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Flow cytometric analysis of mouse hepatocyte ploidy

II. The development of polyploidy pattern in four mice strains with different life spans

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Summary. The development of liver ploidy in mice aged up to 24 months was investigated by flow cytometry in four mouse strains. A mathematical procedure was applied for correction of flow cytometry histograms. In two of the mouse strains, C3H and DBA, both cellular and nuclear ploidy proceed in the same way. The octoploid cell with two tetraploid nuclei is the most numerous cell type in adulthood. On the other hand, strain NZB and the out-bred strain NMRI show at the corresponding age a higher proportion of diploid cells with strikingly low proportions of 4c cells. In addition, high values of 16c cells and nuclei are present in NMRI. In all strains the proportion of binucleate hepatocytes is in the same range (60%). However, the strains differ in ploidy classes of binucleate cells. Development of liver polyploidization does not depend on life span of the specific strain.

Key words: Flow cytometry – Life span – Liver, mouse – Polyploidy

The ploidy pattern of hepatocytes is both age- and species-dependent (review: Grundmann and Seidel 1969). An increasing DNA content in growing liver cells is found not only in human beings (Swartz 1956; Adler et al. 1981; Watanabe and Tanaka 1982) but also in rats (Marquardt and Gläss 1957; Nadal and Zajdela 1966a, b) and mice (Siess and Stegmann 1950; Inamdar 1958; Epstein 1967; Shima and Sugahara 1976; Digernes 1980). The ploidy patterns reported, both for cells and nuclei of rodent liver, therefore depend on the species and strains investigated and on experimental methods (review: Carriere 1969). For instance, by counting the chromosomes in metaphase plates after partial hepatectomy, an incorrect distribution of ploidy levels might be found since the ploidy classes show a particular behaviour after this operation (Grundmann and Bach 1960).

The aim of this investigation is to ascertain, by flow cytometry, the undisturbed DNA pattern during the lifetime of mice, including that of strains with different life expectancies.

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Materials and methods

Animals. Pairs of the following mice strains were purchased to enable us to establish our own breeding colonies: DBA/2Han and the out-bred NMRI/Han from Zentralinstitut für Versuchstierzucht, Hannover, FRG, NZB/Cr/BOM and C3H/Tif/BOM$_f$ from Bomholtgård, Ry, Denmark, C3H/Law from REP, Rijswijk, Netherlands.

In the course of this study, no distinction was made between lines C3H/Law and C3H/Tif/BOM$_f$ of strain C3H.

Mean life spans (averaged over sexes) for the different strains in days are:
C3H: 791 (Smith et al. 1973), 633 (Stutman 1974),
DBA: 710 (Storer 1966), 702 (Goodrick 1975), 570 (Smith et al. 1973),
NZB: 275 (Stutman 1974),
NMRI: 595 when conventionally reared (Lörcher, personal communication).
All animals were housed conventionally in Makrolon®-cages on wood shavings. They received standardized diet and tap water ad libitum.

Liver cell preparation. In total 112 mice of the four strains, aged 1, 2, 3, 6, 9, 12, 18, and 24 months, were used for this study. In each age-group four mice were sacrificed, except when older than one year when only two or three animals were taken. All livers were perfused (Severin et al. 1984) between 9–11 a.m. in order to avoid possible circadian fluctuations.

Staining and flow cytometry. Cellular DNA was stained selectively by a mixture of ethidium bromide and mithramycin (Zante et al. 1976). For measurement of isolated nuclei, the cell plasma was proteolysed by pepsin before staining. The DNA of 10000 to 30000 cells or nuclei from each liver was measured using a home-built cytometer, resembling the ICP 11 (Phywe, Göttingen). DNA distributions were evaluated according to Severin et al. (1984).

Results

No significant differences between males and females with regard to the ploidy pattern of the hepatocytes or the pro-

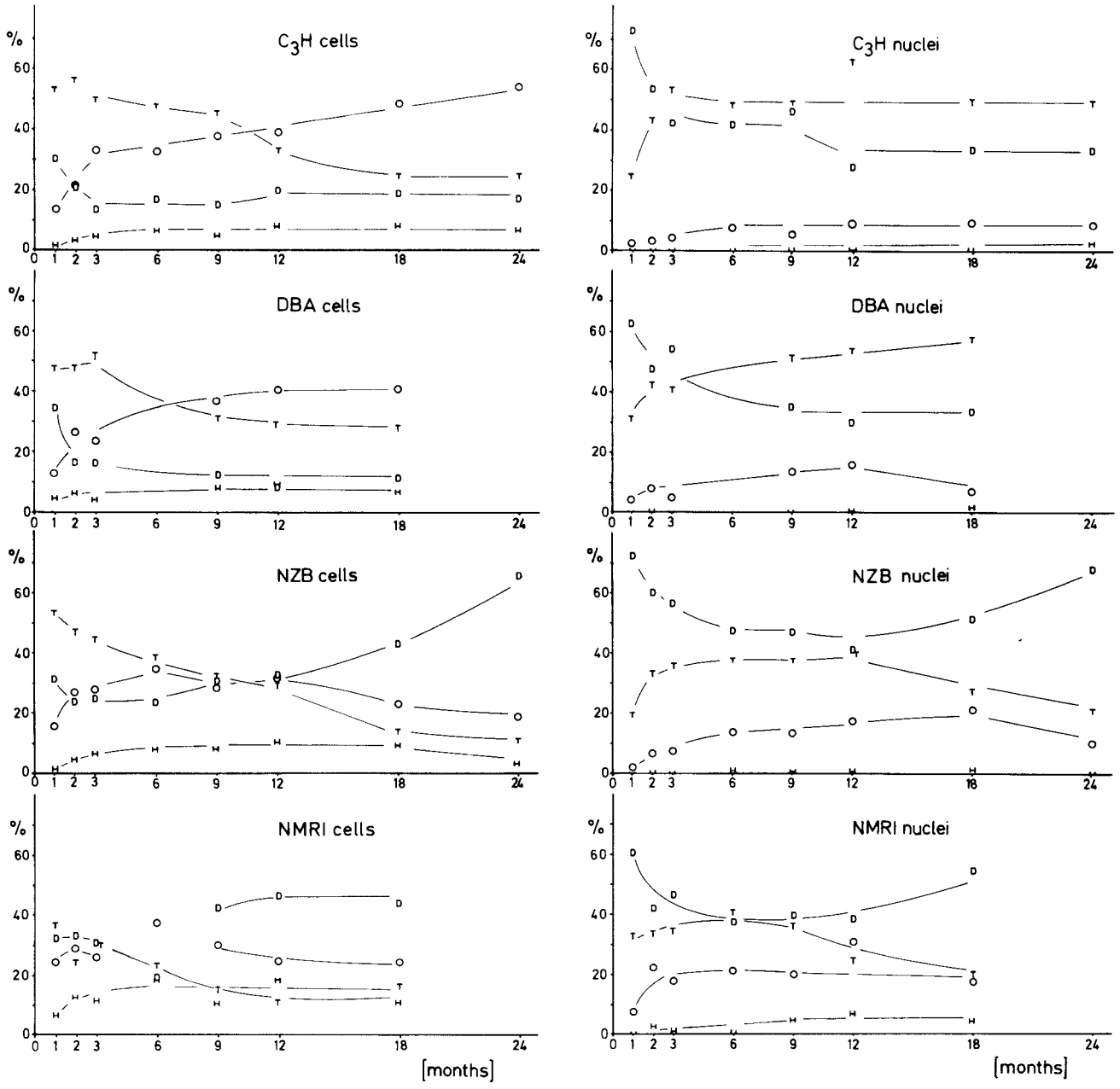

Fig. 1. Time course of relative frequency of liver cell ploidy classes. Mouse strains C3H, DBA, NZB, and NMRI; cells (on the left) and nuclei (on the right), abscissa: age in months. *D* diploid (2c), *T* tetraploid (4c), *O* octoploid (8c), *H* hexadekaploid (16c)

portion of binucleate cells could be detected. A prevalence of tetraploid values (Fig. 1) is observed, when comparing the proportions of the ploidy patterns of liver cells from strains aged between 1–6 months. The octoploid cell (with two tetraploid nuclei) is the "normal" hepatocyte in one-year-old animals of strains C3H and DBA both of which have a similar life span. The ploidy in strain NZB increases differently. Although this strain shows the highest DNA content in liver cells at the age of 6–12 months, in older animals diploid cells predominate. The proportion of diploid cells is, from the second month, 10–20% higher than in the other two above-mentioned strains. In young NZB animals, the number of diploid nuclei declines less markedly and increases after one year. This increase is disproportionately large, causing a relative decrease of the polyploid DNA classes of nuclei and cells. The out-bred strain NMRI shows a ploidy pattern completely different from that of the in-bred strains. The diploid values show a time course similar to that of the NZB mice. However, in NMRI the characteristic differences are the extremely high values of 16c cells and nuclei and the low 4c values. Any discontinuities in the time course, especially in this strain, seem to be an artifact caused by the greater biological variability of this strain. The proportion of binucleate hepatocytes is similar in all the mouse strains investigated here and shows no gross changes between 1–18 months (see Fig. 2). The average value is 56% for NZB, 59% for DBA, and 61% for both NMRI and C3H with a standard deviation of about 10% for the particular age groups. However, differences between all ploidy classes of binucleated cells exist,

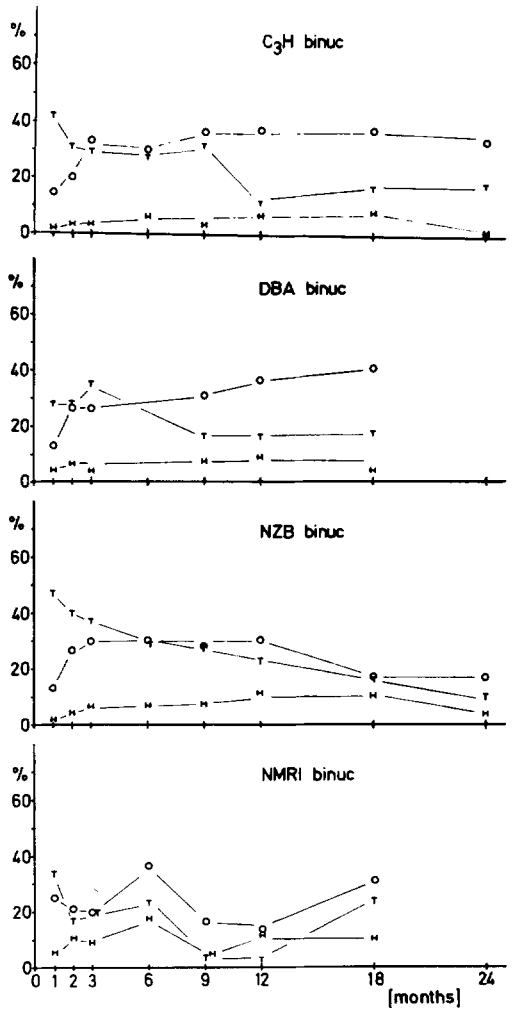

Fig. 2. Time course of relative frequency of the nuclear ploidy classes in binucleate hepatocytes. For symbols see Fig. 1

and are age-dependent. The strains DBA and C3H show similar behaviour in polyploidization of their binucleate cells. The strong fluctuations of ploidy values characteristic of strain NMRI are also found in their binucleated cells. In order to show how reliable our data are that demonstrate the dependence of ploidy on age, the standard deviations of the median DNA values presented in Figs. 1 and 2 are listed in Table 1.

Discussion

It is still unclear to what extent the diploid cells measured flow cytometrically are really parenchymal cells and not sinusoidal cells (Digernes and Bolund 1979; Severin et al. 1984). Sinusoidal cells account for about 30% of the total number of cells in the mouse liver. Nevertheless, we included the first (diploid) peak in our analysis because we wanted to compare several mouse strains, the livers of which were prepared in exactly the same way. Diploid values differed greatly, both between the different mouse strains and, as expected, between the age groups.

The first polyploid cell appears when a considerable number of binucleate cells of the next lower ploidy class has accumulated (Nadal and Zajdela 1966a; Brodsky and Uryvaeva 1978). Polyploid cell classes are formed by acytokinetic mitosis (resulting in a binucleate cell) and a subsequent simultaneous mitosis of the two nuclei of this cell. Fusion of the two mitotic spindles takes place, resulting in two cells of the next higher ploidy class (Beams and King 1942). During the polyploidization process, some predominant cell classes characteristic of this period can be expected. However, we have not been able to demonstrate the temporary accumulation of nuclei of particular ploidy. Also, the observed proportion of binucleate cells does not fluctuate periodically in a mode corresponding to this theory. Nevertheless, the marked increase of the number of tetraploid nuclei in the third month followed by the increase of the number of octoploids in the next interval in strain C3H, and the similar behaviour of the diploid and tetraploid nuclei of DBA and NMRI-mice in the same period, suggests that this process occurs.

Some authors (e.g. Vahs 1979) believe that the polyploidization of the mouse liver is terminated at the age of two months, the beginning of sexual maturation. However, we have found a pronounced ploidy increase even beyond the sixth month in all strains. Noara (1975) has pointed out that the ploidy pattern is not correlated to age but to the development stage of the animal, marked by the body weight. A correlation between DNA values and body weight can be confirmed by experiments in our laboratory (unpublished results), although we cannot correlate these two parameters unequivocally, since body weight depends on stage of development. A steady increase of nuclear ploidy up to the twelfth month is observed in all strains and is followed by a decrease in the number of 4c and 8c nuclei. Only in old animals of strain C3H is nuclear ploidy constant. In this strain, a decrease in the number of binucleate 4c cells is compensated by an increase in the number of 8c cells with two nuclei. The measured fluctuations in ploidy levels appear most markedly in NMRI, and make a definite judgement difficult. However, such high proportions of 16c cells in young mice have not been found in any other strain investigated.

The most pronounced change with time in the ploidy pattern of NZB mice is the increase in diploid cells from 23% in the sixth month to 66% in the 24th month. As

Table 1. Standard deviations of ploidy classes within strains (neglecting age)

Strain	Number of mice	Cells				Nuclei				Binucleate cells		
		2c	4c	8c	16c	2c	4c	8c	16c	4c	8c	16c
C3H	23	6.09	5.09	5.57	1.97	8.12	7.85	3.30	0.67	15.83	5.85	27.97
DBA	13	8.92	8.55	7.22	2.74	9.39	6.33	3.77	1.08	17.89	11.66	9.16
NMRI	26	11.79	6.80	6.10	5.19	8.67	6.91	5.15	2.04	32.39	15.46	25.49
NZB	20	7.04	4.70	5.21	2.41	6.95	7.63	4.55	0.70	25.33	16.61	16.06

far as we know, this phenomenon has not been reported before. The diploid nuclei are mostly mononucleate, because the proportion of tetraploid cells decreases from 29% to 10% during the same period. The average life span of NZB is nine months. Old NZB mice fall ill with autoimmune hemolytic anemia, nephritis with hypertension and generalized vascular alterations (Staats 1981). The increase in the number of diploid liver cells at this age is possibly connected with these diseases.

There are two arguments for the average life span of inbred mice having little or no influence on the physiological and biological fitness of the organism. Firstly, most inbred strains of laboratory animals carry typical defects causing diseases characteristic of the respective strain, dying without a cytochemically detectable premature aging process. Secondly, the life span is determined not only by genetic but also by environmental factors. The durations of life determined by several authors (see Materials and methods) differ considerably, in part because of variations in rearing conditions but also when determined repeatedly by the same authors. Therefore, the great difference in development and the degree of liver polyploidization between NZB and the other three strains are probably not caused by the comparatively low life span of this strain.

Besides different life spans, different values of polyploidy patterns in rodents have been published. The work of Schulte-Herrmann et al. (1976) contains a possible interpretation of this. The authors reveal that the livers of specified pathogen-free animals are up to 20% larger, and contain more DNA because of an increased hepatic nuclear ploidy than livers of germ-free rats. They conclude that infections by environmental microflora influence liver size and ploidy.

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