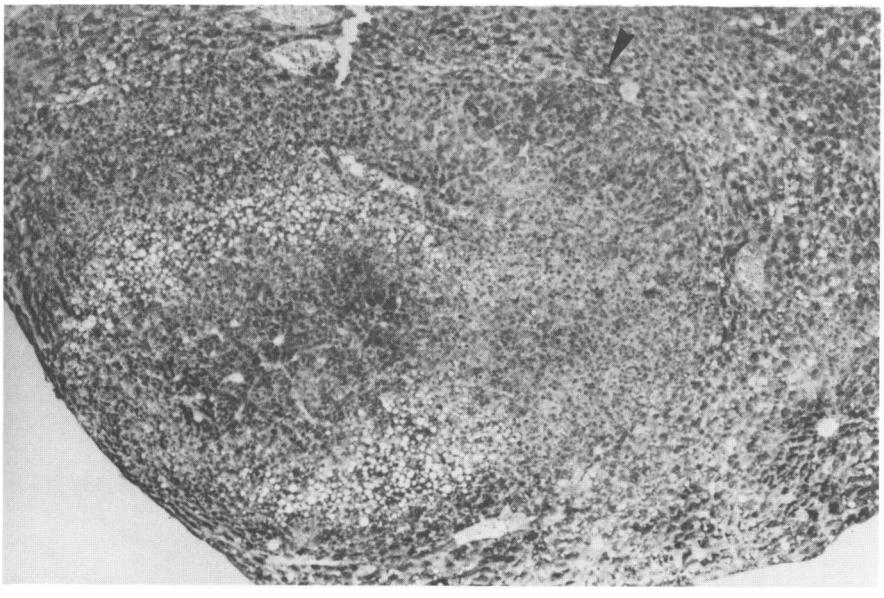

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Figure 1—Focal basophilia of hepatocytes, some of them in mitosis (male, 6 months). (H&E, × 400) **Figure 2**—Sharply defined nodule of hyperbasophilic hepatocytes (male, 7 months). (Toluidine blue, × 100) **Figure 3**—Hepatocyte nodule formed of a mixed population of glycogen-storing cells in the center of the nodule, hyperbasophilic cells in part of the periphery (*arrow*), and a large number of cells which have undergone fatty change (female, 9 months). (H&E, periodic acid–Schiff, × 40)

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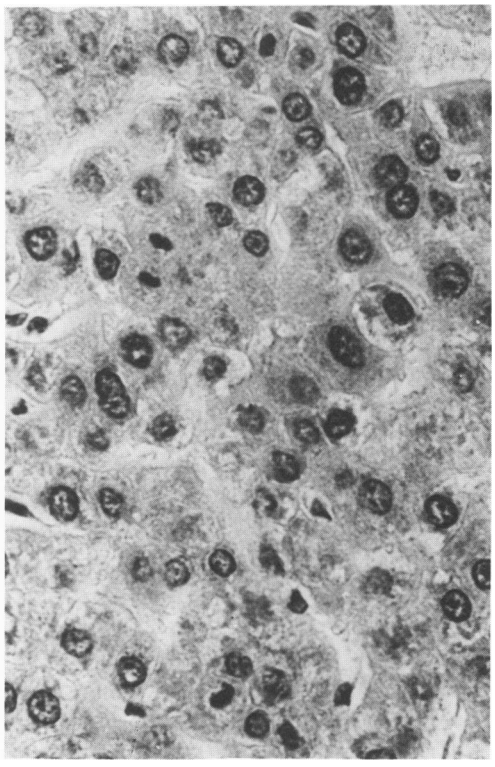

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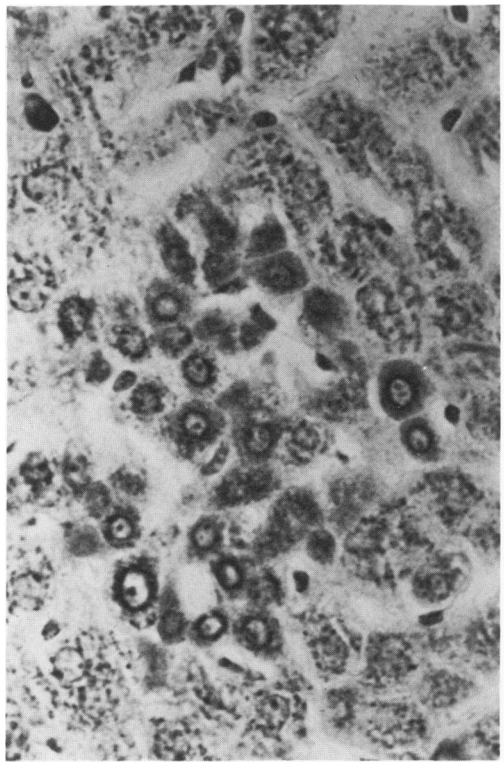

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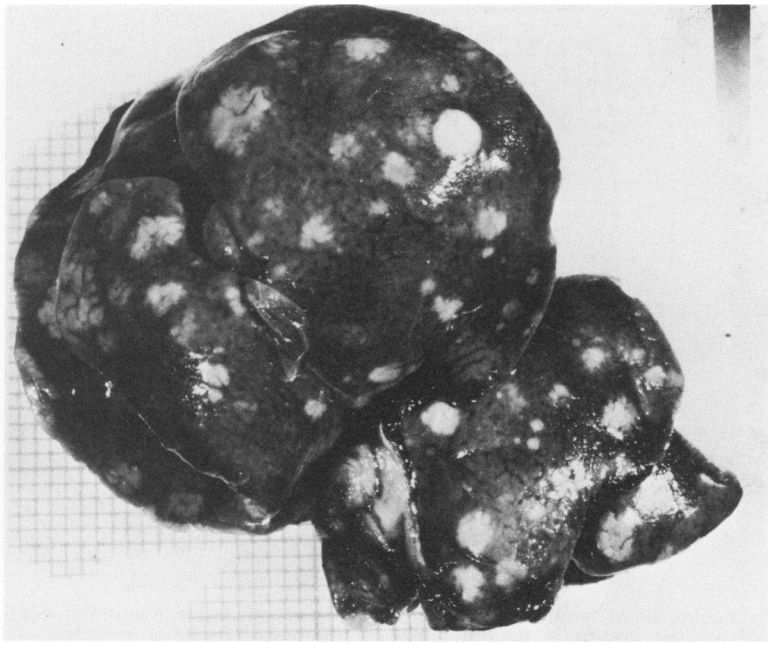
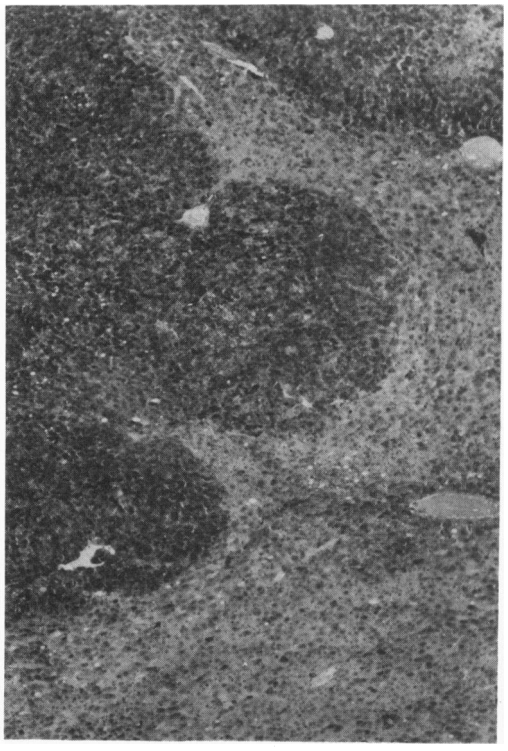
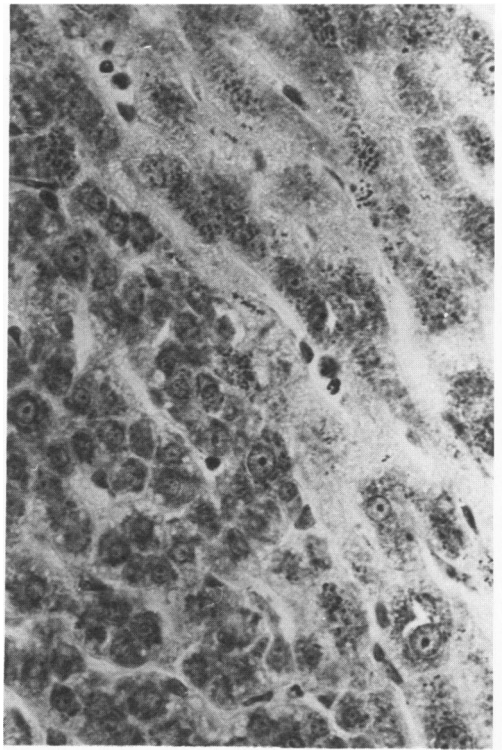


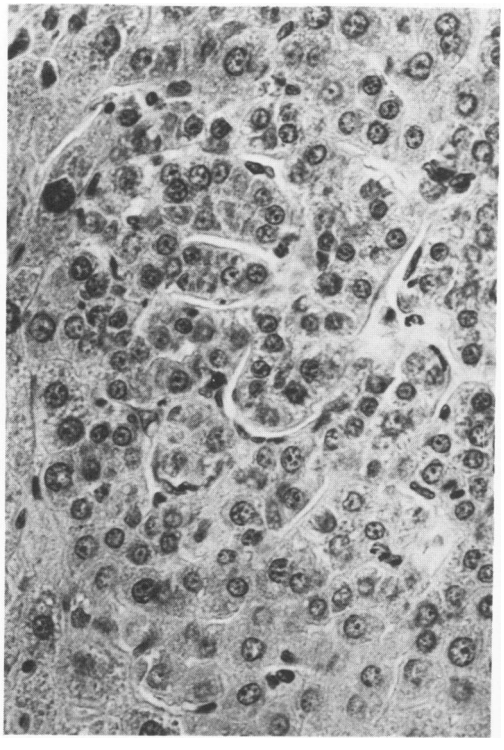
Figure 4—Large foci of acidophilic hepatocytes with some degree of pleomorphism of nuclei and scattered mitotic figures (female, 14 months). (H&E, × 400)

Figure 5—Foci of hyperbasophilic hepatocytes in the same animal as in Figure 4. (Toluidine blue, × 400)

Figure 6—Liver of male, 25 months: multiplicity in size of nodules.







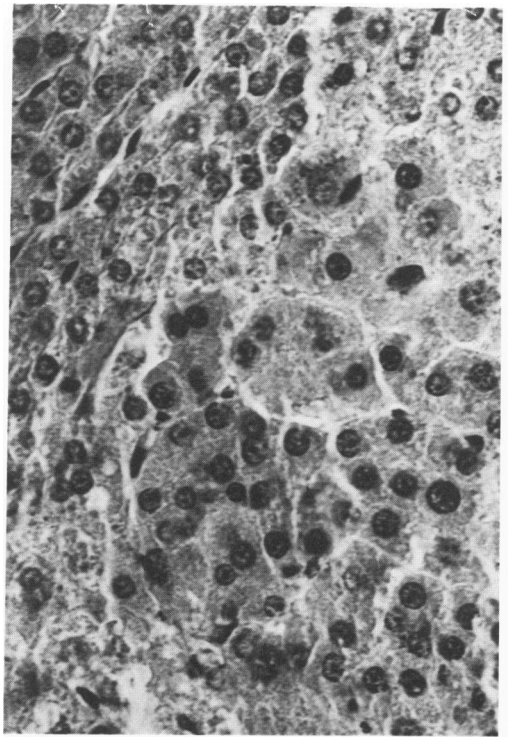


Figure 7—Retention of glycogen in preneoplastic nodules in liver of starved animal (male, 28 months). (Periodic acid-Schiff, × 32) **Figure 8**—Basophilic nodule in liver of aged animal. Note abundance of pigment granules in hepatocytes adjacent to nodule (male, 29 months). (Toluidine blue, × 320) **Figure 9**—Focus of atypical growth in center of basophilic nodule (male, 27 months). (H&E, × 320) **Figure 10**—Acidophilic hepatocytes presenting ground-glass cytoplasm in nodule from liver showing malignant changes elsewhere (female, 38 months, which is also represented in Figures 11 through 14). (Methenamine silver, × 320)

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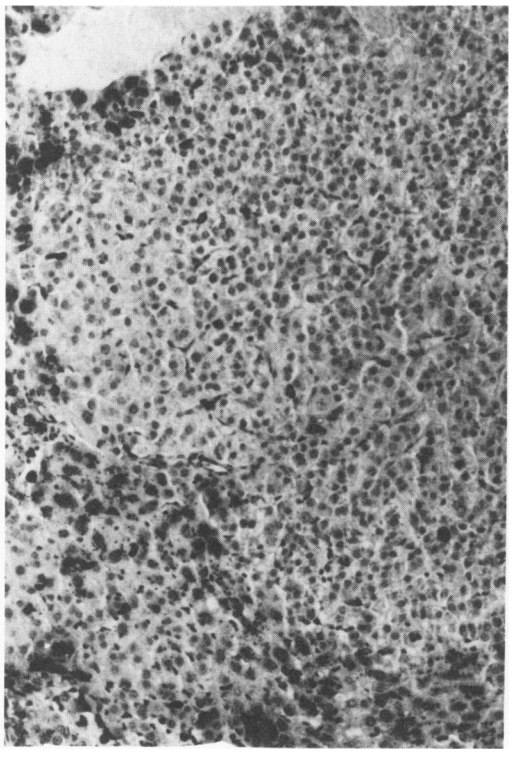

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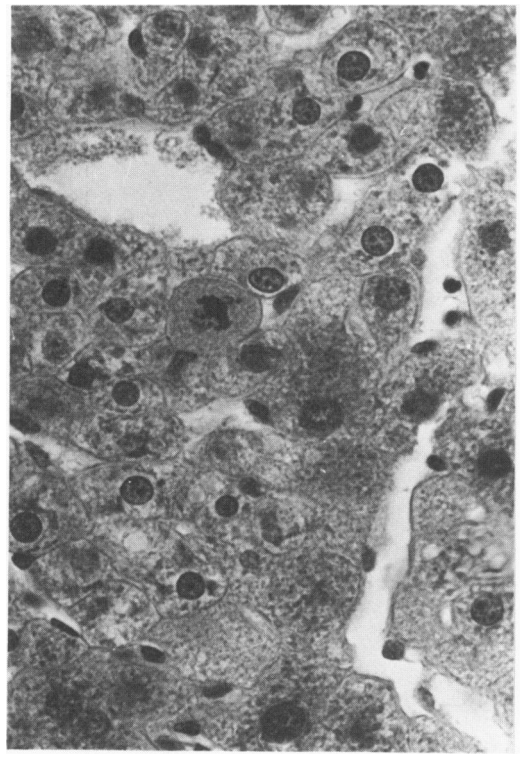

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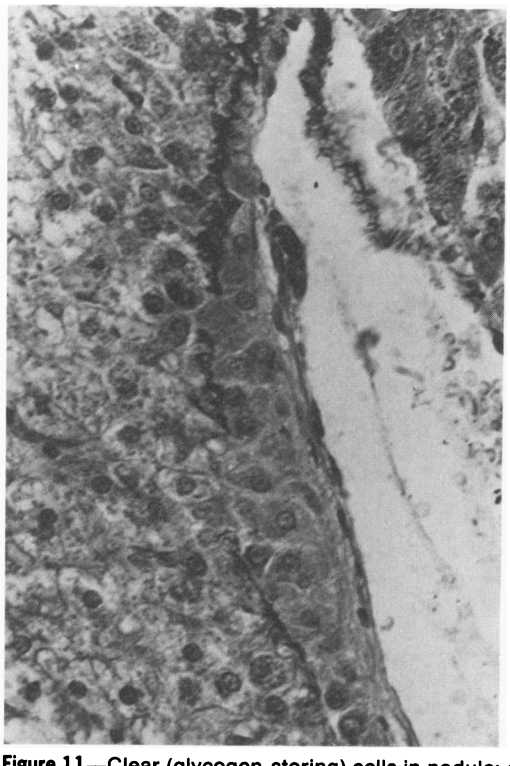

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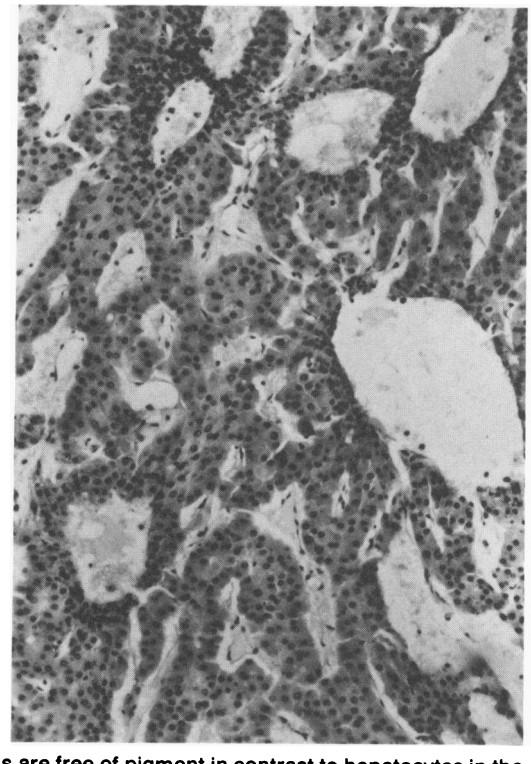

Figure 11—Clear (glycogen-storing) cells in nodule; cells are free of pigment in contrast to hepatocytes in the surrounding parenchyma containing abundant lipofuscin and a certain amount of hemosiderin. (Methenamine silver, × 100) **Figure 12**—Area from hepatocellular carcinoma. (H&E, × 400) **Figure 13**—Tumor penetration of hepatic vein. (Weigert's elastica-van Gieson, × 320) **Figure 14**—Peliosis in hepatocellular carcinoma. (Methenamine silver, × 80)