Rat Liver Parenchymal Cell Function during Azo Dye Carcinogenesis

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SUMMARY

Since azo dye hepatocarcinogenesis involves the parenchymal cells, rose bengal chromoexcretion, a property of the parenchymal cells, was investigated in rats during feeding of 4-dimethylaminazobenzene, a carcinogen, and 2-methyl-4-dimethylaminoozobenzene (2-Me-DAB), a noncarcinogen. These studies showed that both hepatic rose bengal uptake and excretion are reduced in rats fed the carcinogen. In rats fed the noncarcinogen, however, there was a normal hepatic rose bengal uptake and only a slight increase in the excretion of rose bengal. These results were related to a decrease in rat liver parenchymal cell function during azo dye hepatocarcinogenesis and reflected the changes in liver morphology produced by the carcinogen.

MATERIALS AND METHODS

Male Wistar rats (200 gm) were fed a basal diet low in protein (12, diet 3), containing 0.06% DAB or 2-Me-DAB. A control group received the basal diet alone. After the experimental diets were fed, laparotomy was performed at various intervals under Nembutal anesthesia in rats of all three groups. The common bile duct was exposed, ligated at about 10 mm from the duodenum, and cannulated by inserting a 26-gauge needle (to which was fitted a polyethylene tube, I.D. 0.018 in.) approximately 5 mm above the ligature. One milliliter of a solution of radioactive rose bengal in saline, containing 1 mg of the dye with a radioactivity of 5 mc, was injected in the right femoral vein. Blood samples (10 μl) were collected from the tail vein over a period of 3 hr. Samples of bile (5 μl) were collected from the cannula during the same period. The samples were plated for radioactivity measurements and rose bengal concentrations calculated.

RESULTS

A typical semilog plot of rose bengal concentration in bile and blood of a normal rat, during a period of 2 hr. after I.V. injection of 1 mg of rose bengal, is shown in Chart 1. A rapid rise in rose bengal concentration in
The rate constant of this uptake, according to standard kinetics, is given by the following equation:

\[ K = \frac{0.693}{T_{1/2}} \]

where \( T_{1/2} \) is the time required for the concentration to fall to half the initial value. In a normal rat (Chart 1), \( T_{1/2} \) is 3.5 min. and \( K \) is 0.2 min.\(^{-1}\). This signifies that every minute two-tenths of the rose bengal remaining in the blood is being removed. For a group of ten normal rats (200 gm. av. wt.), the values of \( T_{1/2} \) ranged from 3.5 to 4.5 min. and \( K \) was 0.155–0.20 min.\(^{-1}\).

The first part of the blood rose bengal concentration curve for one rat of each group, after 2 weeks of the three experimental diets, is given in Chart 2. The \( K \) values for the rats fed basal diet alone and basal diet plus 2-Me-DAB were equal to that obtained for the normal rat. For the rat fed DAB, \( K \) was 0.042. This means that after 2 weeks of DAB the hepatic rose bengal uptake was five times slower than in the control rat. This different effect of DAB and 2-Me-DAB is also apparent in Chart 3 where the \( K \) values for different periods of feeding are plotted. Each point represents one rat. After 2 days of DAB feeding, the blood clearance of rose bengal decreased and continued to decrease until the end of the experiment (90 days). Rats fed basal diet alone showed a rose bengal clearance within the normal range except for a period of 1 week between the 1st and the 2d week. The significance of this decreased clearance is uncertain. Rats fed 2-Me-DAB showed a normal rose bengal uptake in spite of greater individual variations.

These differences in rose bengal hepatic uptake after DAB or 2-Me-DAB feeding are reflected in the excretion curves of Chart 4, which show maximum rose bengal...
diets at different periods. The concentration of excreted rose bengal in the bile of rats fed the three experimental diets was always lower than in rats fed the basal diet. The rose bengal concentration, after 1 week of DAB, was normal, and then decreased appreciably to a minimum at approximately the 4th week. A slow rise toward basal values followed. The rats fed 2-Me-DAB appeared to excrete the dye at a higher concentration than the controls.

Animals fed azo dye partially excreted it in the bile. Chart 5 shows the absorption spectra for bile, diluted 1:50 in formic acid, collected prior to rose bengal injection, for one rat of each group. The absorption spectra of DAB and 2-Me-DAB in formic acid are also shown. The wavelength of maximum absorption of bile in rats fed the azo dyes corresponded to the wavelength of maximum absorption of the free dye in formic acid. By using the optical density of bile at this wavelength (510 μm), tentative figures may be ascribed to azo dye concentration in bile, since the molar extinction coefficients in formic acid were 0.0525 cm⁻¹ for DAB and 0.0605 cm⁻¹ for 2-Me-DAB. The optical densities of bile, after different periods during the experimental diets, are shown on Chart 6. Rats fed 2-Me-DAB showed a higher concentration of azo dye in the bile than did rats fed DAB. Apparently, the dynamics of azo dye excretion in bile was also different in the two groups fed such dyes. A high concentration of azo dye was attained in the bile of rats fed 2-Me-DAB after 1 week; but the concentration of azo dye in rats fed DAB increased gradually to reach a maximum between the 3rd and the 4th week, after which it slowly decreased. In rats fed the basal diet alone, bile diluted 1:50 in formic acid showed negligible absorption at 510 μm.

The changes which were noted in liver function, during DAB feeding, were accompanied by changes in the lobular distribution of basophilia. In normal liver (Fig. 1), basophilia was relatively homogeneous. After 1 week of DAB feeding (Fig. 2), there was a loss of basophilia around the central vein; regions of hypobasophilia extended toward each other and completely surrounded the portal spaces (Fig. 3). After 5 weeks of DAB feeding the architecture of the liver was markedly modified by bands of necrotic tissue surrounding the portal spaces (Fig. 4). Portal regions which have maintained their basophilia become the site of tumor formation after abnormal regeneration (6).

**DISCUSSION**

Carcinogenic and noncarcinogenic azo dyes behaved differently toward rose bengal uptake by the liver. The carcinogenic azo dye DAB decreased to a great extent the rose bengal uptake, whereas the noncarcinogenic azo dye 2-Me-DAB produced no change (Charts 2 and 3). Apparently, abnormal liver function, after DAB feeding, is related to the carcinogenic effect of DAB. The decreased liver function became evident on the 2nd day of DAB feeding. Maximum effect occurred at 21 days and was continued until the experiment was terminated (90 days). It was shown that progressive degeneration of liver parenchyma occurs during the first 30 days of azo dye hepatocarcinogenesis (17, 18, 20). The effect of DAB on liver function could therefore be attributed to early damage of parenchymal cells, since rose bengal uptake and excretion requires a normal-functioning parenchyma. Moreover, it was shown (Figs. 2–4) that hypobasophilia and necrosis modify the morphology of liver. Maximum necrosis occurs at 30 days, which coincides with maximum DAB binding to parenchymal cell proteins (8, 13). It appears, therefore, that minimum rose bengal uptake at 21 days and minimum rose bengal excretion at 28 days are manifestations of the diminished function of liver parenchyma due to DAB binding.

In view of the possible role of mitochondria in liver excretion (5) and the fact that 2-Me-DAB is known to increase mitochondrial population (1, 2) it was felt that this might explain the difference in rose bengal excretion which was noted in rats fed the noncarcinogen 2-Me-DAB.

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**CHART 6.** Optical density, at 510 μm, of bile, diluted 1:50 in concentrated formic acid, for rats of the three groups following different periods during diets. Each point represents one rat.

**CHART 5.** Absorption spectra of bile, diluted 1:50 in concentrated formic acid, for one rat on each of the experimental diets for 14 days (—). Absorption spectra of 2-Me-DAB (2 × 10⁻⁴ m) and DAB (0.85 × 10⁻⁴) in concentrated formic acid (-----).

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Nevertheless, two other conditions must be considered which could lead to a decreased rose bengal uptake: (a) bile stasis preventing elimination of rose bengal from the parenchymal cells and resulting in a diminished uptake of rose bengal from the bloodstream, and (b) lowered hepatic blood flow.

(a) Bile stasis is presently not being considered as an explanation for the low rose bengal uptake, since Mirvish and Gillman have shown that there is an increased bile flow during DAB hepatocarcinogenesis (14). But low hepatic blood flow (b) above cannot readily be set aside. It has been shown that early in carcinogenic azo dye feeding there occurs a stimulation of the reticuloendothelial system (9). In view of the importance of endothelial and Kupffer cells in hepatic blood flow (19), further investigation is necessary before one can eliminate the part played by the hepatic blood flow following DAB feeding in rats.

Apparently, rose bengal uptake and excretion by liver are reduced during the early stage of DAB hepatocarcinogenesis. This change in liver function may be attributed to early damage of parenchymal liver cells. No such effect is produced by 2-Me-DAB, a noncarcinogenic azo dye.

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