Aspects of Experimental Hepatocarcinogenesis

PART II. HYPERPLASTIC NODULES

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SUMMARY

This article describes the light and electron microscopic features of 4 well-differentiated hyperplastic nodules which arose in the cirrhotic livers of rats fed *p*-dimethylaminoazobenzene (*p*-DAB). The cells of which they were composed were similar in most respects to those described in Part I of this series. An increase in free cytoplasmic ribosomes, prominence of the Golgi apparatus in the perinuclear area, microfilament bundles and abnormal microbodies were the most notable features observed. It is concluded that the 4 nodules and the 3 microscopic foci described in Part I may represent different stages of the same proliferative process. The possible precancerous significance of the nodules remains to be established.

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The later stages of administration of *p*-dimethylaminoazobenzene (*p*-DAB) are characterised by the development of a cirrhosis in which some of the cirrhotic nodules are composed of vacuolated liver cells while others exhibit varying grades of basophilia.[1] In recent years attention has been focused on lesions composed of deeply basophilic cells, the so-called hyperbasophilic cells, as possible sites of neoplastic transformation.[2] These lesions have been shown to be composed of cells which are highly dedifferentiated on electron microscopy.[3] A problem which therefore remains to be resolved is whether the well-differentiated trabecular type of liver cell carcinoma which can be produced by *p*-DAB also arises from this lesion. If this is not the case, alternative histogenetic pathways should exist. The object of the present study was to see if any hyperplastic nodules could be identified as possible precursor lesions in the development of such carcinomas. This article describes the light and electron microscopic features of 4 hepatocellular nodules which have been isolated from the precancerous cirrhotic livers. The interest of these particular lesions lies in the fact that the cells of which they were composed were similar to those of the microscopic foci described in Part I of this series.[4]

EXPERIMENTAL METHOD

The animals used were the same as those described in Part I.[4] The procedure adopted to locate the lesions which form the basis of this article was to dissect small, greyish nodules, no more than 3 - 4 mm in diameter, from the surfaces of the cirrhotic livers with a sharp razor blade. The nodules were bisected and one half was fixed in formalin for histological study while the other half was cut into small fragments less than 1 mm in diameter which were fixed in Karnovsky's fixative for electron microscopy.[5] This tissue was then postfixed in 2% OsO_4 in 0,1M phosphate buffer and embedded in Epon. Sections were stained with uranium and lead salts.

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RESULTS

Light Microscopy

The majority of the nodules examined were composed of pale, vacuolated liver cells. Since these could not be recognised as hyperplastic, they were excluded from the

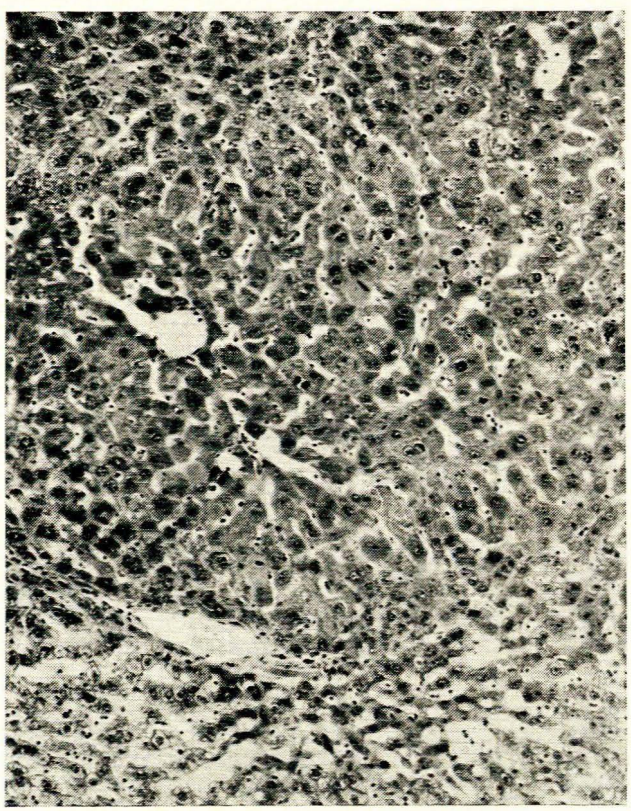

Fig. 1. Non-encapsulated hyperplastic nodule (H. and E. × 500).

study. Other nodules which consisted of deeply basophilic atypical cells closely packed together were also excluded. Several nodules diagnosed as small liver carcinomas will be described in a future article.[6]

Two of the 4 nodules were encapsulated but the remaining 2 were only partially demarcated by fibrous tissue septa and the liver cells in the nodules merged with those in the parenchyma surrounding them (Fig. 1). The liver cells comprising the 4 nodules were larger than normal liver cells, with a moderately basophilic cytoplasm which also occasionally contained a little glycogen. The cells were arranged in trabeculae with intervening sinusoids. Several mitotic figures, some of which were abnormal, were present (Fig. 2).

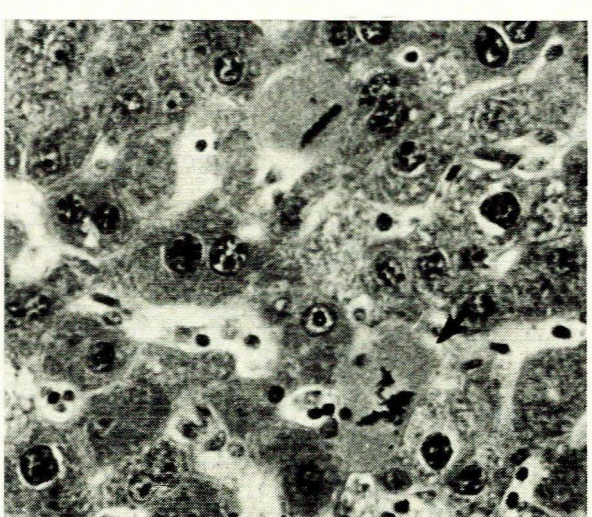

Fig. 2. Enlarged view of same nodule showing cytological detail and abnormal mitotic figure (arrow) **(H. and E. × 1 600).**

Electron Microscopy

The liver cells comprising the 4 nodules closely resembled those of the microscopic foci described in Part I[4] (Figs 3 and 4). The cells were large, with prominent nuclei and nucleoli. The cisternae of the rough endoplasmic reticulum (RER) were mostly wrapped around the mitochondria, but the smooth endoplasmic reticulum (SER) was hardly detectable. The mitochondria were usually normal, but some abnormal shapes were seen. Numerous free ribosomal aggregates were present throughout the cytoplasmic matrix. A feature of many cells was the presence of groups of Golgi bodies close to the nucleus rather than around the bile canaliculi. Some cells contained moderate amounts of glycogen, but mostly there was little or none. Normal

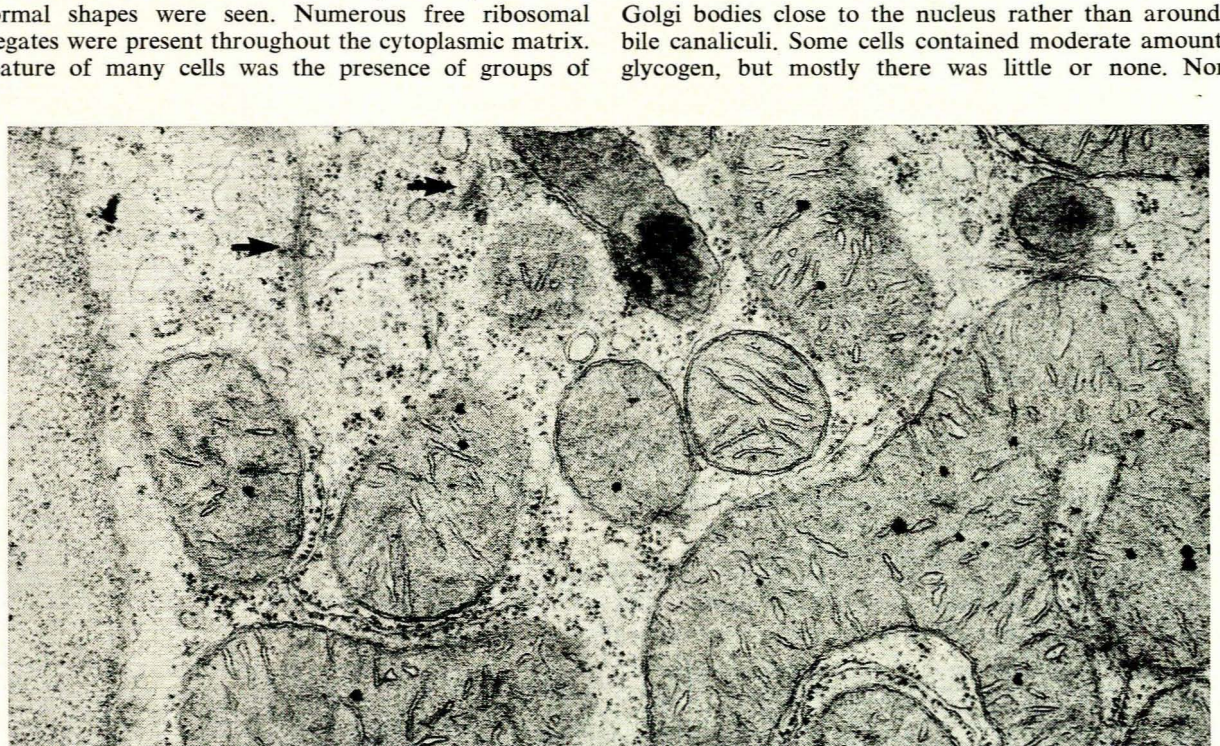

Fig. 3. Increased numbers of free cytoplasmic ribosomes, microfilament bundles (arrows) **and an elongated microbody are shown in this micrograph (× 35 000).**

microbodies were always present and abnormal forms were probably more frequently encountered than was the case in the smaller foci (Part I). Microfilaments were prominent in many cells. The cytoplasmic membrane with its desmosomes and bile canaliculi showed no significant alterations. Few lysosomes and autophagic vacuoles were present.

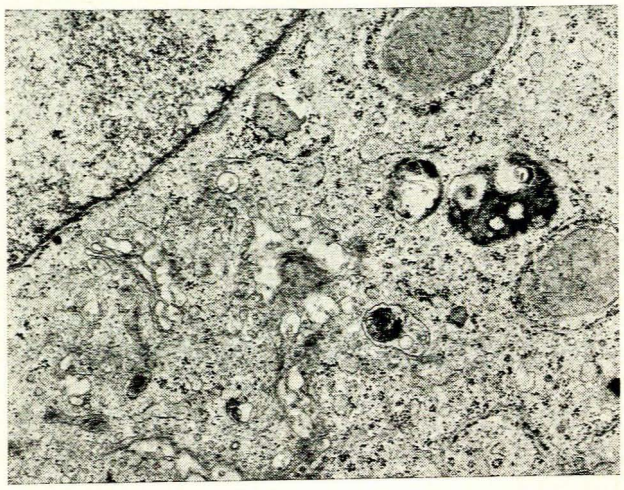

Fig. 4. Prominent Golgi bodies in the perinuclear area (× 30 000).

DISCUSSION

In the cirrhotic livers of animals exposed to *p*-DAB, the hyperplastic nodules display a wide range of histological appearances and those described in this article are comparatively uncommon under present experimental conditions. The large, moderately basophilic liver cells in these nodules showed increased mitotic activity compared with those surrounding the nodules. Therefore the nodules could reasonably be termed hyperplastic. At the ultrastructural level the cells were similar in most respects to those described in early hyperplastic foci (Part I),[4] although cells containing helical polysomes appeared to be absent from the larger nodules.

The only additional feature which requires comment in view of its frequency concerns the abnormal microbodies. Morphological alterations in these organelles have been reported in regenerating liver[7] and following the administration of a variety of toxic agents.[8] Since they have on occasions also been observed in degenerate hypobasophilic parenchymal cells outside the nodules, their occurrence in the nodules appears to have no special meaning. The significance of the other ultrastructural features has been discussed in the earlier article and will not be repeated here, but it was thought that the microscopic lesions were probably not of a simple reparative nature, but seemed rather to represent specific foci of carcinogen-induced hyperplasia. The same conclusion was reached concerning the nature of the nodules described in this article.

According to presently available evidence, some hyperplastic nodules composed of atypical liver cells may be precursor lesions in the development of frank carcinomas,[9-11] and these have been shown to possess some, but not all, of the ultrastructural characteristics of malignant tumours induced by the same agents.[10] As far as the hyperplastic nodules described in this article are concerned, one may point to the fact that features such as prominent bundles of microfilaments and perinuclear Golgi bodies are more characteristic of neoplastic than normal liver cells (see Part I). In addition it will be shown in the final article in this series (Part V) that they may also be seen in small, fairly well-differentiated hepatocellular carcinomas induced by *p*-DAB, which developed in the livers of the same animals from which the nodules described in this article were taken. It can be seen, therefore, that there are some ultrastructural characteristics which are shared by the hyperplastic nodules, the small microscopic foci, and the early liver cell carcinomas. Not surprisingly, some differences in structure were also apparent. These observations do at least raise the possibility, though there is as yet no proof, that the hyperplastic nodules and small foci are lesions which precede the development of well-differentiated hepatocellular carcinomas. The evolution of small, well-differentiated hyperplastic foci and nodules into well-differentiated carcinomas has been described at the light microscopic level,[9] and the present experimental results are in keeping with this observation. It seems probable that the poorly differentiated, so-called 'hyperbasophilic foci' give rise to the more common, less well-differentiated liver cell carcinomas.[12]

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