

Aspects of Experimental Hepatocarcinogenesis

PART V. ULTRASTRUCTURAL MORPHOLOGY OF EARLY HEPATOCELLULAR CARCINOMAS

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SUMMARY

A study has been made of 6 hepatocellular nodules which arose in the cirrhotic livers of rats fed *p*-dimethylaminoazobenzene (*p*-DAB). Four of the nodules which were composed of well-differentiated liver cells were non-invasive, but 2, although indistinguishable from the 4 in other respects, showed limited signs of infiltration of adjacent parenchyma. With the electron microscope, the liver cells comprising the nodules were found to be fairly well differentiated and contained normal microbodies, desmosomes and bile canaliculi. It was concluded that the 6 nodules represented early, well-differentiated carcinomas, although 4 were in a non-invasive stage.

S. Afr. Med. J., 48, 1335 (1974).

The majority of well-differentiated carcinomas induced by *p*-dimethylaminoazobenzene (*p*-DAB) tend to be solid and rather poorly differentiated tumours. Their ultrastructural features have been studied by Svoboda¹ and also by Ma and Webber.² It is well-recognised too that *p*-DAB administration can result in the formation of well-differentiated carcinomas of trabecular type.^{3,3} In addition to these tumours, the livers of animals fed *p*-DAB may contain small circumscribed tumour-like nodules which commonly give rise to difficulties in the diagnosis of hyperplasia versus neoplasia.³ These relatively rare lesions are composed of comparatively well-differentiated liver cells and may be encapsulated. They have been referred to as 'early neoplasms'.⁸ During an investigation of the carcinogenic effects of *p*-DAB, 6 examples of this lesion were encountered and form the basis of this report.

MATERIALS AND METHODS

The animals used were the same as those referred to in the first and second articles in this series.^{4,5} They weighed 100 g each at the onset of the experiment. The methods used to locate and prepare the nodules for light and electron microscopic study have been described in Part II.⁵ The 6 nodules described in this article measured 3-4 mm in diameter and were removed from the livers of animals killed between 14 and 20 weeks after commencement of

carcinogen administration. In order to establish as conclusively as possible whether the nodules were invasive or not, serial sections were cut from the formalin-fixed and also Epon-embedded tissue and examined with a light microscope.

RESULTS

Light Microscopy

The 6 nodules resembled each other very closely. The liver cells comprising them were larger than normal hepatocytes (Fig. 1). The cells possessed moderately basophilic cytoplasm and prominent nuclei and were arranged in cords with intervening sinusoids. Occasional mitotic figures were noted. A thin fibrocellular capsule surrounded each of the nodules. In 4 of the nodules it was not possible to show any evidence of invasion in both wax- and Epon-embedded sections, but in 2 of the nodules, small foci of invasion of surrounding parenchyma were detected (Fig. 2).

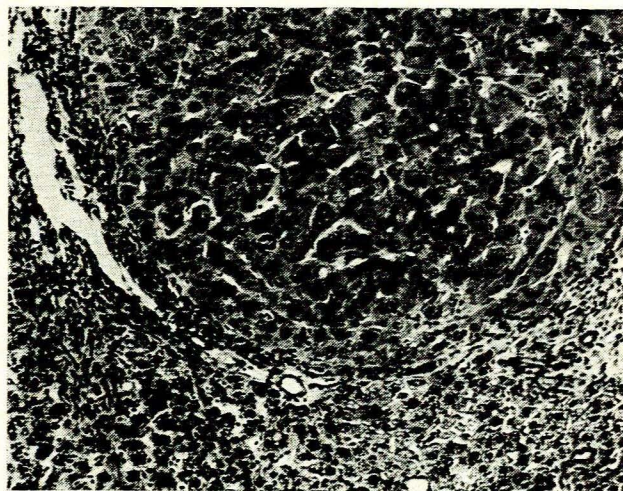


Fig. 1. Well-differentiated non-invasive hepatocellular carcinoma (H. and E. \times 400).

Electron Microscopy

The 6 nodules were identical on ultrastructural study. The majority of tumour cells had large, round nuclei, often with somewhat crenated margins and compact dense

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Date received: 14 February 1974.

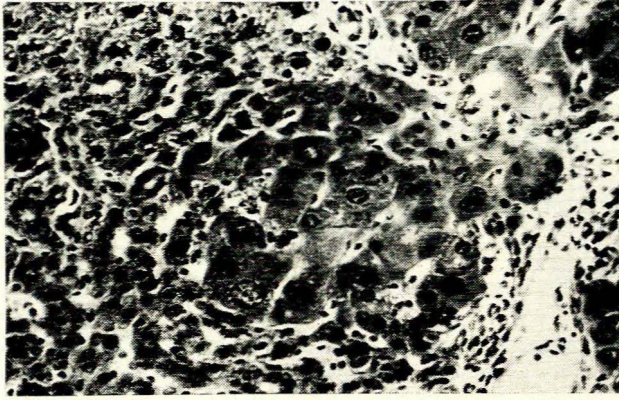


Fig. 2. A focus of infiltration of parenchyma surrounding one of the tumours (H. and E. $\times 800$).

nucleoli (Fig. 3). The degree of the development of the rough endoplasmic reticulum varied in different cells but most commonly the cisternae were rather sparse and closely related to mitochondria. Less frequently, the cells contained large numbers of long cisternae which occupied large areas of the cytoplasm (Fig. 4). Free ribosomes were present in increased numbers. The smooth endoplasmic reticulum was poorly represented in all cells. Mitochondria were generally plentiful and large, with numerous short cristae and normal granules. Bundles of microfilaments were noted in most cells. Golgi bodies tended to be located in the vicinity of the nucleus rather than the bile canaliculi, which were hard to find but present in all nodules. Micro-

bodies with crystalloids were always observed. The cells usually contained little glycogen, but a few examples of 'membrane glycogen arrays' containing large glycogen rosettes were found (Fig. 5). The plasma membrane showed numerous interdigitations and desmosomes.

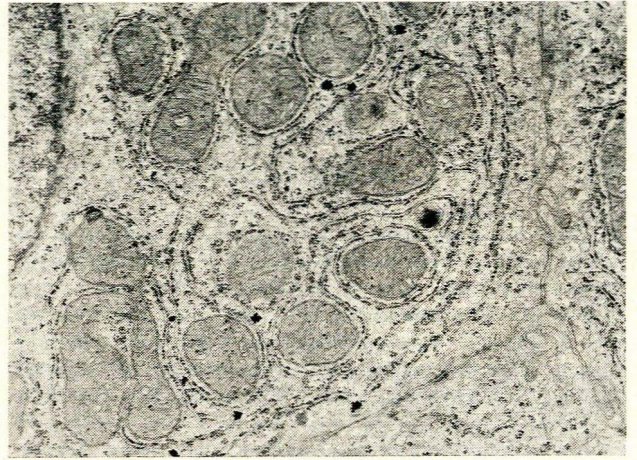


Fig. 4. Tumour cell with abundant rough endoplasmic reticulum ($\times 12\ 000$).

DISCUSSION

Apart from the obvious signs of infiltration of adjacent tissue in 2 of the nodules, the 6 nodules were indistinguish-

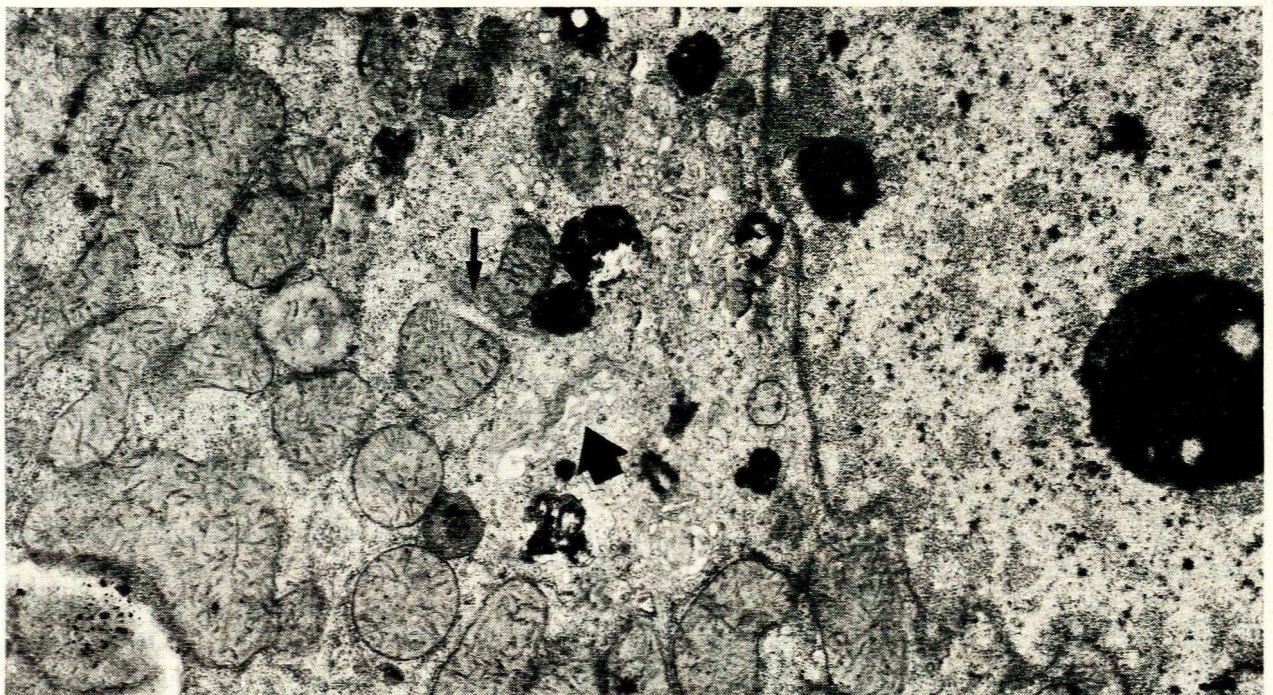


Fig. 3. Portions of 2 tumour cells. Microfilaments (small arrow) and Golgi apparatus (broad arrow) are indicated, while microbodies and desmosomes are also present ($\times 15\ 000$).

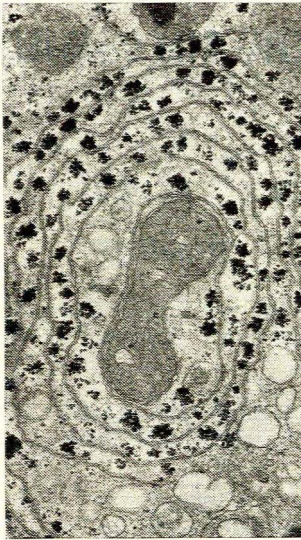


Fig. 5. Membrane glycogen array ($\times 15\ 000$).

able from each other on light and electron microscopic study. It consequently seems reasonable to infer that all 6 nodules are fundamentally similar and therefore neoplastic, although 4 of the tumours were apparently in a non-invasive stage of their development. It is obvious that conclusive proof of the neoplastic nature of the 4 non-invasive nodules would require transplantation to other animals.

As far as the fine structure of the tumours is concerned the occurrence of abundant rough endoplasmic reticulum, microbodies, desmosomes and bile canaliculi have all been observed in carcinomas of the better differentiated or trabecular variety induced by 3-methyl-4-dimethyl-aminoazobenzene.² Mitochondria of experimentally-induced hepatomas may show considerable variation in morphology,⁶ but in the more slowly growing tumours the mitochondria tend to be large, whereas in the more de-differentiated, rapidly growing tumours they tend to be smaller, with fewer cristae. The occurrence of 'membrane glycogen arrays' similar to those seen in the tumour cells have been described in rat hepatomas^{7,8} and also in human carcinomas.⁹ It is of interest that in the present experi-

mental hepatomas the tumour cells generally contain very little glycogen elsewhere, and this supports the hypothesis that the membrane arrays are actively engaged in glycogen synthesis.¹⁰ The existence of small carcinomas less than 5 mm in diameter has previously been reported.¹¹ These tumours failed to transplant although they were morphologically similar to the large carcinomas. It was suggested that they could be compared with the 'carcinoma *in situ*' elsewhere in the body. It seems probable that the non-invasive tumours in this series fall into a similar category. The available literature relating to the histogenesis of azo dye-induced liver carcinomas makes no mention of a non-invasive stage,¹²⁻¹⁴ but the findings in this article suggest that an underdetermined but probably small percentage of liver cell carcinomas may be preceded by such a non-invasive phase.

Current evidence favours the so-called hyperbasophilic foci as the source of azo dye-induced hepatocarcinomas.¹⁵ These foci are composed of cells which show a significant degree of de-differentiation at the ultrastructural level and have largely lost any resemblance to normal liver cells. They do not possess microbodies, desmosomes or bile canaliculi.¹⁵ It seems likely that such lesions would give rise to equally poorly-differentiated carcinomas. In contrast to these lesions, the present investigations have demonstrated that the azo dyes result not only in the formation of small, comparatively well-differentiated carcinomas but in various proliferative or hyperplastic lesions which are composed of fairly well-differentiated cells.^{4,5} It is not unreasonable to postulate that the hyperplastic lesions described in Parts I and II of this series may represent some of the precursor stages in the development of the tumours described in this article.

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