Aspects of Experimental Hepatocarcinogenesis

PART III. IRON OVERLOAD AND HEPATOCARCINOGENESIS

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SUMMARY

In order to investigate the possible influence of iron overload in the liver on the carcinogenic action of *p*-dimethylaminoazobenzene (*p*-DAB), a group of 40 rats was fed 4% ferric citrate in maize until the livers became quite markedly siderotic. *p*-DAB was then fed to the animals. No difference was observed between the experimental and control groups with regard to the final number of liver tumours which were produced. In the siderotic animals the fibrous tissue septa were occasionally broader than is usually the case in the controls and a prominent bile ductal response was sometimes evoked. It is concluded that iron overload does not significantly influence the action of a fairly potent carcinogen such as *p*-DAB.

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In haemochromatosis the incidence of primary carcinoma of the liver is said to range from 11,5% to 42,9%,[1-5] while in non-pigmentary cirrhosis the incidence is usually accepted as being much lower.[2] The reason for the high rate of malignant degeneration in haemochromatosis is not clear, though the iron itself may obviously be a factor. In contrast to this, the belief is generally held that in the indigenous Black races of South Africa iron is not an important factor in the development of primary liver carcinoma.[6-8] While there is no evidence to refute this hypothesis, the possibility that iron excess may aid the induction of tumours by other carcinogenic agents cannot as yet be completely excluded, especially in view of the known association of carcinoma and haemochromatosis. It has previously been shown[9] that iron overload may be a decisive factor in determining the effects of a weak hepatotoxic agent, e.g. acetamide. In these experiments the acetamide (a known carcinogen in some strains of rats) was able to elicit the formation of small, tumour-like nodules only in the presence of hepatic siderosis, while in the absence of excess liver iron it proved to be relatively innocuous even when administered for prolonged periods. In order, therefore, to explore the effects of iron overload on the action of a more powerful carcinogen, *p*-dimethylaminoazobenzene (*p*-DAB) was employed. This is known to be able to consistently produce a high incidence of liver cancer in the local strain of rats.

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EXPERIMENTAL METHOD

A group of 50 male rats of a locally bred albino strain was used. The weight of each animal at the onset of the experiment was 100 g. The animals were initially fed a maize diet to which ferric citrate had been added in a proportion of 4% by weight. This feeding continued for 2 months. A number of biopsies were done at this time and confirmed that the livers were siderotic. Ferric citrate administration was then stopped, and *p*-DAB feeding commenced, 0,05% (w/w) of the dye being mixed with the maize. Five animals were sacrificed at monthly intervals for the first 4 months after the start of the carcinogen diet. The remaining 30 animals remained on the carcinogen diet for a total of 20 weeks, after which they remained on a maize diet until the end of the experiment at 40 weeks.

When the animals died or were killed, a full autopsy was done. Tissue was removed from 2 or 3 lobes of the liver and processed for histological study by conventional methods. Sections were stained with haematoxylin and eosin, Perls' stain for iron, and with collagen and reticulin stains.

Since this experiment was undertaken at the same time as an investigation on a much larger series of 100 rats, in which the rats were killed at weekly intervals, it was considered that the latter would adequately serve as controls for the present experiment. The dosage of carcinogen and the basic maize diet were identical in the two groups.

RESULTS

Experimental Animals

By the end of the experiment at 40 weeks, 20 of the 30 animals had developed liver tumours (70%). These included liver cell carcinomas of trabecular type, undifferentiated solid carcinomas and adenocarcinomas, some of which were mixed tumours. The distribution of these three main tumour types were similar to that normally produced by using the present experimental methods (40%, 40%, 20%). The cirrhosis which accompanied all the tumours resembled that produced under 'iron-free' or control conditions, except that in about a quarter of the animals the fibrous tissue septa tended to be wider and bile duct proliferation was often more conspicuous (Fig. 1). The parenchymal cell changes, both degenerative and regenerative, were virtually identical in the two groups. By the end of the experiment the liver cells were almost totally iron-free; most of the iron present was contained

in large iron-laden macrophages in the fibrous tissue septa.

During the first 3 - 4 months of carcinogen administration, when the liver cells still contained iron in the cytoplasm, there was usually little quantitative or qualitative difference in the response of the iron-laden liver when compared with the controls.

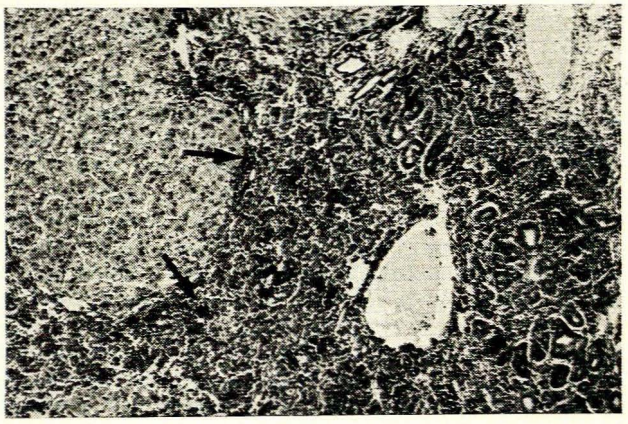

Fig. 1. A cirrhotic nodule (upper left) **is surrounded by a large mass of proliferated bile ducts. Many iron-laden macrophages are present** (arrows) **(H. and E. × 500).**

Controls

Of 40 animals which survived until the end of the experiment, 28 developed liver tumours (70%).

DISCUSSION

The possible role of excess iron in the production of liver disease is still controversial. It was demonstrated by Witzleben and Chaffey[10] that in the iron-laden liver the level of glucose-6-phosphatase actually was reduced compared with controls and, moreover, certain hepatotoxins, e.g. bromobenzene, could produce a greater fall in enzymes in such livers when compared with controls. There is also evidence to suggest that while massive iron overload in the liver *per se* does not lead to any gross degree of hepatic damage,[11] the combination of excess iron and an additional toxin, e.g. ethionine, may result in an increase in the severity and rapidity of development of cirrhotic changes in the livers of rats.[12] However, these findings have also been challenged.[13] It may be noted, too, that Bantu siderosis can be associated with hepatic fibrosis and on occasions a cirrhosis of portal type.[14] Though the position is therefore far from clear, it does seem that excess iron is not entirely innocuous. Its effects may depend on highly specific circumstances which still remain to be accurately defined, an opinion which has already been voiced.[10]

With this background, and in view of the high incidence of hepatic siderosis in the Black races of South Africa and the known association of primary liver cancer and haemochromatosis, the problem of the possible ancillary role of iron in the pathogenesis of malignant disease seemed worth re-examining.

In order to investigate this problem, Dunn[15] fed rats ethionine and gave them iron-dextran injections. Although the livers developed siderosis, no increase in the numbers of liver tumours was observed. In experiments performed by Williams and Yamamoto,[16] siderosis was induced in rats by the feeding of 8-hydroxyquinoline (HQ) with or without added ferrous gluconate, and the animals were then fed various carcinogens. In those animals fed the carcinogen plus HQ the incidence of liver cancer was lower than in those animals fed the carcinogen only. Experiments such as these are confusing, however, since the HQ could be antagonistic to the carcinogens.

The present studies have now shown that prior iron overload has failed to influence the carcinogenic effects of *p*-DAB. There was no difference in the percentage of animals which ultimately developed liver cancers or in the stage at which they appeared when these were compared with the controls. It should be noted that for most of the period during which the carcinogen was fed (20 weeks), iron was actually present in the liver cells, though in decreasing amounts. At the end of the experiment the liver cells contained little or no stainable iron. In the present experiments some increased collagen and reticulin formation did occur, but this was not surprising since it has been postulated that iron may stimulate fibrogenesis.[17] Differences between the effects of *p*-DAB and ethionine in this regard may be due to dietary or other influences, e.g. the strain of animals used.

In an earlier study it was reported that iron overload could apparently enhance the action of a weak carcinogen, acetamide, provided the iron was given in large doses at the same time as the carcinogen.[9] This potentiating effect of iron is not demonstrable when more powerful carcinogens are used. The findings may therefore shed some light on the fact that Bantu siderosis is not of special significance in tumour formation. Any possible enhancing influence which iron could exert would simply not be detectable in a population group in which there is already a high incidence of the disease, and which is presumably exposed to powerful carcinogenic influences, e.g. aflatoxin.

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