Aspects of Experimental Hepatocarcinogenesis

PART I. EARLY HYPERPLASTIC FOCI

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SUMMARY

This article, the first of a series of 5, describes the light and electron microscopical features of early foci of parenchymal cell hyperplasia which developed in the livers of rats fed the carcinogen *p*-dimethylaminoazobenzene (butter yellow). The cells in the foci possessed increased numbers of free cytoplasmic ribosomes, prominent perinuclear Golgi bodies and bundles of microfilaments. These features suggested that the cells were not merely regenerative in nature but represented a definite carcinogen-induced proliferative response.

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Since the carcinogenic properties of certain azo compounds were first recognised,[1] these agents have been extensively employed in studies of experimental liver cancer in rats. *p*-Dimethylaminoazobenzene (*p*-DAB, butter yellow) was selected for use in the present work on the grounds that a comprehensive body of knowledge concerning its biochemical and morphological effects has already accumulated,[2-4] and under present experimental conditions it produces a high percentage of well-differentiated liver cell carcinomas in the local strain of rats.

The relevance of the azo dye/rat experimental system to the disease so prevalent in the indigenous Black races of Southern Africa may legitimately be questioned. Apart from the fact that the azo dyes are not implicated in human pathology, there are certain morphological differences in the human and experimental cancers, the latter tending to be more variable in structure and, from the functional point of view, bile formation does not occur. Nevertheless, the better-differentiated liver cell carcinomas in particular closely resemble human carcinomas in appearance and growth characteristics, including metastatic behaviour.

The purpose of the present work was to explore a number of aspects of the development of liver cancer in rats which in the past have received little or no attention.

While it is widely accepted that hyperplasia commonly precedes the development of both human and experimental liver cancer,[5] very little is known about the earliest stages of this process. It would, for example, be desirable to know whether the early changes were non-specific in nature, or whether there were any morphological criteria which might suggest that they were implicated in the subsequent formation of hyperplastic nodules and possibly tumours.

The first article in this series describes the light and electron microscopical features of early hyperplastic lesions which developed following administration of the carcinogen. The remaining articles in this series are: Part II—Hyperplastic nodules; Part III—Iron overload and hepatocarcinogenesis; Part IV—Changes in the liver following cessation of carcinogen administration; Part V—The ultrastructural morphology of early hepatocellular carcinomas.

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EXPERIMENTAL METHOD

One hundred male rats of a locally bred albino strain were used. They were fed a basic maize diet to which 0,05% w/w of *p*-DAB was added for periods of up to 20 weeks. The animals were sacrificed at weekly intervals after they had been placed on a normal diet for 2 - 5 days. Since the foci of hyperplasia were only microscopical in size, they could therefore not be recognised on gross inspection of the liver. The only method which could be adopted to locate them was as follows: small blocks of liver tissue, about 1 mm in diameter, were removed at random from all the lobes of the liver (at least 10 blocks from each liver). Fixation for ultrastructural study was achieved by immersion in 2% OsO_4 in 0,1M phosphate buffer. After dehydration they were embedded in Epon. One micrometre section was cut from each block and stained with toluidine blue. After a prolonged search, 3 possible foci were located. These blocks were then sectioned for electron microscopy. Sections were stained with uranium and lead salts and were examined in a Siemens Elmiskop IA electron microscope.

RESULTS

Light Microscopy

The livers were obviously cirrhotic and many carcinomas had developed by the end of the experiment.

In both haematoxylin and eosin-stained paraffin sections and the Epon-embedded sections stained with toluidine blue, the 3 foci were composed of islands of lightly basophilic cells which contained only traces of cytoplasmic glycogen (Figs 1 and 2). The cells were slightly larger than those surrounding it. Occasional mitotic figures were seen.

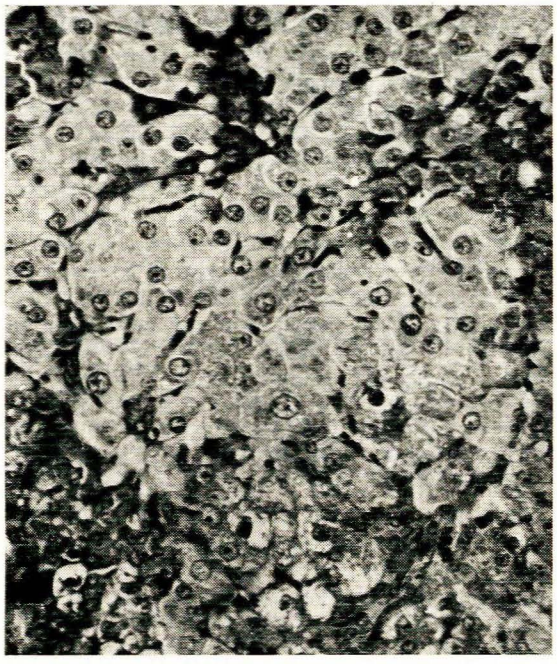

Fig. 1. Early hyperplastic focus (PAS stain × 400).

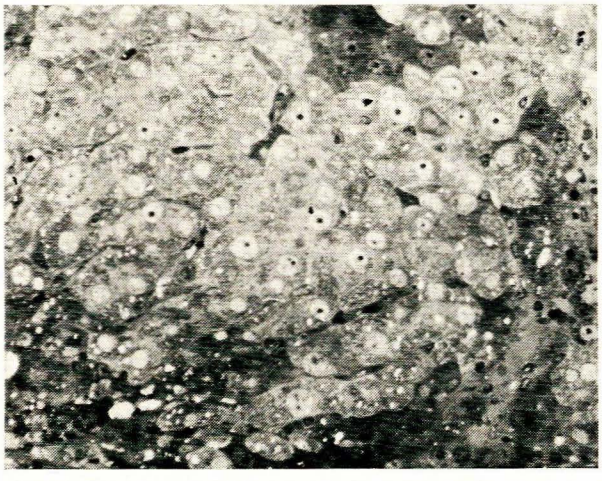

Fig. 2. Early hyperplastic focus. Epon-embedded section (toluidine blue × 300).

Electron Microscopy

The ultrastructure of the typical cell which occurred in each of the nodules is illustrated in Fig. 3. The nuclei were enlarged, with a round or slightly ovoid shape and slightly crenated outline. Nucleoli were moderately hypertrophied.

The rough endoplasmic reticulum (RER), which was quite well represented in most cells, was composed of cisternae which more often than not were closely applied to the mitochondria. Parallel arrays were rarely present. The smooth endoplasmic reticulum (SER) was poorly developed. The cytoplasmic matrix contained fairly numerous polysomal aggregates, whereas glycogen was sparse. The mitochondria were of normal size and appearance, with a notable exception that in some cells, the intramitochondrial granules were greatly reduced in number or absent. It was a common feature of these cells that many prominent Golgi bodies were located close to the nucleus rather than in the vicinity of the bile canaliculi. Microbodies which were sometimes of an abnormal shape were always present, as were bile canaliculi and desmosomes. Bundles of microfilaments were often seen close to the nuclear margin or elsewhere.

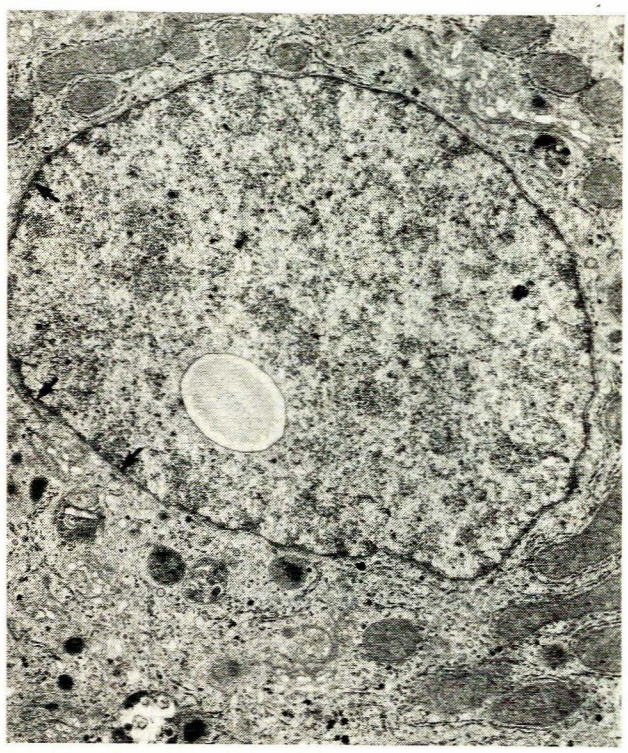

Fig. 3. Typical cell of hyperplastic foci. Arrows indicate microfilament bundles. Several Golgi bodies can be identified (× 8 000).

In one of the nodules approximately one-third of the cells studied presented a rather different appearance (Fig. 4). The RER was composed of very long cisternae, and the

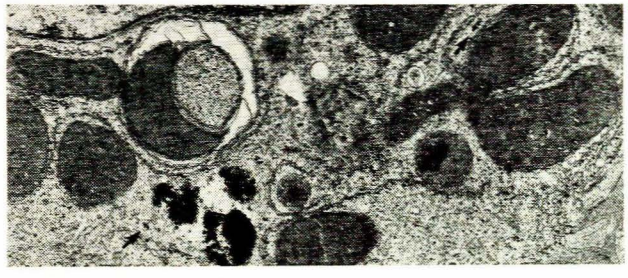

Fig. 4. Second cell type from one of foci showing 2 helical polysomes (arrows) **(× 30 000).**

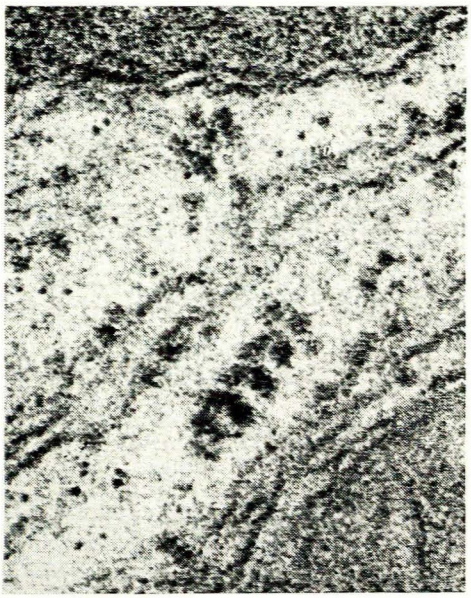

Fig. 5. Enlarged view of one of the helical polysomes seen in Fig. 4 (× 150 000).

mitochondria were small with a dense matrix and normal granules. The cytoplasmic matrix mostly contained single ribosomes, and the rather infrequent polysomes present were exclusively of the helical type (Figs 4 and 5). In the plane of section one could usually count 6 - 8 ribosomes in the structure.

Liver parenchyma surrounding the nodules usually showed a conspicuous increase in the SER, while the RER was extensively disorganised and often visibly degranulated.

DISCUSSION

The early stages of *p*-DAB carcinogenesis are characterised by degenerative changes in the parenchymal cells.[6] Many cells become vacuolated and hypobasophilic, and with the electron microscope this can be attributed to dispersal of the RER with a variable loss of ribosomes. A great increase in SER is usually seen. Cells showing these changes persist as long as the carcinogen is administered, and many of the cells surrounding the small cellular foci were of this type. These changes are largely attributable to the toxic action of the carcinogen.

At a slightly later stage, basophilic material begins to reaccumulate in parenchymal cells, firstly in isolated cells and then as small islands.[7,8] Ultimately, larger hyperplastic lesions composed of cells with a varying degree of cytoplasmic basophilia are formed. The problem has been to decide whether the foci of basophilic cells of the type described are merely regenerating to replace those lost by focal necrosis, or whether they conceivably represent a very early proliferative stage of a process which could ultimately result in a tumour.

The liver cells comprising them were appreciably altered from the normal hepatocytes and they also differed completely from those surrounding the foci. The increased number of free ribosomes is indicative of an accelerated rate of protein synthesis.[9] The unusual location and prominence of the Golgi bodies are more characteristic of certain neoplastic cells,[10,11] but they are not evidently features of regenerating liver cells.[12,13] A similar location for these organelles has been described in embryonic hepatocytes.[14,15] Small bundles of microfilaments are rarely found in normal liver cells and they are most common in the vicinity of the bile canaliculus. It will be shown that bundles of microfilaments occur with increased prominence and frequency in larger hyperplastic nodules[16] and also in early hepatocellular carcinomas.[17] The significance of the helical polysomes which occurred in some of the cells of one of the nodules is obscure. These curious polysomal structures have been reported under a wide variety of conditions.[18] They have been reported in the liver after the injection of other liver carcinogens, e.g. lasiocarpine,[19] but have not yet been described with the azo dyes. Despite their unusual configuration, their amino acid uptake is said to be normal.[18] A paucity of intramitochondrial granules has been described in some liver cell carcinomas.[10]

The over-all ultrastructural characteristics of these cells are therefore distinct from liver cells undergoing physiological division, e.g. following partial hepatectomy. On the contrary, some of the features are more characteristic of embryonic hepatocytes and even tumour cells. The tentative conclusion is that they are probably not merely reparative in character but represent a specific response to the ingestion of the carcinogen and that they are probably the starting point in the formation of larger hyperplastic nodules.[20] It will be shown in Part II of this series that cells with an essentially similar ultrastructure have been found in 4 larger hyperplastic nodules isolated from the precancerous liver.[16]

It should be emphasised that the small foci described are quite different from the so-called 'hyperbasophilic foci', since the latter are composed of de-differentiated cells which have largely lost any ultrastructural resemblance to normal liver cells.[21]

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