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Notes on the Early Effect of 3′-Me-DAB Feeding on Parenchymal Cells of Rat Liver[1),2)]

By

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(With 3 Text-figures and 2 Tables)

It has been accepted that rat liver is heterogeneous for ploidy of parenchymal cells (Sulkin, 1943; Naora, 1957; Alfert and Geschwind, 1958; Honda and Makino, 1964). In adult rats, about 80 per cent of hepatic cells were polyploid, the remaining nearly 20 per cent of cells being diploid. On the other hand, Honda and Matsuzawa (1965) have reported that diploid cells differ from polyploid cells in sensitivity to a carcinogen, 3′-methyl-4-dimethylaminoazobenzene and that the diploid cells are more resistant to azo-dye than the polyploid. There is, however, obtained no information for the evidence that the diploid parenchymal cells undergo damage by the azo-dye in any early stage after the application of the chemicals. In this connection the present experiment was undertaken with speical concern to an early effect of 3′-Me-DAB administration on cell division of the liver parenchymal tissue as a part of serial work of a similar nature.

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Materials and methods: Wistar-King-A rats, both sexes, 11 to 12 week-old and 135-180g in body weight were fed *ad libitum* a basal diet containing 0.06 per cent 3′-Me-DAB (3′-methyl-4-dimethylaminoazobenzene). Control rats were fed the basal diet alone.

On the 5th day of azo-dye administration, the animals were partially hepatectomized, and fed normal diet. They were killed about 48 hours after the operation during 10–12 a.m. in order to minimize the effects of the diurnal variation on mitotic frequency. The subsequent procedures were the same as those described in the previous paper (Honda and Matsuzawa 1965).

The frequency of diploid and polyploid cells, as well as the mitotic index were determined on the basis of metaphase figures.

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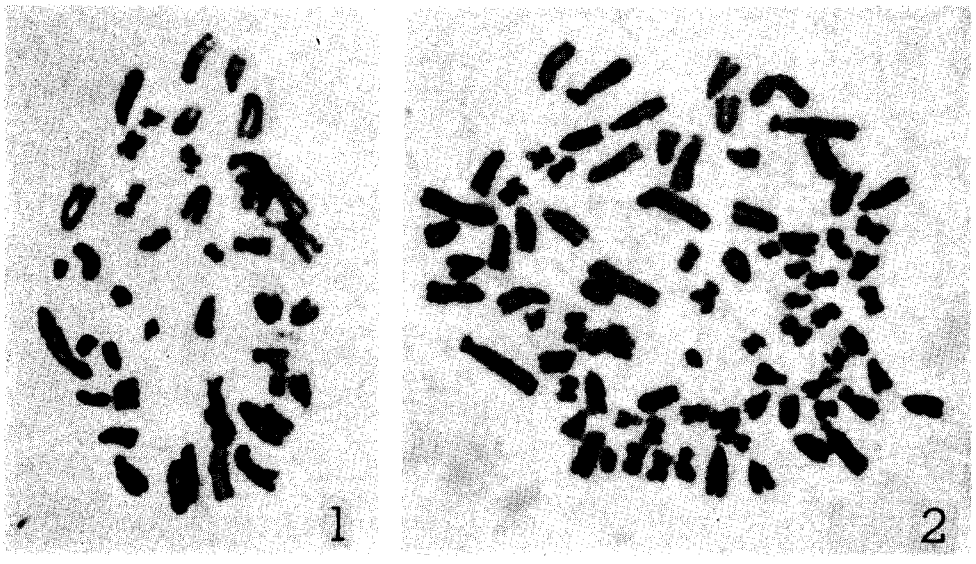
Results

Frequency values of diploid and polyploid metaphase cells and the mitotic indices obtained in the experiment are given in Table 1. Referring to the data in the table, it is apparent that averaged frequencies of regenerating normal liver tissues following partial hepatectomy were 31.4 per cent for diploid cells and 68.6 per cent for polyploid ones. Mitotic indices of regenerating adult livers were 2.0 per cent on an average with a range from 1.3 to 2.3 per cent. It is of particular interest that in normal rat livers, there is no remarkable change in the frequency of

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

Rat no.	Body weight	Sex	Age	Ploidy frequency of metaphase cells		Mitotic index	No. of cells obs.
				diploid	polyploid		
1	145 g	M	10 wks	34.7%	65.3%	1.3%	176
2	160	M	10	28.6	71.4	2.3	262
3	270	M	12	24.7	75.3	2.2	150
4	180	M	13	37.5	62.5	2.2	208
			Average	31.4%	68.6%	2.0%	



Figs. 1–2. Photomicrographs of cells in regenerating rat livers following partial hepatectomy. 1: diploid cell. 2: polyploid cell.

diploid and polyploid cells at metaphase, though mitotic indices varied to a considerable extent. This seems to imply that both diploid and polyploid cells of normal livers divided in proportion to their frequency values in parenchymal cell populations.

Table 2. Frequency values of diploid and polyploid metaphase cells and mitotic indices in regenerating liver tissues of rats fed azo-dye for five days

Rat no.	Body weight	Sex	Age	Period of azo-dye feeding	Ploidy frequency of metaphase cells		Mtitotic index	No. of cells obs.
					diploid	polyploid		
1	135 g	F	12wks	5 days	64.7%	35.3%	0.51%	203
2	150	M	11	5	60	40	0.55	105
3	150	F	12	5	59.7	40.3	0.8	119
4	150	M	12	5	62.5	37.5	0.9	160
5	180	F	12	5	52	48	1.2	125
				Average	59.8%	40.2%	0.79%	

Table 2 provides frequency values of diploid and polyploid metaphase cells and mitotic indices in regenerating livers of partially hepatetomized rats which had been fed azo dye for five days. The frequency values of polyploid cells decreased strikingly in azo dye fed rats: diploid cells showed 59.8 per cent in average frequency, while the frequency of polyploid cells was 40.2 per cent on an average. Mitotic indices showed also a marked decrease. It is apparent that the frequency values of diploid and polyploid cells showed a remarkable change along with the change of mitotic indices. This indicates apparently that polyploid cells were injured more heavily by azo-dye than diploid cells.

On the basis of the above data obtained it is possible to estimate the damage of diploid cells. The relative values of mitotic frequencies of both diploid and polyploid cells to those of non-treated rat livers may be represented by the following equations:

$$\mathrm{Drf} = \frac{\mathrm{Ot}\cdot\mathrm{Qt}}{\mathrm{Oc}\cdot\mathrm{Qc}} \times 100$$

$$\mathrm{Prf} = \frac{\mathrm{Pt}\cdot\mathrm{Qt}}{\mathrm{Pc}\cdot\mathrm{Qc}} \times 100$$

Drf: relative frequency of diploid metaphase cells. Prf: relative frequency of polyploid metaphase cells. Ot and Oc: per cent of diploid metaphase cells of rat livers treated and non-treated by azo-dye, respectively. Pt and Pc: per cent of polyploid metaphase cells of rat livers treated and non-treated by azo-dye. Qt and Qc: mitotic indices of rat livers treated and non-treated by azo-dye.

Cell damages were estimated in diploid and polyploid cells based on the above

formula, and the results are graphically shown in Figure 3. The relative frequency values of both diploid and polyploid cells at metaphase to control values strikingly decreased in the rats fed azo-dye: approximately one quarter of polyploid cells, and three quarters of diploid cells in the liver parenchymal tissues divided in response to partial hepatectomy. Evidence obtained seems to indicate that the mitotic activity of polyploid cells is repressed to a great extent under the effect of the carcinogen, in comparison with diploid cells.

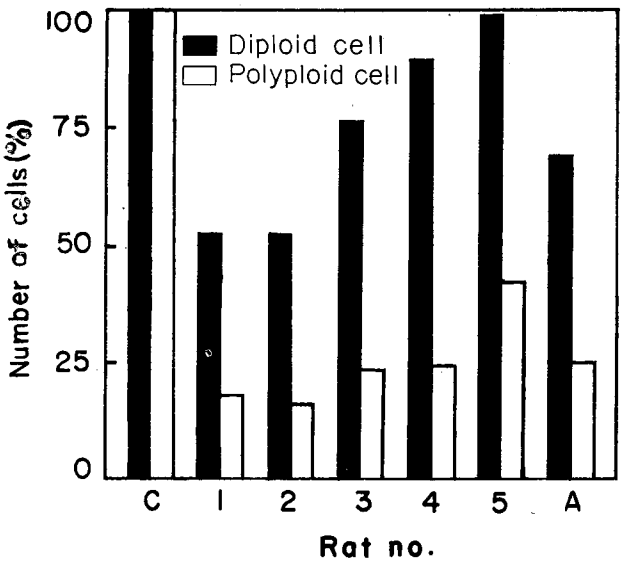


Fig. 3. Histogram showing relative frequency values of diploid and polyploid metaphase cells in partially hepatectomized rat livers administered with azo-dye for five days to those of non-treated rat livers. C: control values in non-treated rat livers. A: average values in rats treated by azo-dye.

Discussion

In a former paper, Honda and Matsuzawa (1965) have shown that diploid liver parenchymal cells of rats differ consistently from polyploid cells in the sensitivity to 3′-Me-DAB. The results of the present study supplement the above feature. Further, the present study has provided the result that about three quarters of diploid cells divide in response to partial hepatectomy after 5 day administration of azo-dye, as shown in figure 3.

Based on the direct measurement of interphase cell nuclei of adult rat livers, Sulkin (1943) reported that in centrolobular area, there is a sharp increase in frequency of octoploid and 16 ploid nuclei (hyperploid), comparing to those of the whole liver tissues. In this connection, it is particularly significant to know that

the progressive degeneration of liver parenchyma and decreased liver function occurs first in centrolobular area during the early stages of azo-dye carcinogenesis (Daoust and Molnar, 1964; Watters and Cantero, 1965). Watters and Cantero (1965) reported that liver function of uptake and excretion of rose bengal decreased evidently on the 2nd day, and that the maximum effect occurred on the 21st day of DAB feeding. Disintegration of parenchymal cells lying in pericentral area was especially apparent at the early stages of azo-dye feeding showing a maximum on the 30th day (Daoust and Molnar, 1964), with changes in the lobular distribution of basophilia. Watters and Cantero (1965) showed that after one week of DAB feeding, there was a loss of basophilia around the central vein. All the above reported features seem to be a series of closely related nature and suggest a possibility that in the early period of azo-dye feeding, parenchymal polyploid cells lying in the centrolobular region undergo damage by the carcinogen, resulting in a loss of basophilia, as well as in a decrease of uptake and excretion of rose bengal in the centrolobular region of liver.

Summary

The frequency of diploid and polyploid cells at metaphase and the mitotic index of hepatic cells were studied in regenerating livers of partially hepatectomized rats which had been fed azo-dye for five days. Information was obtained that, in the early stages of azo-dye feeding polyploid parenchymal cells underwent damage to a great extent under the influence of the carcinogen, in comparison with diploid cells. Discussion was made on the centrolobular distribution of polyploid cells, their sensitivity to azo-dye, progressive degeneration of liver parenchyma, and decreased liver function during the early stages of azo-dye carcinogenesis.

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