Cytological and Histochemical Studies on Regenerating Livers of Rats with Special Reference to Age[1),2),3)]

By

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(With 6 Text-figures and 1 Table)

Recently, Honda (1964), and Honda and Makino (1964) reported the change in chromosomal ploidy in regenerating rat-liver cells with age. On the other hand, Matsuzawa (1964b) found that the liver esterase activity occurred first in centrolobular cells and gradually in periportal cells in the course of post-natal development of rats, with together an increase of the total activity.

The present study was undertaken in a hope to inquire into any possible relationship between the chromosome ploidy of hepatic cells and the esterase activity by means of histochemical study and electrophoresis.

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Materials and methods: Both male young (2–3 week-old, weighing 20–30g) and adult rats (7–12 week-old, weighing 115–130g) of Wistar-King A strain were used for study. Partial hepatectomy was carried out following the method of Higgins and Anderson (1931). The animals were killed two days after partial hepatectomy.

For chromosome study, very thin marginal strips of the regenerating lobes were removed and minced with scissors into fine pieces. The minced tissues were immersed in McCoy's medium according to Sasaki's technique (Sasaki 1961). Drops of the minced specimens were placed on clean slides and squashed following to the water treatment acetic dahlia squash method.

For histochemical study, normal liver tissues 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 for esterase following Burstone's technique (1956).

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For electrophoretic study, both intreated and regenerating liver tissues were homogenized with an equal amount of water, and centrifuged 20 minutes at 15,000 x g. Electrophoresis of extracts was carried out following a modified method of Ogita (1962). Agar gel, 0.7–0.9mm in thickness, was prepared by pouring 50 ml of the following mixture onto a 12 cm × 16 cm glass plate: agar (Difco Bacto agar), 0.35 g: polyvinylpyrrolidone, 1.0g: glycerin, 2.5 ml: gum arabic, 0.25g: potassium phosphate buffer, pH 6.8, ionic strength 0.015, 50 ml.

The plate was connected at each end use being made of filter paper, to a buffer tray containing phosphate buffer of ionic strength, 0.05. The sample was absorbed by filter paper (Toyo filter paper No. 50) measuring 15 mm × 2.0 mm, and then placed on agar gel.

Electrophoresis was carried out at 2.0 mA per cm for 90–120 minutes at 4°. The plate was then stained for esterase with a substrate medium containing 20 ml of potassium phosphate buffer (pH 6.8; ionic strength 0.05), 1 ml of 1% α-naphthyl acetate solution in acetone and 25 mg of naphthanil diazo blue B.

Results

Cytological observation:

Table 1 provides data on the mitotic frequency of diploid and polyploid cells in regenerating liver in relation to the age and body weight. The majority of polyploid cells observed were tetraploid.

In young rats, the vast majority of metaphase cells, more than 90 per cent in frequency, was diploid, polyploid cells being very few in occurrence (Table 1). In adult rats, the frequency of diploid cells decreased markedly in regenerating liver tissues, with a concomitant increase of polyploid cells. The average frequency value of polyploid cells was 67.3 per cent (Table 1, Figs. 1 and 2).

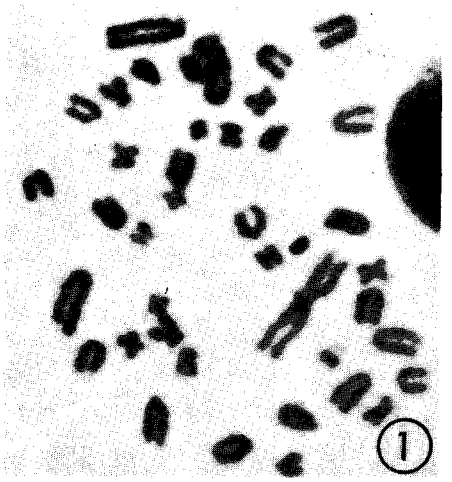
Table 1. Frequency of diploid and polyploid metaphase cells in regenerating livers of young and adult rats

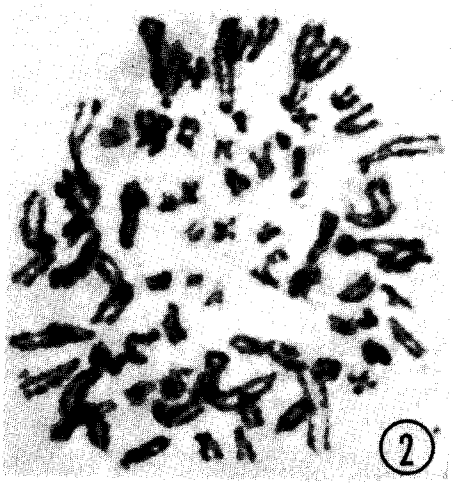
	Rat No.	Age	Body weight	Sex	Ploidy frequency of metaphase cells		No. of cells observed
					diploid	polyploid	
Young	Y 1	2 wks	20 g	♂	93.0%	7.0%	243
	Y 2	2	25	♂	96.5	3.5	200
	Y 3	3	25	♂	96.4	3.6	250
Adult	A 1	7 wks	115 g	♂	35.6%	64.4%	163
	A 2	12	130	♂	29.9	70.1	458
	A 3	12	120	♂	32.9	67.1	170

Histochemical observation:

It was found that esterase activity in the liver increased with age of rats. In young rats, the esterase activity was shown only in central areas of hepatic lobules, while the activity in adult rats was striking in both central and periportal

areas, the former being rather active (Figs. 3 and 4). Evidence obtained consistently agreed with that observed in the previous study (Matsuzawa 1964b).





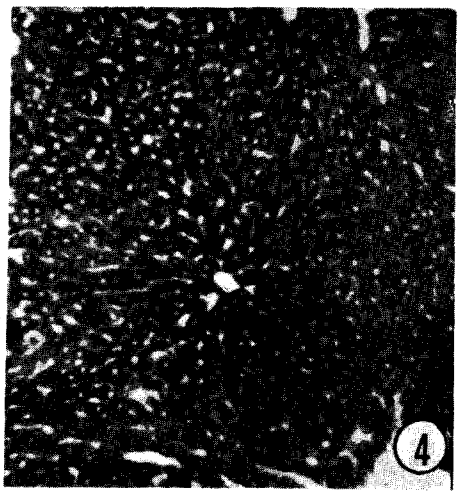


Fig. 1. Diploid metaphase cell.
Fig. 2. Polyploid metaphase cell.
Fig. 3. Esterase. Young rat liver. ×100.
Fig. 4. Esterase. Adult rat liver. ×100.

Zymograms of esterase:

Seven to eight bands with esterase activity could be separated electrophoretically in agar gels at pH 6.8 from water extracts of normal young and adult rat livers (Fig. 5). They were designated as A to F in the order of decreasing electrophoretic mobility to the cathode, and as G, and H to the anode. Band C′ in the liver of

normal young rats, exhibited the most intense activity of esterase, and band A′ ranking next in the level of the enzyme activity. Band B was completely missing in young rats.

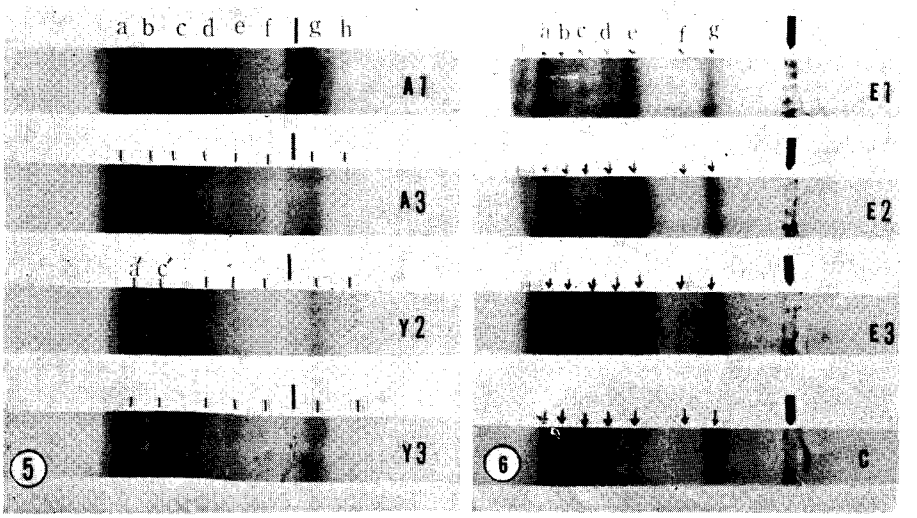


Fig. 5. Pattern of esterase isozymes in liver tissues of Wistar-King A rats. A1 and A3 obtained from adult rats. Y2 and Y3 obtained from young rats.

Fig. 6. Pattern of esterase isozyme in liver tissues of young Fischer rats. E1, 2, and 3, obtained from adrenalectomized rats. C, obtained from normal rat.

In the liver of adult rats, band C showed lower activity of esterase than did band A. The esterase activity in band H decreased in comparison with that of young rat livers.

No significant changes in the pattern of esterase isozymes were detected between normal and regenerating liver tissues.

Discussion

The results of the present study have revealed that, in regenerating rat livers, there is a marked difference in ploidy chromosome pattern between young (2–3 week-old) and adult rats (7–12 week-old). Diploid cells were predominant in young rats, while in adult rats polyploid cells appeared in more than 65 per cent of metaphase cells. Similar evidence was reported by Naora (1957), Alfert and Geschwind (1958), and Post *et al.* (1960) on the basis of the spectrophotometric measurement of DNA contents or the direct measurement of nuclear size in resting hepatic cells. All the features derived by different authors by different techniques in intact or regenerating livers are in fine agreement with each other in relation to the ploidy pattern. This fact may be in favor of lend support to the assumption that

the frequency of polyploid cells in observed mitotic cell populations of regenerating livers would reflect the frequency of polyploid cells in resting cell populations of intact livers.

The pattern of esterase isozymes obtained by means of agar gel electrophoresis in the present study is not identical with that obtained by others through starch gel technique (Hunter and Burstone 1960, Paul and Fattell 1961). Such a discrepancy may be ascribed to the difference of gels or rat strains used, because it was demonstrated in the authors' laboratory that the pattern of esterase isozymes in normal liver differed in different strains of rats or mice.

Markert and Møller (1959), Flexner *et al.* (1960) and Hunter *et al.* (1964) reported the effect of age on the pattern of lactic dehydrogenase isozymes and esterase isozymes in livers of guinea pigs and mice. The present study has successfully supplemented the above feature in rats. It was found that regenerating rat liver showed the isozyme pattern identical to that of normal livers. This supplements the results presented by Hunter *et al.* (1964) in the mouse liver. The simultaneous change in ploidy and esterase isozyme pattern may be of special interest. The decrease of esterase activity in band H in adult rats might possibly be attributable to the disappearance of diploid cells.

The esterase activity in periportal cells of adult rats, but not of young rats, is also histochemically interesting in connection with the change of isozyme pattern. The evidence presented seems to suggest a possible difference in molecular form of esterase between central and periportal cells.

One of the present authors (Matsuzawa, 1964a) reported that, in rat livers, glucose-6-phophsatase activity was more affected by adrenalectomy in centrolobular cells than in periportal cells. The present study, revealed that in liver tissues the activity of esterase as well as the pattern of esterase isozymes shifted after adrenalectomy (Fig. 6). It seems very possible that some hormones may control the distribution of the enzyme in hepatic lobules. Further investigation along this subject is now in progress.

Summary

The present study deals with the alteration in chromosomal ploidy and esterase activity in the liver of rats with age.

In the livers of young rats (2–3 week-old), the vast majority of metaphase cells were diploid, while in adult rats (7–12 week-old) polyploid metaphase cells increased in number with concomitant decrease of diploid cells.

Histochemically, the esterase activity in untreated young rats livers was found in a few layers of cells around central vein, whereas normal adult livers showed a striking increase of the activity, especially in periportal areas of each lobule.

The pattern of esterase isozymes in the liver tissue was quite different between young and adult rats.

The pattern of esterase isozyme in the liver altered concomitantly with the

change of chromosome ploidy during the post-natal developmental stages of rats.

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