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Letter to the Editor

Appearance of Iron Free Hyperplastic Hepatocytes Following Ferric Nitrilotriacetate and 2-Acetaminofluorene Sequential Administration*

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THE DEVELOPMENT of rat liver tumors induced by chemicals is often preceded by the appearance of several subpopulations of transformed hepatocytes which are considered as precursors of cancer cells; they show distinctive morphological, histochemical and biological properties such as loss of single cell plates, starvation resistant glycogen storage, enzyme deficiency [glucose-6-phosphatase (G-6-Pase), ATPase etc.] or increase [γ-glutamyltranspeptidase (GGT)], enhanced rate of proliferation [1–3]. In addition, hepatocytes deprived of cytoplasmic iron have been observed both in liver tumors [4] and in preneoplastic lesions [5], suggesting a disturbance in the iron uptake or metabolism by the transformed cells.

In this letter I report the presence, lobular distribution and behaviour of iron-deprived hepatocytes in rats made siderotic by ferric nitrilotriacetate (Fe-NTA) administration and treated thereafter with 2-acetaminofluorene (2-AAF).

Male Sprague–Dawley rats 4 weeks old were used; they were treated with daily i.p. injections of Fe-NTA (0.5 ml/100 g body wt) for 30 days [6]. Then they were fed 2-AAF (0.05% in diet) for 20 days; subsequently half the animals were returned to the normal diet for an additional 20 days, whereas the others continued to receive the carcinogen. During this time i.p. injections of Fe-NTA were given every two days to all the animals. Controls received Fe-NTA or 2-AAF according to the same schedule.

Animals were killed every 10 days, some of them after 36 hr starvation to deplete glycogen. The liver was quickly sliced by hand; alternate slices were rinsed in saline and immersed in the Perls solution for the gross identification of the larger iron-free lesions according to Williams *et al.* [7]; soon after color development the adjacent unstained slices were fixed in Carnoy fluid or in 10% buffered formalin and embedded in paraffin. The sections were stained with haematoxylin and eosin, Perls method for iron and PAS method for glycogen. In some instances cryostat sections were also prepared for histochemical demonstration of G-6-Pase and GGT. The mitotic index was determined by scoring 5000 cells or more either within or outside the iron-deprived areas.

After one month of Fe-NTA administration Perls reactive granules were present within all the hepatocytes, irrespective of their lobular position; bile duct cells were negative; Kupffer cells only scarcely involved. The mean size of the iron-containing granules was consistently smaller in the centrilobular cells than in those around the portal tracts. At the end of the treatment (i.e. 70 days later) the siderosis in the livers of control animals was of great extent and remarkable homogeneity. Such a finding is in agreement with the results reported by Awai *et al.* [6]. The modified treatment schedule here employed did not induce significant iron deposition within the islets of Langerhans and the onset of a clear-cut diabetic status.

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When siderotic animals were treated with 2-AAF while still receiving parenteral iron a light to moderate iron loss by the centrilobular hepatocytes was observed within the second week of poisoning, so that most of these cells contained tiny iron granules. This could be an aspecific toxic effect of 2-AAF, because the drug omission from the diet caused iron reappearance in a few days.

On the other hand small groups of hepatocytes completely devoid of iron began to appear in the peripheral portions of the liver lobule and later increased. At the end of the intoxication period some of them gave rise to hyperplastic nodules devoid of laminar arrangement, which sometimes compressed the surrounding parenchyma. Their growth could be explained with the observed increase in the mitotic index (0.55 vs 0.00% of the surrounding tissue). Iron-free hyperplastic areas with similar mitotic indexes were present, even if to a lesser degree, in the animals recovering from 2-AAF administration. Thus, the aspecific drug toxicity cannot explain the absence of stainable iron from these lesions. Furthermore, most iron-free hepatocytes showed starvation-resistant glycogen storage; also G-6-Pase activity was often low or absent whereas the appearance of GGT activity was more irregular.

All these results are consistent with those previously reported by Williams *et al.* [7–9], which used a different, more time consuming, experimental schedule to induce an hepatic siderosis with an heavy involvement of cells other than hepatocytes; the time was sharply curtailed, however, when iron overload was achieved by subcutaneous injections of iron dextran [9, 10].

In conclusion, the early appearing perilobular hepatocytes devoid of iron can be considered as transformed, preneoplastic ones. The disturbance in the iron metabolism, which possibly reflects the disappearance of membrane receptors [11], can be used as a marker in the very early stages of liver carcinogenesis, offering also a tool for the preparation of pure cell populations in viable form.

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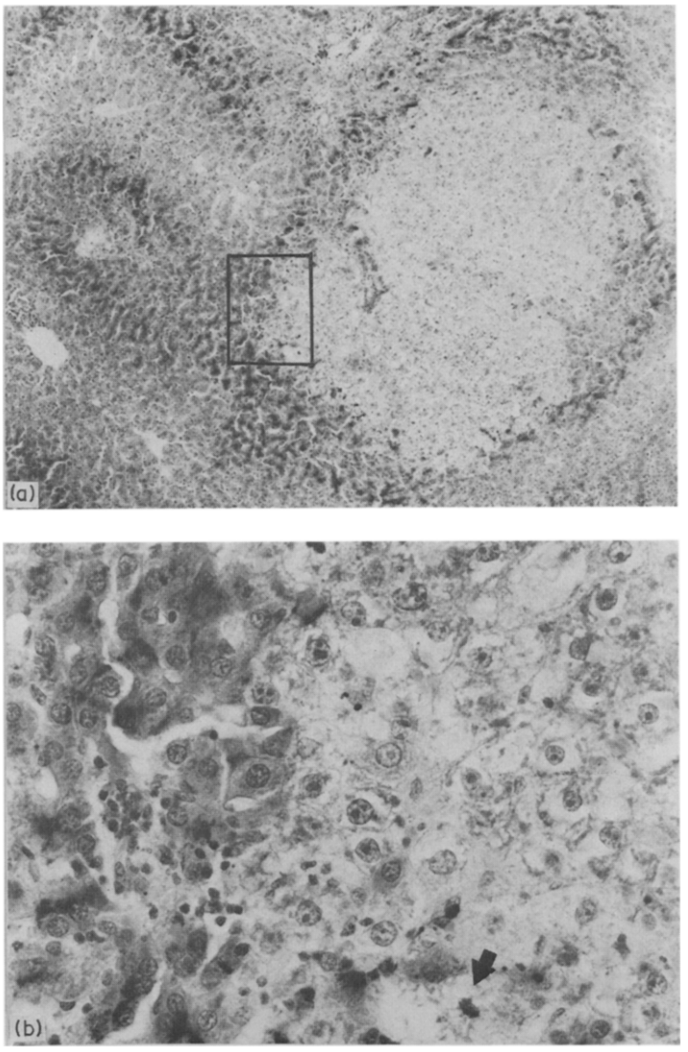

Fig. 1. (*a*) *An early hyperplastic liver nodule devoid of iron* 40 days *after* 2-AFF *administration; the hepatocytes of the outer third of the lobule show heavy iron deposition* (×45). (*b*) *Iron-free hepatocytes without laminar arrangement; some of them are vacuolated, partly by glycogen dissolution; arrow; mitosis* (×280). (*Perls reaction counterstained with carmalum*).