A Histochemical Study of Succinic Dehydrogenase Activity in Rat Liver Cancerogenesis Induced by 3-Methyl-4-Dimethylaminoazobenzene

The changes in the dehydrogenases and cytochrome enzyme systems in liver tumours induced with azo dye are extensively investigated biochemically[1]. Relatively few histochemical studies on these enzyme systems in azo dye cancerogenesis and tumours have been carried out[2-4]. The results obtained are still controversial on some points. In order to contribute to the problem of the histogenesis of azo dye-induced rat liver tumours, we performed experiments on the histochemical detection of succinic dehydrogenase activity in the course of rat liver cancerogenesis induced by 3-methyl-4-dimethylaminoazobenzene (3-Me-4-DAB), as well as in primary liver tumours induced by this cancerogen.

Experiments were carried out with albino rats weighing 100–120 g, fed a semi-synthetic diet containing 0.06% 3-methyl-4-DAB[5]. Control animals were fed the same diet with the cancerogen omitted. Control and experimental animals were killed at 9 a.m. on the 15th, 30th, 60th, 90th, 120th and 150th days after the beginning of the experiment. Sections of the left lobe of the liver and sections of the liver tumours were processed according to the method of CHELTON and SCHNEIDER[6].

The morphological changes in the liver in cancerogenesis were the same as those observed by a number of other authors[5,7]. In the first 15 days of cancerogen feeding, there were no changes in the succinic dehydrogenase pattern characteristic for the normal liver: high enzymatic activity in liver parenchyma cells and no detectable activity in cholangioepithelial cells. At the end of the first month, a marked decrease was recorded in succinic dehydrogenase activity of liver parenchyma cells in the intermediate portions of the liver lobules (Figure 1). To a lesser degree, such changes were observed in liver sections of control animals. In our experimental series a diffuse growth of cholangioepithelial cells was noticed towards the 60th day, forming greyish nodules. In single cases larger nodules of 5-6 mm in diameter were observed having a stroma characteristic for cholangiomas. In these cases differentiation of cholangiofibrotic areas from cholangiomas proves difficult. Differing from normal bile duct cells, a positive succinic dehydrogenase reaction was observed in the bile ducts of cholangiofibrosis, as well as in cholangioma nodes originating from them (Figure 2). The connective tissue stroma shows a negative reaction, while the hyperplastic areas of liver parenchyma cells display a high succinic dehydrogenase activity which does not differ from that of the normal liver parenchyma (Figure 3). Towards the 120th day, single and multiple

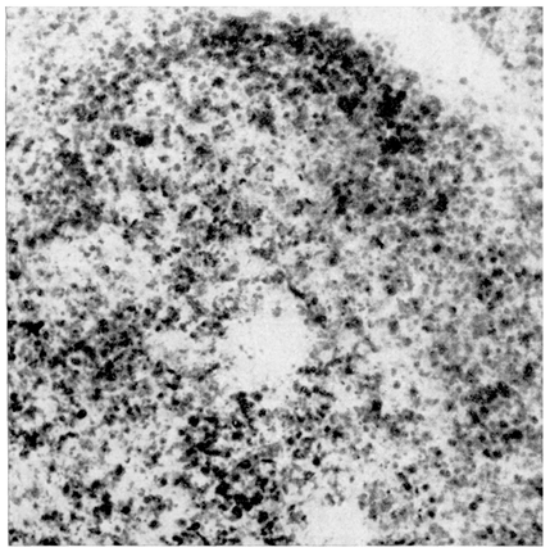

Fig. 1. Decrease of succinic dehydrogenase activity in the intermediate portions of liver lobules. 60 days of 3'-Me-4-DAB. ×100.

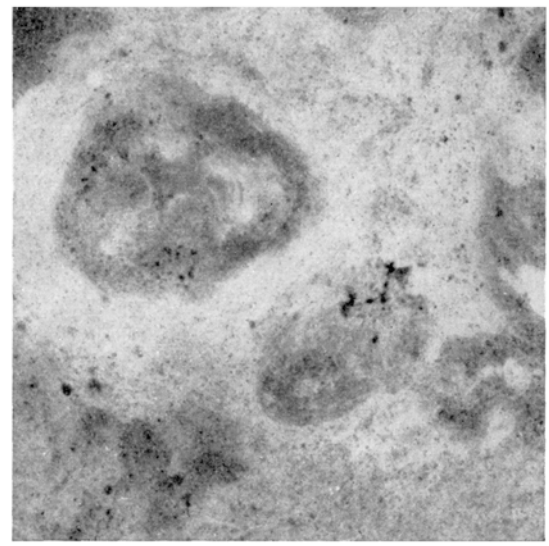

Fig. 2. Cholangiofibrosis with strongly positive succinic dehydrogenase reaction in bile ducts. ×100.

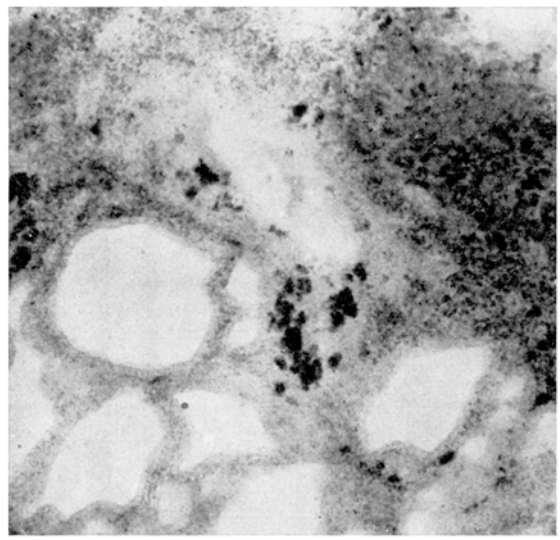

Fig. 3. Hyperplastic liver parenchyma nodule, positive reaction in parenchyma cells. Negative reaction in cystic bile ducts. ×100.

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[3] B. PEARSON and V. DEFENDI, Cancer Res. *15*, 593 (1955).
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large tumour nodes occurred which, according to their histological structure, may be divided into hepatomas and cholangiomas. In the tumours with cholangioma structure, a positive reaction for succinic dehydrogenase activity is observed in accordance with the results of PEARSON et al., obtained by a different method[3]. Formazan crystals are detected in the outgrown ducts, succinic dehydrogenase activity being increased with the number of layers of the epithelium of their walls as well as with increasing of cellular cytoplasm. In the large cystic formations, no positive reaction is observed (Figure 3) with the exception of some rare cases, when the epithelium starts growing and destroying the structure of the cysts and the adjacent tissues. Differing from cholangiomas, solid tumours of the hepatoma type show a negative succinic dehydrogenase reaction (Figure 4). In single cases (3 animals), we observed solid tumours with nuclear histogenesis, which showed a positive succinic dehydrogenase reaction – less intensive than that of normal liver parenchyma. Probably these tumours represent a transition to a hepatocarcinoma, which later on may display a negative succinic dehydrogenase reaction. The possibility exists too that these tumours are cholangiocarcinomas in a stage of dedifferentiation and parallel loss of succinic dehydrogenase activity.

Fig. 4. Solid tumour node of the hepatoma type, no succinic dehydrogenase activity. Positive reaction in the adjacent liver parenchyma. ×100.

The results of our experiments show that, in the course of azo-dye induced rat liver cancerogenesis, the succinic dehydrogenase activity in parenchyma and bile duct cells follow a definite pattern. In typical hepatoma, the activity is markedly lower than in normal parenchyma cells and in hyperplastic parenchyma cell nodules. The negative reaction in normal bile duct cells becomes positive in cholangiofibrosis and cholangiomas with a possible subsequent decrease in more dedifferentiated cholangiocarcinomas.

The 'tissue dilution artefact' as commented on by JONES et al.[8] should be taken into account in the evaluation of biochemically obtained data, but our histochemical results confirm the biochemically obtained results, showing quantitative changes in liver succinic dehydrogenase activity in azo-dye cancerogenesis.

Résumé. L'auteur a étudié histochimiquement l'activité de la succine déhydrogénase lors de la cancérogénèse expérimentale du foie des rats induite par le 3'-méthyl-4-diméthylaminoazobenzène. On a observé une augmentation de l'activité enzymatique dans les stades précancéreux (cholangiofibrose et hypertrophie parenchymateuse) suivie d'une diminution et disparition de cette activité dans les tumeurs développés.

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Progressive Localization of Neutral-Red Positive Region with Morphogenesis in *Limnaea*

The classical method of vital staining in experimental embryology was given a sharp impetus by REVERBERI et al. REVERBERI[1] had, for example, pointed out a fruitful method of detecting the localization of mitochondria in Ascidian eggs by means of vital staining with Janus green. This and many other attempts to follow the reorientation and redistribution of tangible substances, which go hand in hand with chemodifferentiation, have been ably summed up by REVERBERI[2]. It is obvious that the changing pattern of localization of mitochondria (which houses the many important enzymes) and other substances, following fertilization, closely reflects the changing substratum which culminates into chemodifferentiation.

We have therefore followed this method, which REVERBERI et al. have so fruitfully employed in the case of marine molluscs and ascidia, in our work on fresh water mollusca, stained with Janus green and the supra vital stain, neutral red. As neutral red in very dilute solution has absolutely no toxic effect on *Limnaea* embryos (in fact, they can be kept in neutral red for 48 h and still hatch out into living snails), it is a particularly convenient agent for this sort of work. The *Limnaea* embryos are, however, far from being ideal material for study, namely, glass-clear embryos with little or no colouring matter. Actually, the fresh water molluscs, including *Limnaea*, have a regiment of natural colouring substances, as COMFORT[3] has pointed out, some U-V fluorescing components of which are manufactured, as we have found[4], before hatching. Nevertheless, the visible natural colour in the embryos is not too intense to mask the brilliant hue due to neutral red. Thus a worthwhile study can be carried out until just after hatching, when the outer shell begins to thicken.

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