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Morphological clues for the diagnosis of small hepatocellular carcinomas

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Summary. Histological features of 44 cases of small hepatocellular carcinoma (HCC) were examined and compared with those of large regenerative nodules. The highly differentiated type of HCC most often occurred in nodules which were less than 2 cm in diameter. Noticeably, in 9 out of 15 such cases (60.0%), tumour cells were arranged in trabeculae of almost normal thickness (normotrabecular pattern). These trabeculae, however, showed variable nuclear crowding, occasional microacinar formation, and increase in cytoplasmic basophilia. It is emphasized that the presence of this triad may be a very reliable indicator for the histological identification of early HCC, especially in examining limited material such as a biopsy specimen. However, cellular and structural atypia becomes more prominent in nodules which are larger than 2 cm.

Key words: Hepatoma – Hepatocellular Carcinoma – Liver Neoplasms – Biopsy

Introduction

Nodular lesions occurring in the liver, including small hepatocellular carcinomas (HCC), are now more often detected and followed-up because of recent advances in image diagnosis (Okuda 1981; Shinagawa et al. 1984). These nodular lesions are classified on the basis of their histological findings. Although there is a comparative cytological study between small and large HCC nodules (Nakanuma et al. 1981) it seems that generally applicable criteria for histological diagnosis of small HCC have not yet been established. Accordingly, we have examined small HCCs (less than 4 cm) in relation to actual tumour size and compared the findings with those in large regenerative nodules in order to focus on their differences. In this paper, some diagnostic features of small HCC will be described.

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Materials and methods

During the period from 1975 to 1985, 51 cases with small nodular hepatic lesions (less than 4 cm in diameter) were surveyed at Chiba University Hospital and affiliated hospitals. They had been detected by ultrasonography and had undergone histological examination. Of these, 4 cases (2 needle biopsy and 2 surgical cases) were shown to be large regenerative nodules according to our criteria, as will be described later. Forty-four cases were diagnosed as hepatocellular carcinoma (HCC), consisting of 9 needle biopsy, 26 surgical, and 9 autopsy cases (Table 1). The diagnosis of HCC was made based upon the existence of an invasive growth pattern, namely vascular invasion, capsular invasion, or replacing growth (Nakashima et al. 1982) in the surgical and autopsy cases. In the present survey, the lesions having none of these abnormalities were categorized into regenerative nodules as will be described later. The biopsy cases were selected from those presenting clinical evidence of progressive tumour enlargement (e.g., about twice in diameter within 2 years; Fig. 1A and 1B), or undergoing subsequent tumour excision or autopsy (Table 2). On the latter occasions, invasive tumour growth could be demonstrated upon histological exami-

Table 1. Liver specimens

Group (Tumour size)	No. of cases	Histological specimens		
		Biopsy	Resection	Autopsy
Group 1 (–2 cm)	15	6	7	2
Group 2 (2–3 cm)	19	2	14	3
Group 3 (3–4 cm)	10	1	5	4
Total	44	9	26	9

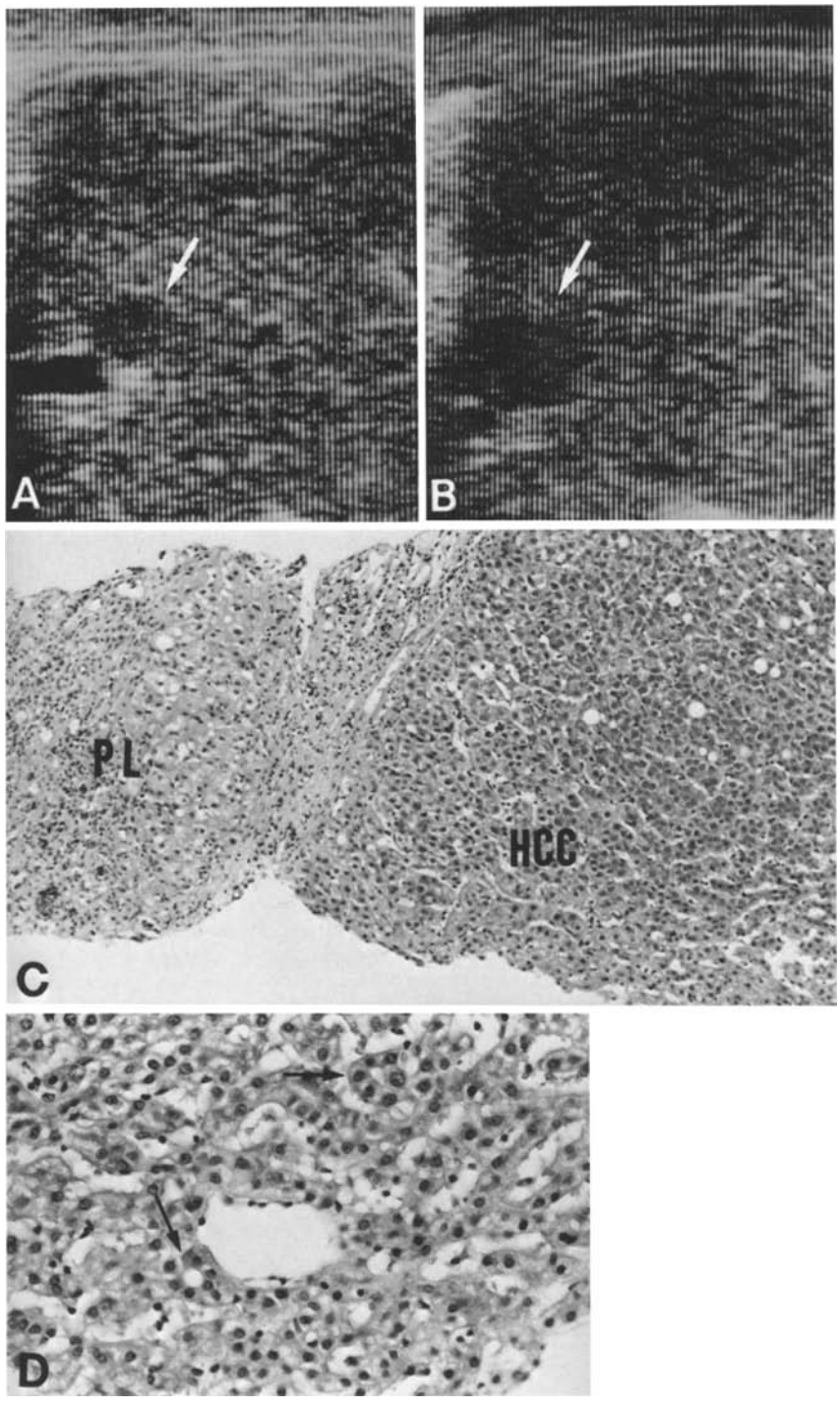

Fig. 1A–D. A case of small HCC in which tumour growth was demonstrated clinically. **A** Round 10 × 10 mm hypoechoic lesion (*arrow*) detected by ultrasonogram. **B** Ultrasonogram after 20 months exhibiting definite tumour growth (19 × 14 mm, *arrow*). **C** Biopsy specimen from a nodule shown in Fig. 1A. When compared with a pseudolobule (PL), the tumour (HCC) shows distinctive cytoplasmic basophilia and nuclear crowding. Tumour cell trabeculae are still 1–2 cells thick (normotrabecular). This specimen had previously been evaluated as a borderline lesion (H and E, × 86). **D** Another portion of the tumour shown in **C**. Note microacinar formation (*arrows*). (H and E, × 194)

nation. Three additional biopsy cases presenting some atypical changes suggestive of HCC were excluded because they did not show the diagnostic criteria described above. For comparative histological examination, a biopsy specimen was also obtained from the extranodular liver parenchyma.

All the nodules were found during follow-up examination of patients who had an underlying chronic liver disease, either overt cirrhosis or the pre-cirrhotic stage of chronic hepatitis. In addition, 6 cases had 2 nodules and 7 cases had 3. No instance of de novo HCC developing in the normal liver was therefore included in our survey.

For controls, 19 large regenerative nodules were available which occurred in cirrhotic livers and measured 0.5–2.0 cm. The diagnosis was validated by the following criteria; no growth during clinical observation (Fig. 2A and 2B) and/or absence of distinctive changes between the nodules and extranodular liver parenchyma (Fig. 2C). None of these nodules presented any findings suggestive of invasive growth. The controls

Table 2. Small hepatocellular carcinoma diagnosed by biopsy

Case No.	Tumour size at detection (cm)	Clinical course (Tumour size, cm)	Biopsy findings	
			Cellular differentiation	Histological type
1	1.0	20M (1.9).	I	N
2	1.1	1M, Resection.	I	m
3	1.9	31M (10.0), Autopsy (Cancer death).	II	N
4	1.2	5M, Resection.	I	N
5	1.9	24M (4.0), Irradiation.	I	N
6	1.7	24M (4.6); 31M, Autopsy (Hepatic failure).	II	Scirrhous
7	2.9	2M, Resection.	II	m
8	2.8	3M, Resection.	II	m
9	2.0	23M (4.0), Biopsy.	III	m

All but Case 9 were biopsied at the time of detection. M = month(s); N = normotrabecular type; m = microtrabecular type; ( ) = cause of death

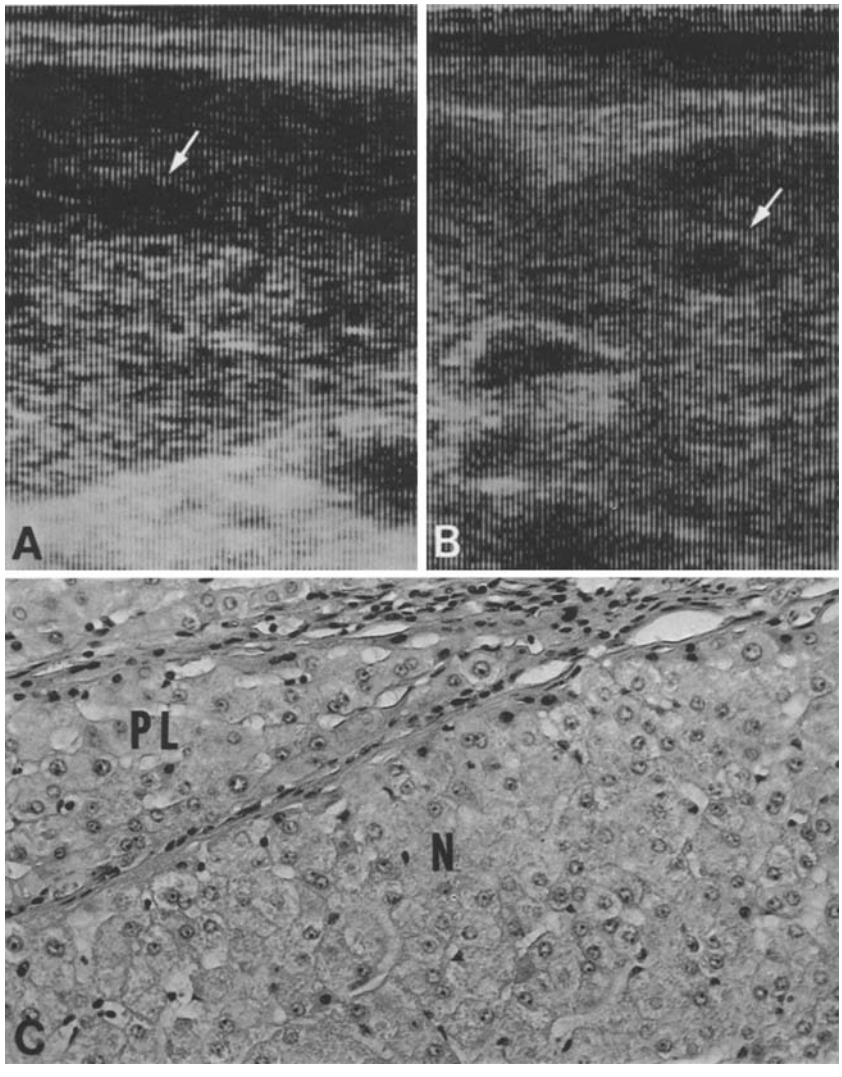

Fig. 2A–C. A large benign regenerative nodule (13 × 7 mm) occurring in a patient with liver cirrhosis. **A** The nodule was first detected as a hypoechoic lesion (*arrow*). **B** After 18 months, size of the nodule (*arrow*) is unchanged. **C** Resected specimen corresponding to Fig. 2B shows no distinctive histological difference between the nodule (N) and neighboring pseudolobule (PL). No invasive growth is seen. (H & E, ×194)

consisted of the aforementioned 4 clinically detected cases and 15 autopsy cases which were selected from our previous files of autopsied livers.

Tissue blocks were fixed in 10% formalin and routinely stained with haematoxylin-eosin and silver impregnation. Masson's trichrome was used for the evaluation of capsular invasion and the Weigert's elastic fiber stain for vascular invasion of tumours. Simple haematoxylin stain was applied to reveal cytoplasmic basophilia.

HCC nodules were divided into the following 3 groups ac-

Table 3. Cellular differentiation and histological type

	No. of case (biopsy)	Cellular differentiation				Histological type			
		I	II	III	IV	N	m	M	Others
Group 1	15 (6)	7 (4)	8 (2)	0	0	9 (4)	5 (1)	0	1[a](1)
Group 2	19 (2)	3 (0)	13 (2)	2 (0)	1 (0)	3 (0)	13 (2)	2 (0)	1[b](0)
Group 3	10 (1)	0	7 (0)	3 (1)	0	0	7 (1)	3 (0)	0

[a] scirrhous
[b] anaplastic

N = normotrabecular; m = microtrabecular; M = macrotabecular

cording to their size: less than 2 cm (group 1), 2–3 cm (group 2), and 3–4 cm (group 3) in diameter.

The individual groups were examined in regard to cytological differentiation according to Edmondson and Steiner's classification (1954) (Tables 2 and 3). Highly differentiated HCC resembling normal hepatocytes was categorized into grade I. In grade II, mild but definite nuclear atypism was observed. Prominent nuclear atypism and pleomorphism with the appearance of many multinucleated giant cells were characteristic of grade III HCC. Grade IV HCC was rather uniformly composed of anaplastic tumour cells with very scanty cytoplasm. The grading was made based upon the predominant feature of a nodule.

Histological typing was also made according to the WHO (Gibson 1978) and Peters' (1976) classifications. In addition, we tentatively divided the trabecular type of HCC into normotrabecular (1–2 cells thick), microtrabecular (3–7 cells thick), and macrotrabecular (more than 8 cells thick) subtypes.

Variable cytological and histological findings, which have been known to be common manifestations of HCC, were listed (Table 4) and the appearance rate of the individual items in each group was examined. This was principally made in comparison with the findings in extranodular sites.

With regard to the differential diagnosis between the smallest HCC group (group 1, 15 nodules) and large regenerative nodules (19 nodules), the diagnostic validity of the listed items was examined by evaluating sensitivity, specificity, and overall accuracy of each element (Table 5). When the number of nodules containing a certain change was P in group 1 and P' in control, the values were expressed by the following calculations:

$$\text{Sensitivity} = \frac{P}{15} \times 100$$

$$\text{Specificity} = \frac{19 - P'}{19} \times 100$$

$$\text{Overall accuracy} = \frac{P + (19 - P')}{15 + 19} \times 100$$

Some items, which were most frequently observed, were then selected and their diagnostic importance was also assessed (Table 6).

Results

The number of cases included in each group and the origin of the liver specimens are shown in Table 1. Six out of 9 biopsied cases later underwent hepatic surgery or autopsy (Table 2). In general there was a close similarity in regard to the histological findings between the biopsy and the corresponding surgical specimens. In 2 biopsy cases diagnosed as HCC becaue of progressive tumour growth (Fig. 1A an 1B), histological examination revealed the presence of some distinctive abnormalities such as nuclear crowding, cytoplasmic basophilia, and microacinar formation (Fig. 1C and 1D). In contrast, in 2 biopsied and in 2 resected nodules that displayed no enlargement, there was no significant atypical change (Fig. 2C).

The result of the grading in relation to tumour size is shown in Table 3. Although grade II predominated in each group, grade I could be seen in 7/15 (46.7%) of the nodules included in group 1. It was rare in group 2 and virtually absent in group 3.

As shown in Table 3, almost all cases belonged to the trabecular type of HCC. In group 1, normotrabecular subtype was seen in 9/15 (60.0%), while microtrabecular and macrotrabecular varieties became prevalent in the cases with a larger tumour size. The association between the tumour size and trabecular thickness was statistically significant (chi square test; $p < 0.005$). Silver impregnation revealed that the normotrabecular HCCs had a well preserved reticulin framework while it was more or less obscured in the micro- and macrotrabecular varieties. It was thus suggested that the silver impregnation was unavailable for the differential diagnosis between HCCs of the normotrabecular type and benign large regenerative nodules. The capillaries surrounding the thickened trabeculae showed a tendency to form a characteristic dilated channel (Nakanuma et al. 1980). In the plump trabeculae, individual cell size was often reduced but nuclear size was unchanged or even enlarged, ensuing in an increase in nucleocytoplasmic ratio. Irregular nuclear transfiguration also became prominent.

Scattered microacinar formation was rather common in each group. Although in no instance

Fig. 4. Cytological and structural atypism found in small hepatocellular carcinoma

	No. of cases	Nuclear crowding	Microacinar formation	Cytoplasmic basophilia	Thickening of nuclear membrane	Irregular nuclear shape	Peripheral nuclear localization	Prominent nucleoli	Anisokaryosis	Mallory bodies
A. Small HCCs										
Group 1	15	14 (93.3%)	11 (73.3%)	13 (86.7%)	3 (20.0%)	9 (60.0%)	1 (6.7%)	3 (20.0%)	0 (0%)	0 (0%)
Group 2	19	18 (94.7%)	15 (78.9%)	19 (100%)	13 (68.4%)	16 (84.2%)	2 (10.5%)	13 (68.4%)	2 (10.5%)	2 (10.5%)
Group 3	10	10 (100%)	9 (90.0%)	9 (90.0%)	7 (70.0%)	8 (80.0%)	0 (0%)	7 (70.0%)	4 (40.0%)	0 (0%)
Total	44	42 (95.5%)	35 (79.5%)	41 (93.2%)	23 (52.3%)	33 (75.0%)	3 (6.8%)	23 (52.3%)	6 (13.6%)	2 (4.5%)
B. Controls										
	19	2 (10.5%)	0 (0%)	2 (10.5%)	1 (5.3%)	6 (31.6%)	1 (5.3%)	1 (5.3%)	4 (21.1%)	0 (0%)

Table 5. Diagnostic validity of each factor: comparison between group 1 and controls

	Nuclear crowding	Microacinar formation	Cytoplasmic basophilia	Thickening of nuclear membrane	Irregular nuclear shape	Peripheral nuclear localization	Prominent nucleoli	Anisokaryosis
Sensitivity (%)	93.3	73.3	86.7	20.0	60.0	6.7	20.0	0
Specificity (%)	89.5	100	89.5	94.7	68.4	94.7	94.7	78.9
Overall accuracy (%)	91.2	88.2	88.2	61.8	64.7	55.9	61.8	44.1

did a pseudoglandular pattern predominate, it was seen to be present in 6 cases as an appreciable tumour element.

As shown in Table 4, nuclear crowding and increased cytoplasmic basophilia were most commonly observed, the average incidence in the 3 groups being 95.5% and 93.2% respectively. Microacinar formation (79.5%) and irregularity in nuclear shape (75.0%) were second in incidence. In group 1, these changes were more frequently observed when compared with the other items. The term "microacinar formation" indicated a subtle change derived from mild dilatation of bile canaliculi (Figs. 1C and 3) and did not comprise overt pseudoglandular formation. This change occurred only sporadically and was usually unrelated to cholestasis. Thickening of the nuclear membrane and enlargement of nucleoli were frequent in groups 2 and 3 but not in group 1.

Peripheral nuclear localization, which occurs in small basophilic cords and is considered to be a possible sign of small HCC (Kondo 1985) and anisokaryosis were less commonly observed in each group. In 2 cases of group 2, marked intracytoplasmic accumulation of Mallory bodies was seen.

Some indicators available for the distinction between well differentiated HCC, which was mostly included in group 1, and control nodules are shown in Table 5. In the control nodules, mild nuclear crowding and increased cytoplasmic basophilia were independently observed in 2 cases (incidence = 10.5%, Table 4; specificity = 89.5%, Table 5). No microacinar formation could be detected. Irregularities in nuclear shpae were not uncommon (6 cases, incidence = 31.6%, Table 4; specificity = 68.4%, Table 5). Usually, however, the change was prominent in the hepatocytes undergoing degenerative processes. Anisokaryosis and enlargement of nucleoles were found in an area compatible with liver cell dysplasia (Anthony 1976; Anthony et al. 1973). The appearance of the other items was rare in the controls. It was thus revealed that three elements–increased cytoplasmic basophilia, nuclear crowding, and microacinar formation–showed an overall accuracy in excess of 88% (Table 5). The diagnostic importance of these three items, especially when taken together, is shown in Table 6. In group 1, 13 cases had more than two items (+2 = 3 cases; +3 = 10 cases: sensitivity, 13/15 = 86.7%) while none of the control cases did (specificity, 100%). Second, irregularities in nuclear shape were also noticeable (Table 5). Thickening of nuclear membrane and enlargement of nucleoli showed a high specificity but low sensitivity. The significance of intracytoplasmic Mal-

Fig. 6. Diagnostic validity of three major findings[a]

	No. of positive items		
	1	2	3
No. of nodules			
Group 1	15	13	10
Controls	4	0	0
Diagnostic validity			
Sensitivity	100%	86.7%	66.7%
Specificity	78.9%	100%	100%
Overall accuracy	88.2%	94.1%	85.3%

[a] nuclear crowding, increased cytoplasmic basophilia, and microacinar formation

lory bodies could not be evaluated owing to their rarity.

Discussion

Currently minute nodular lesions of the liver can be detected clinically and are often presented for cytological and/or histological examination (Ohto et al. 1980; Tao et al. 1984; Tatsuta et al. 1984). It seems from the clinicopathological aspect that one of the most important issues is the distinction between highly differentiated HCC and large, discrete regenerative nodules occurring in cirrhotic livers. The definition of small HCC has been made arbitrarily by several investigators (Okuda et al. 1977; Yamazaki et al. 1981; Hsu et al. 1985) and variabilities in histological as well as cytological manifestations have rarely been discussed in connection with tumour size, especially in the examination of biopsied samples. We therefore divided small HCC nodules into three groups according to their size and compared their morphological manifestations. All of these nodules had overt signs of HCC such as progressive enlargement or invasive growth pattern. In studying these selected cases, we were able to reveal a combination of some characterstic features, a probable sign of early HCC.

For example, group 1 (less than 2 cm) had well differentiated cytological appearances (grade I or II of Edmondson and Steiner's classification) and tended to be arranged in normotrabecular fashion. In contrast, in group 3 (3–4 cm), a grade I appearance no longer predominated and the basic microtrabecular pattern was seen to be mixed with macrotrabecular or pseudoglandular elements. In the normotrabecular type of small HCC, the trabeculae were usually unaccompanied by canalicular dilatation of sinusoidal capillaries. Nakanuma et al. (1980) have made a similar observation and stated that sinusoidal dilatation occurred in larger HCC nodules. We are inclined to believe that this normotrabecular pattern is a prototype of early HCC.

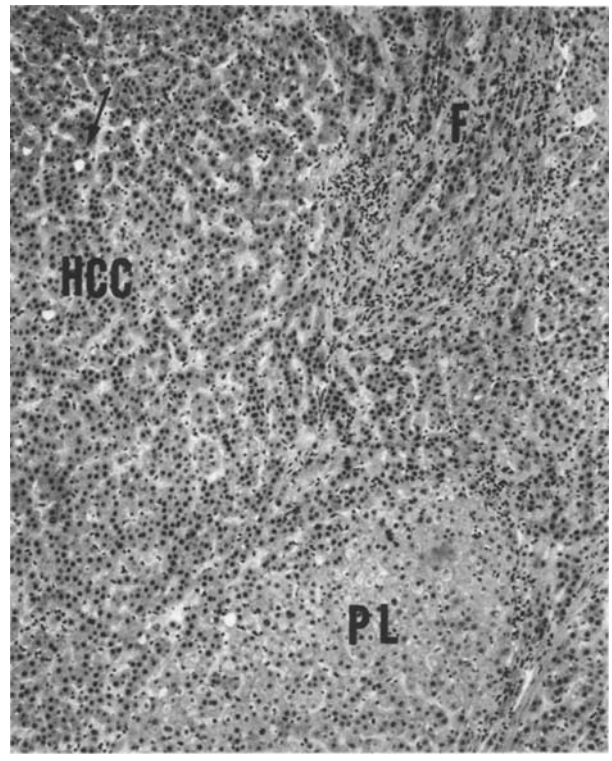

Fig. 3. Resected specimen of a small HCC (15 × 12 mm). This uncapsulated tumour (HCC) involves an adjacent pseudolobule (PL) directly replacing benign hepatocytes. Fibrous tissue (F) is also involved. Basophilic tumour cells are arranged in normotrabecular fashion and display occasional microacinar formation (*arrow*). (H & E, × 70)

The present study also suggests that variable cytological as well as structural alterations diagnostic for HCC did not appear randomly but rather in a sequence following tumour growth. Of these, nuclear crowding and increased cytoplasmic basophilia were the most frequently encountered abnormalities in group 1, the incidence of which was quite distinctive when compared with control nodules (Table 4). Scattered microacinar formation was the second most frequent change. Such a change unassociated with cholestasis was rarely encountered in controls and hence should be regarded as a probable sign of malignant transformation. Other cellular atypia became more prominent in tumours larger than 2 cm. For instance, the incidence of anisokaryosis was 40% in group 3.

As far as the histological diagnosis of small HCC is concerned, the importance of various find-

ings could not be equally assessed. Conversely, special emphasis should be placed upon a few selected items which were often seen in highly differentiated, possibly early HCC but which rarely occurred in controls. In other words, the items with high sensitivity, specificity, and overall accuracy might be of diagnostic importance. It was thus concluded that nuclear crowding, increased cytoplasmic basophilia, and microacinar formation were regarded as a "triad" for diagnosing early HCC. In our series, all of the nodules containing more than two items of the triad were indeed HCC. It seems, therefore, that even in a limited biopsy specimen, the identification of highly differentiated HCC may well be possible if there exist at least two components of the triad. In contrast, none of the control nodules had a combination of the changes included in the triad.

It is assumed from the present result that early HCC nodules are composed largely of trabeculae of almost normal thickness; they are gradually transformed into micro- or macrotrabecular or pseudoglandular patterns with tumour growth and tend to present increased cellular atypia, a familiar feature of advanced HCC nodules.

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