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Occurrence of Hyperploid Cells in the Regenerating Rat Liver¹⁾

By

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(With 5 Text-figure)

As is well known, partial extirpation of the liver in rats and mice is followed by rapid mitotic multiplication of cells in the remaining part resulting finally in the restoration of the organ. Chromosome study of the regenerating liver cells has revealed that in the hepatic cells, polyploidy is not uncommon (Beams & King 1942, Sato 1950, Tanaka 1953a, 1953b, Makino & Tanaka 1953). The purpose of this short paper is to give some information on the occurrence of high polyploidy in the liver cells of adult rats during restoration after partial hepatectomy.

Before going further, the author wishes to acknowledge here his indebtedness to Dr. T. H. Yosida for his kind suggestions and guidance in the course of this work. Gratitude is also expressed to Prof. S. Makino for his invaluable advice and for going through the manuscript.

Material and method

Pure and mixed strain rats were used for study; they were the Wistar, Wistar-King A, Wayne-pink-eyed yellow and in pure Japanese stock rats. Nine animals, ranging in age from three to eighteen months, furnished the material; they were anaesthetized and the median and left lateral lobes of the liver were removed in each. This represents about 30-40 percent of total liver mass. The rats were sacrificed at various intervals after operation, such as at 24, 48, and 72 hours. The preparations for the study of the chromosomes were made according to the simple squash technique after Yosida and Ishihara (1956).

Observations

1) *Frequency of hyperploid cells in the regenerating liver*: In the regenerating livers observed in the nine experimental animals, high polyploid cells were very frequent in occurrence; their occurrences showed no particular relation to age, sex or strain of rats. As shown in Table 1 tetraploid cells were highest in frequency in seven individuals under study. On the other hand, diploid cells were most frequent in two individuals (Nos. 3 and 5). The hyperploid cells higher than octoploid did not exceed tetraploid cells in frequency. In rats No. 2 and 8, how-

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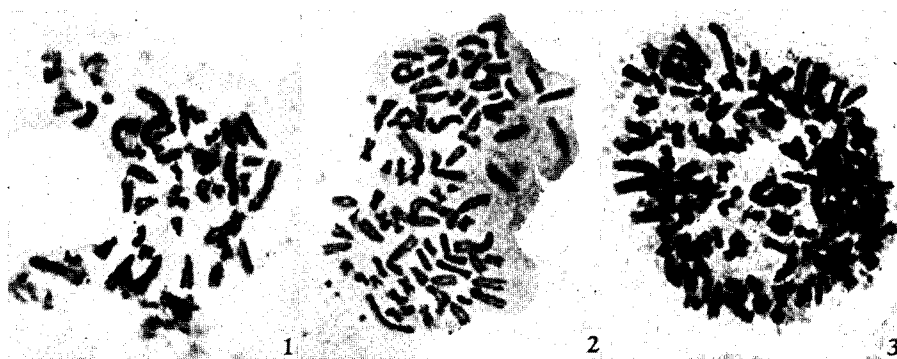
Dedicated to Prof. Tohru Uchida in honor of his 60th birthday.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 13, 1957 (Prof. T. Uchida Jubilee Volume).

Table 1. Frequency of ploid cells in the course of liver restoration after partial extirpation

Ind. No.	Sex	$\pm 2n$	$\pm 4n$	$> \pm 4n$	No. of cells obs.	Strain	Age (months)	Hrs. after oper. (hours)
1	♂	42.0(%)	44.0(%)	14.0(%)	50	W	12	48
2	♀	18.59	45.45	35.95	242	W	8	48
3	♂	44.00	38.0	18.00	50	Wayne	5	24
4	♂	40.0	42.0	18.00	50	Wayne	5	72
5	♂	43.24	32.43	24.32	37	WKA	12	72
6	♂	34.00	46.00	20.00	50	WKA	18	72
7	♂	30.86	52.00	17.20	175	WKA	12	48
8	♀	18.00	44.00	38.00	50	mix.	3	48
9	♀	30.00	44.00	24.00	50	mix.	4	48

ever, the hyperloid cells higher than $8n$ were more frequently found than diploid cells. It is thus apparent that the majority of liver cells here observed were hyperloid cells as far as the scope of the present observations is concerned.



Figs. 1-3. Photomicrographs of the chromosomes from regenerating rat liver cells. 1; $2n$ cell. 2; $4n$ cell. 3; $8n$ cell.

2) *Cytological features of the hyperloid cells*: Though the exact chromosome number could not be determined in the hyperloid cells, it is apparent that they range in number from $8n$ to $32n$. Any possible relationship between the size of cell body and the number of chromosomes was sought for in the liver cells. It was found that in $2n$, $4n$ and $8n$ cells, the increase of cell size was roughly proportional to the chromosome number (Figs. 1-3), though the size of the chromosomes was rather invariable according to the degree of polyploidy. Further, the hyperloid cells higher than $8n$ showed no remarkable increase of cell size. The chromosomes of the hyperloid cells are of slender shape, with an appearance like daughter chromatids occurring in the diploid cell (Figs. 4-5). Most of those hyperloid cells were approximately similar in size to tetraploid or octoploid cells.

Remarks

Beams & King (1942), Biesele (1944), Wilson & Leduc (1948, 1950a), Tanaka (1953a, 1953b) and Makino & Tanaka (1953) reported that many polyploid cells appeared in the hepatic cells during the period of liver restoration. The results of the present observations have shown that although there is a wide variation in chromosome number, the cells in the hyperploid range ($4n$ to $32n$) are extremely frequent in the regenerating liver cells of rats after partial extirpation. The present author seems to be the first to report the occurrence of hyperploid cells ranging from $8n$ to $32n$ in the restoring rat liver. The occurrence of such hyperploid cells was again confirmed by the examination of DNA-content (Leuchtenberger and Schrader 1951, Naora 1951, Thomson and Frazer 1944, *etc.*).



Figs. 4-5. Photomicrographs of the chromosomes from regenerating rat liver cells. 4; probably $16n$ cell. 5; probably $32n$ cell.

So far as hyperploid cells involving $8n$ to $32n$ chromosomes are concerned, the size of the cell and the size of individual chromosomes seem not correlated with an increase of the chromosome number. The present observations suggest that in the regenerating rat liver there occur two types of polyploid cells; in the one type an increase of the chromosome number causes an increase of the cell size, and in the other no such relationship occurs.

Geitler (1939) was first to report a method of doubling chromosome numbers without breakdown of the nuclear wall which was called "endomitosis" in the somatic cells of insects. Several authors have stated that the production of the hyperploid cells in regenerating liver cells has connection with endomitosis. Biesele, Poyner and Painter (1942), and Wilson and Leduc (1950) have claimed endomitosis as the mechanism of the formation of the hyperploid cells.

Summary

The present paper describes the results of a cytological investigation of polyploid hepatic cells in the restoring liver of rats after partial extirpation. The

numerical data here obtained are shown in Table 1. It is evident that high polyploid cells are extremely numerous in occurrence. Though the exact chromosome number could not be determined in the hyperploid cells, the numbers seem to range from $4n$ to $32n$.

The relationship between the size of cell body and the number of chromosomes was examined; in the $2n$, $4n$ and $8n$ cells, the increase of cell size is roughly proportional to ploidy, while hyperploid cells higher than $8n$ show no increase correlational to cell size. It is then evident that an increase of the chromosome number is not always followed by an increase of cell size.

As the mechanism for the production of hyperploid hepatic cells, the duplication of the chromosome number by endomitosis was discussed.

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