Effects of 3′-Me-DAB on Parenchymal Polyploid Cells of Rat Livers[1),2),3)]

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(With 2 Text-figures and 2 Tables)

A considerable amount of work has been done on carcinogenesis in livers of rats fed azo dye. It has been known that the rat liver is a cytologically heterogeneous tissue with regard to ploidy of parenchymal cells. Sulkin (1943), Naora (1957), Alfert and Geschwind (1958) and Honda and Makino (1964) reported that in adult rats, about 80 per cent of hepatic cells were polyploid, the remaining cells being diploid. In this situation, interesting is a possible effect of azo dye on ploidy frequency of hepatic cell populations. Use being made of microspectrophotometry, Stich (1960) studied changes in the ploidy frequency in livers of rats fed azo dye, and noted an increased occurrence of aneuploid cells.

The present study deals with the ploidy frequency of hepatic cells through direct chromosome countings in livers of hepatectomized rats which had been fed azo dye with particular reference to differential effects of azo dye on diploid and polyploid cells.

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Materials and methods: Adult rats of Wistar-King A Strain (both sexes, 1–3 months of age and 65–220g of body weight) were used in this study. The animals were fed *ad libitum* 0.06 per cent 3′-metydl-4-dimethylaminoazobenzene (3′-Me-DAB) in the basal diet used in the Makino laboratory.

After 1, 4 and 6 months of the azo dye administration, animals were partially hepatectomized according to the technique of Higgins and Anderson (1931), and killed about 48 hours after the operation during 10–12 a.m. to minimize the effect of the diurnal variation on mitotic frequency. Very thin marginal strips of the remaining lobe were

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removed and minced with scissors into fine pieces. The chromosome slides were prepared with the following procedure: the minced tissue was treated with 0.5 per cent sodium citrate for 10 to 15 minutes and squashed according to the acetic dahlia squash method. Rats fed the basal diet and hepatectomized served as controls.

The frequency of diploid and polyploid cells was determined on the basis of reliable metaphase figures with the aid of oil immersion lens.

On the other hand, pieces of livers removed were frozen in liquid nitrogen and sectioned 10 micra thick in a cryostat at −20°. Frozen-substituted sections were made according to the method of Chang and Hori (1961, 1962a, b) and stained with Lison's toluidine blue method for ribonucleic acid (1953).

Results

Frequencies of diploid and polyploid metaphase cells in regenerating livers of normal rats after partial hepatectomy are presented in Table 1. Referring to

Table 1. Frequency of diploid and polyploid metaphase cells in regenerating liver tissues of normal rats

Rat no.	Body weight	Sex	Age	Ploidy frequency of metaphase cells		No. of cells observed
				diploid	polyploid	
1	115 g	M	2 mon.	29.9%	70.1%	458
2	105	F	2	33.6	66.4	265
3	175	M	3	31.9	68.1	160
4	290	M	5	18.0	82.0	100
5	290	M	9	25.0	75.0	100
6	245	F	9	18.0	82.0	100
			Average	23.2%	76.8%	

the data in the table it is apparent that in 2 to 9 month-old rats, polyploid cells showed a high frequency in mitotic cell populations. The majority of the metaphase cells (76.8 per cent on an average) were polyploid regardless of the age and body weight of rats, while diploid cells were few in occurrence, being 23.2 per cent on an average.

On the other hand, livers from partially hepatectomized rats showed a very low frequency of polyploid metaphase cells, being 14.4 per cent on an average (Table 2) in comparison with the frequency value in control specimens at corresponding ages, while the frequency of diploid metaphase cells was high showing 85.6 per cent on an average. Livers from rats fed azo dye for one month showed 26.4 per cent polyploid cells and 73.6 per cent diploid ones respectively, on an average. In rats fed azo dye for 4 and 6 months, polyploid metaphase cells strikingly decreased showing 8.3 per cent on an average with a variation from 4.4 to 11.2 per cent. The results presented may imply that polyploid liver cells are influenced more severely

by azo dye than diploid liver cells in regeneration following partial hepatectomy. The influence of azo dye on polyploid cells seems to increase with the passage of time in feeding.

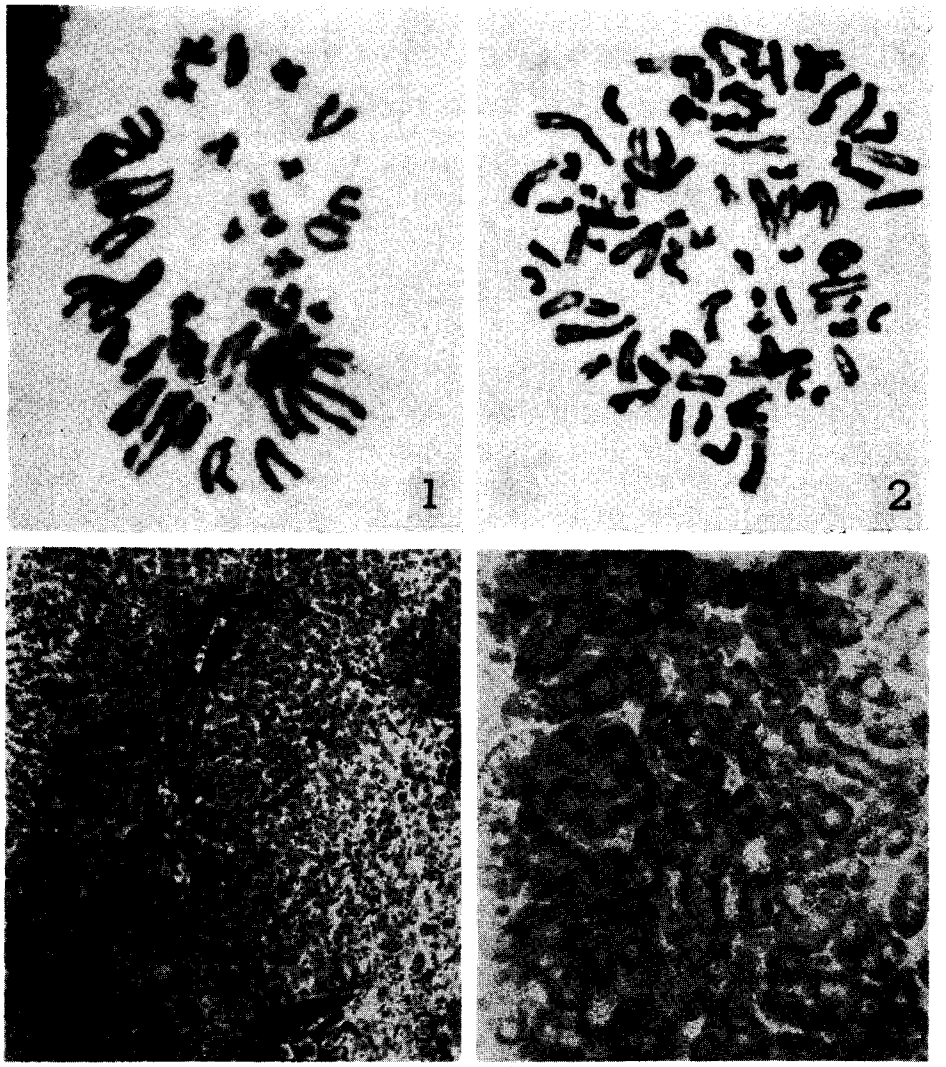
Table 2. Frequency of diploid and polyploid metaphase cells in regenerating liver tissues of rats fed azo dye

Rat no.	Body weight*	Sex	Age*	Period of azo dye feeding	Ploidy frequency of metaphase cells		No. of cells observed
					diploid	polyploid	
1	190 g	M	2 mon.	1 mon.	64.8%	35.2%	71
2	220	M	2	1	86	14	100
3	200	M	3	1	69.5	30.5	200
4	135	F	3	1	74	26	130
				average	73.6%	26.4%	
5	270	M	5 mon.	4 mon.	88.8	11.2	125
6	180	F	5	4	93.4	6.6	75
7	190	F	5	4	95.6	4.4	90
				average	92.6%	7.4%	
8	240	M	8 mon.	6 mon.	89.7	10.3	116
9	190	F	9	6	90.9	9.1	110
				Average	90.8%	9.2%	

*) Age and body weight at partial hepatectomy

Histologically, general features of livers of the rats fed azo dye for one month were apparently normal, except the fact that a slight bile duct proliferation and regenerating cells occurred in periportal areas of hepatic lobules. Those regenerating cells were characterized by a small nucleus and a little amount of the cytoplasm. The staining reaction for toluidine blue was more intense in the regenerating cells than in normal cells. Basophilic clumps were represented by diffused fine granules and distributed in the periphery of the cytoplasm. Similar cytochemical features were reported by some other investigators in rat livers fed azo dye (Orr, 1940; White and Edward, 1943; Opie, 1944; Firminger, 1955; Daoust and Molnar, 1963; Hori, 1965). Livers of rats fed for four months are characterized by histological changes being remarkable by the formation of pseudolobules consisting of regenerating cells. Cholangiofibrosis was hardly observed in such specimens. Degenerating cells contained also basophilic fine granules in the cytoplasm, distributing only in a narrow peripheral area of the cytoplasm. Two rats, no. 5 and 6 showed small nodules of hyperplastic cells. Fine basophilic granules were distributed homogeneously throughout the cytoplasm of those cells. The most drastic changes were observed in no. 5 rat which had been fed the dye for 4 months. In livers from rats fed the dye for six months, hyperplastic cells increased in number

together with highly hyperplastic cells. Cholangiofibrosis was observed in these rats.



Figs. 1 and 2. Photomicrographs of cells in regenerating rat livers following partial hepatectomy. 1: diploid cell. 2: polyploid cell (tetraploid)

Fig. 3. Regenerating liver cells appeared in response to the administration of 3′-Me-DAB (lower left) × 70.

Fig. 4. Same as Fig. 3. High magnification. Basophilic granules appeared in the periphery of the cytoplasm with long axis.

Discussion

Based on microspectrophotometry studies (Naora, 1957; Stich, 1960) as well as on direct measurements of interphase nuclei (Sulkin, 1943; Alfert and Geschwind, 1958), it has been shown that the rat liver is a cytologically heterogeneous tissue with respect to ploidy of parenchymal cells. In adult rats, about 80 per cent of hepatic cells were polyploid with the remaining cells which were diploid. The same tendency was also obtained in a frequency of diploid and polyploid metaphase cells in regenerating livers of partially hepatectomized rats (Honda and Makino, 1964; Matsuzawa and Honda, 1965). Information was provided in the present study that a remarkable difference occurred in frequency of diploid and polyploid metaphase cells between regenerating livers of rats fed azo dye after partial hepatectomy and those of regenerating livers of non-treated rats. In the rat livers treated with azo dye, the frequency of polyploid metaphase cells was quite low being 14.3 per cent on an average, with a high frequency of diploid cells at 85.7 per cent on an average. Similar tendency was obtained by Sitch (1960) in precancerous livers of rats fed azo dye after partial hepatectomy, based on the microspectrophotometric measurement of DNA contents.

Daoust and Molnar (1964) reported that the administration of 4-dimethylaminoazobenzene (DAB) to rats produced cellular damages in livers resulting in the loss of about 50 per cent of the parenchymal cells at early stages. This seems to indicate that a compensatory cell proliferation takes place in response to such cell damages produced by the carcinogen (Daoust, 1962, 1964). Similar evidence has been presented in rats fed 3′-Me-DAB (Price *et al.*, 1952).

The data cited above may imply that diploid and polyploid cells of rat livers differ in the sensitivity to azo dye. It is apparent in the light of the present results that diploid cells are more resistant against the cell damage than polyploid cells, as far as cell division is concerned and that the injured cells, mainly polyploid ones, may become necrotic with final damage. On this basis, it is most probable that diploid cells divide more frequently than polyploid cells after partial hepatectomy.

In reference to data involving primary hepatomas of rats induced by azo dye (Yosida and Ishihara, 1956, 1957; Honda, 1963), a suggestion is possible that most of the primary hepatomas observed might have originated from diploid parenchymal cells, and that in the course of carcinogenesis or after development into hepatomas, the chromosomes might become polyploid.

It remains uncertain that azo dye exerts damaging effects on parenchymal diploid cells during the course of the destruction damage of polyploid cells. This is a subject of further investigation now in progress.

Summary

The frequency of diploid and polyploid cells at metaphase was studied in the liver of rats fed azo dye after partial hepatectomy.

Polyploid cells showed a remarkably low mitotic frequency in livers of rats fed azo dye, with a high frequency of diploid cells. Evidence presented suggests that diploid and polyploid parenchymal cells of rat liver differ in the sensitivity to azo dye.

Histologically, the livers of one month azo dye treated rats was normal in general appearance. Regenerating hepatic cells made their appearance in four months treated livers. In six months treated livers, hyperplastic cells were prominent.

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