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Changes in the Nucleus-Cytoplasm and Nucleolus-Nucleus Volume Ratios in the Liver Parenchymal Cells of the Golden Hamster during Post-Embryonic Development

Zmiany stosunków objętościowych jądrowo-plazmatycznych i jąderkowo-jądrowych komórek miąższu wątroby chomika złocistego w rozwoju pozazarodkowym

Изменение объемных нуклео-цитоплазматических и нуклеоле-нуклеусовых отношений клеток паренхимы печени у золотистого хомячка после эмбрионального развития

INTRODUCTION

Morphometric studies of the cell and its components, performed along with the histological or histochemical methods, create an opportunity of closer determination of the mutual relationships or quantitative changes within the cell, without interference of the subjective element. The liver cells present a convenient material for such studies, and for this reason the liver has become an almost classic object of the research of this type.

Because of the variety of problems resulting from the structure and function of the components of the liver cell, the morphometric investigations undertaken by different authors are limited, as a rule, to a definite stage of the individual development, or to a narrow range of problems. Thus, for instance, Zotter, Kemmer and Sauer (19) studied the changes in the size of the morphotic elements of the liver cells during the first 10 days of the life of the mouse, and Wüstenfeld and Rausch-Oertgen (18) compared the size of the nuclei of the liver cells of the guinea pig before and after birth. Some studies concern the action of chemical compounds: Bader, Stiller, Holland-Letz and Bergleiter (2) analysed the changes in the nucleus and nucleolus volume produced by thioacetamide, and Adhami and Bosse (1), those affected by ether. The material used for the investigations of this type is usually uniform with regard to the age and other characteristics. In the interesting methodical paper by Jerusalem, Eling and Jap (9), attention is paid to the changes which occur in the liver cell during the daily cycle.

The rat liver cells have been the most frequent objecs of morphometric studies; those of the hamster have been investigated much less in this respect, although two items of the list of references can be quoted here: David (4) studied the influence of fasting on the number and size of nucleoli, and Tongiani (17) analysed the changes in the dry mass content of the nucleus and cytoplasm in the liver cells of adult hamsters, as well as changes in the number of binucleate cells during fasting. These papers, however, do not take into consideration the age of the animals. It seemed therefore advisable to undertake an attempt at investigating the changes in the nucleus-cytoplasm and nucleolus-nucleus volume ratios in the liver cells of the golden hamster during post-embryonic development.

MATERIAL AND METHODS

The livers of golden hamsters (*Mesocricetus auratus* Waterh. 1839) of local breeding served as material for the investigations. Each time 3 individuals from the following age groups were studied: 0, 1, 3, 7, 14, 21, 28, 42 days, 2, 3, 4, and 30 months; in addition, one animal aged 6 months was used. In all, 37 hamsters were examined. The animals were always dissected at about 11 a.m. Fresh liver fragments, 3 mm thick, were fixed in Carnoy's fluid for 22 hours, embedded in paraffin and sectioned at 8 μ. After soaking the sections with water by the accepted methods, the preparations selected for cell measurements were stained with a solution of safranine, water blue, orcein and eosin. In order to visualize and measure the nucleoli, the preparations were stained according to Brachet, using pyronin B and methyl green at *pH* 4.8—5.0.

For determination of the frequency of occurrence of binucleate cells in the individual age groups, 350 cells from each animal were scanned in randomly chosen fields of view.

A 15x micrometric eyepiece was used for biometric measurements. The diameters of the cells and of their nuclei were measured using a 40x objective, and the measurements of the nuclei and of their nucleoli were carried out with a HI90x objective.

The diameters of 50 randomly chosen cells and of their nuclei were measured in the liver of each animal. Similarly, the diametres of 50 nuclei and of all nucleoli contained therein were measured in the preparations stained according to Brachet. There were also carried out measurements of 150 binucleate cells and of their nuclei. The diameters thus obtained made it possible to compute the volumes of the cells, nuclei and nucleoli. These data were then subject to statistical analysis.

RESULTS

1. BINUCLEATE CELLS AND AGE

The frequency of the occurrence of binucleate cells in the liver parenchyma of the golden hamster was computed from sections 8 μ thick. During the first days of post-embryonic life, binucleate cells occurred in about 1%. During the first weeks of life, a rapid increase of the percentage of binucleate cells in the liver was observed (Fig. 1); after 3 weeks it reached above 10%, and towards the end of the 4th month of life the percentage of binucleate cells rose to 20%. No further increase of the frequency of the occurrence of these cells was observed at a later age.

2. LIVER CELL VOLUME

The smallest mean volume of the parenchymal liver cell was observed in newborn individuals, where it amounted to $1355 \pm 46.2\ \mu^3$. With the advancing

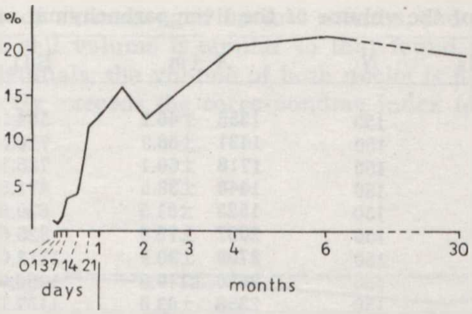

Fig. 1. Frequency of occurrence of binucleate cells in the liver of the golden hamster, correlated with age

age of the hamster, the liver cells increased their dimensions; this increase was especially rapid during the first month of life and, towards the end of that period, reached the mean volume of 2709 ± 90.9μ^3 which is twice the volume of the liver cell in newborn hamsters (Table 1, Fig. 2). The increase of the cell volume with probability $P<0.001$ is confirmed by statistical analysis performed by means of the t-Student test. It appears from the studied material that further increase of the liver cell volume had a slower rate, reaching the mean volume of 3009 ±115.3 μ^3 at the age of 3 months.

Because of the small number of binucleate cells studied (150 cells) it was not possible to carry out full analysis of the volume changes correlated with age. It was only found that binucleate cells in individuals aged from 21 days to 30 months did not show any distinct changes of the volume which might be dependent on the age (Fig. 2). The average volume of these cells was larger by 45% than that of uninucleate cells of adult individuals and amounted to 4375 ±124 μ^3.

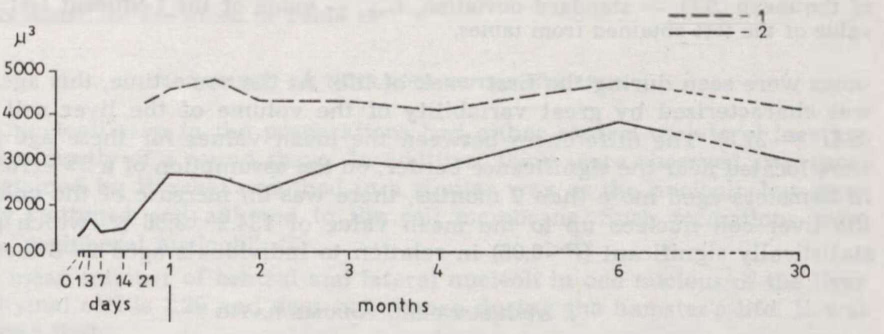

Fig. 2. Changes in the volume of the liver parenchymal cells, correlated with age; 1 — uninucleate cells, 2 — binucleate cells

3. NUCLEUS VOLUME

The volume of the liver cell nucleus, contrary to the volume of the cell, undergoes only slight changes during the life of the animal (Table 2). The smallest volumes of the nucleus were observed in hamsters aged from 14 days to 2 months, their mean value being 124.8 ±3.27 μ^3. Slightly larger nucleus vol-

Table 1. Increase of the volume of the liver parenchymal cell, expressed in μ^3

Age of animals	*N*	X ±*m*	S.D.	*P* <
0 days	150	1355 ±46.2	564.9	
1 day	150	1431 ±58.9	720.5	P < 0.4
3 days	150	1718 ±60.1	735.3	P < 0.001
7 days	150	1448 ±38.5	471.5	P < 0.2
14 days	150	1523 ±51.5	629.8	P < 0.02
21 days	150	2067 ±75.6	925.6	P < 0.001
1 month	150	2709 ±90.9	1112.6	P < 0.001
1.5 month	150	2680 ±119.8	1466.0	P < 0.001
2 months	150	2338 ±93.0	1137.7	P < 0.001
3 months	150	3009 ±115.3	1411.0	P < 0.001
4 months	150	2840 ±99.0	1211.6	P < 0.001
6 months	50	3978 ±252.8	1787.4	P < 0.001
30 months	150	3013 ±121.0	1418.1	P < 0.001

Explanation: *N* — number of cells studied, *X* ±*m* — mean and standard error of the mean, S.D. — standard deviation, *P* — probability of occurrence of accidental differences between the size of the cells of newborn hamsters and that of the other age groups.

Table 2. Changes in the volume of the liver cell nucleus, expressed in μ^3

Age of animals	0—7 days	14 days to 2 months	3—30 months
N	600	750	500
X ±*m*	141.5 ±8.03	124.8 ±3.27	134.2 ±3.66
S.D.	27.81	12.66	11.58
$t_{exp.}$		1.997	2.617
$t_{0.05}$		2.060	2.069

Explanation: *N* — number of cells studied, *X* ±*m* — mean and standard error of the mean, S.D. — standard deviation, $t_{exp.}$ — value of the t-Student test, $t_{0.05}$ — value of the test obtained from tables.

umes were seen during the first week of life. At the same time, this age group was characterized by great variability of the volume of the liver cell nuclei (S.D. = 27.8). The differences between the mean values for these age groups were located near the significance border, on the assumption of a 5% error level. In hamsters aged more than 2 months, there was an increase of the volume of the liver cell nucleus up to the mean value of 134.2 ±3.66 μ^3, which proved statistically significant ($P<0.05$) in relation to individuals aged 2—8 weeks.

4. NUCLEUS-CELL VOLUME RATIO

The comparatively high stability of the nucleus volume, and the age-conditioned increase of the cell volume produce a ratio which distinctly depends on the age of the animal (Table 3, Fig. 3). During the first 3 days of life of the hamster, the nucleus volume is more than 10% of the volume of the whole liver cell. Later on, because of the increasing cell volume, there is observed during the first month of life a decrease of the nucleus-cytoplasm volume ratio down to the mean value of 5.3 ±0.18%. In older animals, the changes of this ratio do not show a distinct correlation with age.

In binucleate cells of animals aged up to 3 months, the ratio of the volume of both nuclei to the cell volume is similar to that found in uninucleate cells (Fig. 3). In older individuals, the volume of both nuclei is 6.2 to 7.0% of the cell volume, which distinctly exceeds the corresponding index for uninucleate cells.

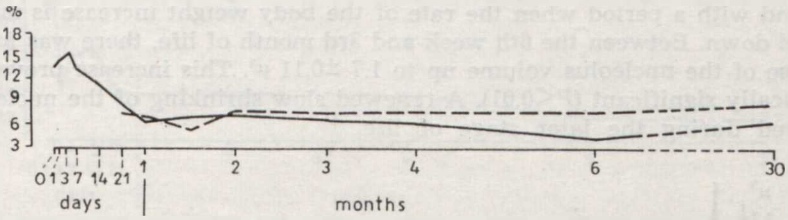

Fig. 3. Nucleus-cell volume ratio, correlated with age; for explanation see Fig. 2

Table 3. Changes in the nucleus — cell volume ratio, expressed in %

Age of animals	*N*	*X* ±*m*	S.D.	*P* <
0 days	150	10.4 ±0.30	3.7	
1 day	150	10.7 ±0.34	4.2	P < 0.6
3 days	150	11.2 ±0.39	4.8	P < 0.2
7 days	150	9.7 ±0.32	3.9	P < 0.2
14 days	150	8.5 ±0.33	4.0	P < 0.001
21 days	150	6.9 ±0.24	3.0	P < 0.001
1 month	150	5.3 ±0.18	2.2	P < 0.001
1.5 month	150	5.6 ±0.23	2.8	P < 0.001
2 months	150	5.9 ±0.18	2.3	P < 0.001
3 monts	150	5.4 ±0.20	2.4	P < 0.001
4 months	150	5.3 ±0.19	2.3	P < 0.001
6 months	50	3.7 ±0.17	1.5	P < 0.001
30 months	150	5.6 ±0.21	2.6	P < 0.001

Explanations are given in Table 1.

5. NUMBER OF NUCLEOLI IN THE NUCLEUS

The nucleoli seen in the preparations had either central or lateral location and were mostly of a round shape. In addition, there were observed structures which stained by Brachet's method in a similar way as the nucleoli, but were strongly flattened and adhered to the cell membrane. Such formations were termed "peripheral nucleoli".

The mean number of central and lateral nucleoli in one nucleus of the liver parenchymal cell is 1.29 and does not change during the hamster's life. It was also found that:

71.83% of the nuclei had one nucleolus,

24.44% of the nuclei had 2 nucleoli,

3.73% of the nuclei had 3 nucleoli.

During the first day of the hamster's life, the mean number of "peripheral nucleoli" in 1 nucleus was 0.46, and, beginning with the third day of life, this index fell to 0.26 and remained on a similar level during the whole life.

6. THE VOLUME OF NUCLEOLI

The sum of the volumes of the nucleoli found in one nucleus distinctly decreases during the first 6 weeks of life of the hamster (Fig. 4), changing from the mean value of $2.9 \pm 0.17\ \mu^3$ in one-day-old individuals down to $1.3 \pm 0.06\ \mu^3$ in 6-week-old animals, which coincides with the beginning of sexual maturation and with a period when the rate of the body weight increase is markedly slowed down. Between the 6th week and 3rd month of life, there was found an increase of the nucleolus volume up to $1.7 \pm 0.11\ \mu^3$. This increase proved to be statistically significant ($P < 0.01$). A renewed slow shrinking of the nucleoli was observed during the later stage of life.

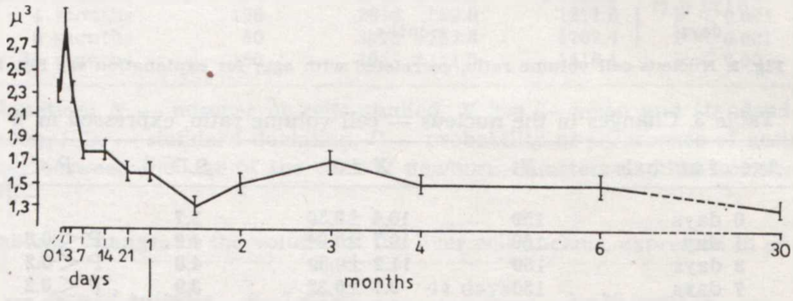

Fig. 4. Changes in the volume of central and lateral nucleoli, correlated with age; vertical lines denote standard error of the mean

Table 4. Correlation between the volume of nucleoli and their number in the nucleus

Number of nucleoli in nucleus	Mean volume of nucleolus μ³	Volume of sum of nucleoli in nucleus μ³
1	1.76	1.76
2	1.12	2.24
3	0.92	2.71

The study of the correlation between the sum of the nucleolus volumes in the nucleus and the number of nucleoli (Table 4) disclosed that the increase of the number of nucleoli in the nucleus from 1 to 3 produces a decrease of the mean nucleolus volume by 47%; on the other hand, when the number of nucleoli increases from 1 to 3, the sum of the nucleolus volumes increases by 54%.

The volume of the so-called "peripheral nucleoli", similarly as their number, shows no perceptible correlation with age, except during the first day of life. The mean volume of these nucleoli in one liver cell nucleus is $0.15\ \mu^3$.

7. NUCLEOLUS-NUCLEUS VOLUME RATIO

The considerable stability of the size of the nucleus, together with the age-conditioned decrease of the size of the nucleoli, accounts for the fact that the mean nucleolus-nucleus volume ratio falls during the first three weeks of life of the golden hamster from $2.3 \pm 0.13\%$ in the newborn to $1.4 \pm 0.05\%$ in

3-week-old animals (Fig. 5) and remains on this level for several months. A tendency to further decrease of the nucleous-nucleus ratio was observed in hamsters aged 6 and 30 months.

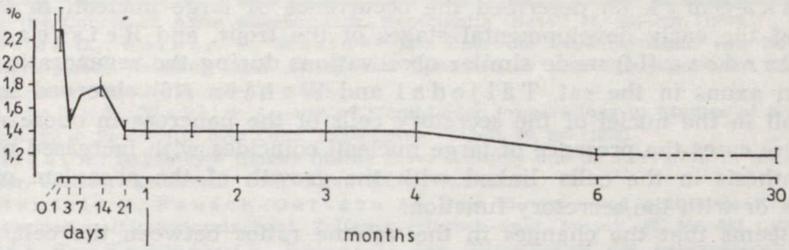

Fig. 5. Changes in the percentage ratios of the volumes of central and lateral nucleoli to the nucleus volume; vertical lines denote standard error of the mean

DISCUSSION

The fact that the number of binucleate cells increases with the age of the hamster, which could be observed in the present study, has an analogue in Geller's (7) observations concerning the rat. The 20% participation of binucleate cells found in the 8 μ sections of the liver of adult hamsters is, in the light of Pfuhl's (13) mathematical analysis, lower than the actual value because part of the cells are split in histological sections. In fact, a higher incidence of binucleate cells in the liver of the golden hamster can be expected; this is also supported by the results of Tongiani (17) obtained from smears of the liver cells.

The strongly increased variability of the dimensions of the liver cell nucleus, which is observed during the first week of the hamster's life, can be explained by the cell divisions which are frequent at that time, and, in this connection, by the occurrence of the small post-division nuclei besides large nuclei from the final stage of interphase or from the initial stage of prophase. This conclusion finds support in the observations of Gauthier (6), who found that the volume of the fibroblast nuclei can be doubled during the inter-division period. A slight increase of the nucleus volume observed in adult and old individuals can be linked to polyploidy of a number of nuclei, described by Mironescu and Dragomir (12) and by Meinders-Groeneveld and James (11).

The increase of the volume of the liver cells during the hamster's life, which is in harmony with the behaviour of these cells in other mammals (5) and of the epithelial cells of the small intestine in the rat (15), results, given the slight variations of the nucleus volume, in the nucleus-cytoplasm ratio being changed in favour of the latter, and seems to be mainly the effect of the accumulation of reserve substances in the cell cytoplasm.

The mean number of 1.29 nucleoli in the nucleus of the liver cell of the hamster found in the present investigations exceeds by 12.5% only that given for the same species by David (4), which is less than a half of the mean nucleolus number found in the liver cells of the rat (4, 12), or 4 or 5 times less than the number of nucleoli reported for some mouse breeds (8).

The large volumes of the nucleoli observed during the first days of life of the hamster and finding their reflection in a high nucleolus-nucleus ratio usually decreased with the decreasing rate of the growth of the body weight. The presence of large nucleoli in the nuclei of the liver cells of the mouse during the first days of life was noted by Kemmer and Zotter (10). Byczkowska-Smyk (3) described the occurrence of large nucleoli in the liver cells of the early developmental stages of the trout, and Reissenweber and Cardoso (14) made similar observations during the regeneration of the neuron axons in the rat. Täljedal and Wahlin (16) observed increased nucleoli in the nuclei of the secretory cells of the pancreas in obese mice. In all these cases the presence of large nucleoli coincides with increased processes of synthesis in the cells linked with the growth of the organism or of its tissues, or with the secretory function.

It seems that the changes in the volume ratios between the cell, nucleus and nucleoli determined in the hamster by the morphometric methods are linked with the slowing down and then with the inhibition of the growth processes in the liver, as well as with the accumulation of reserve materials in the cell cytoplasm.

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STRESZCZENIE

Przeprowadzono morfometryczne badania komórek miąższu wątroby 37 chomików złocistych (*Mesocricetus auratus*) w różnym wieku.

W wyniku badań stwierdzono, że częstość występowania komórek dwujądrzastych wzrasta od 1% u chomików nowo narodzonych do 20% u chomików dorosłych (ryc. 1). Objętość jednojądrzastych komórek miąższu wątroby jest najmniejsza u osobników nowo narodzonych i wynosi 1355 ±46,2 μ^3, w okresie pierwszego miesiąca życia ulega podwojeniu, a u osobników kilkomiesięcznych obserwowano dalszy niewielki wzrost objętości komórek (tab. 1). Średnia objętość komórek dwujądrzastych u osobników dorosłych wynosi 4375 ±124 μ^3 i jest o 45% większa od objętości komórek jednojądrzastych wątroby chomików w tej grupie wieku. Jądro komórki wątroby w ciągu całego życia chomika wykazuje tylko niewielkie zmiany średniej objętości (tab. 2), w rezultacie czego stosunek objętości jądra do objętości komórki ulega zmianie od wartości wynoszącej ponad 10% w pierwszych dniach życia zwierząt do wartości nieco powyżej 5% u osobników dorosłych (tab. 3).

Stwierdzono też, że jądra komórek miąższu wątroby chomika złocistego zawierają najczęściej 1 jąderko (71,83%), stosunkowo często 2 jąderka (24,44%), a niekiedy też 3 jąderka (3,73%). Średnia liczba jąderek przypadająca na jedno jądro nie ulega zmianie w okresie życia chomika, natomiast objętość jąderek w jądrze posiada najwyższe wartości w pierwszych dniach po urodzeniu (2,9 ±0,17 μ^3 u osobników jednodniowych). W okresie kilku tygodni życia objętość jąderek zmniejsza się do połowy (ryc. 4). Powoduje to zmianę wskaźnika jąderkowo-jądrowego od wartości 2,3% w pierwszym dniu życia pozazarodkowego do 1,2% u chomików starych (ryc. 5).

РЕЗЮМЕ

Авторы провели морфометрические исследования клеток паренхимы печени 37 золотистых хомячков (*Mesocricetus auratus*) разного возраста.

В результате исследований установлено, что частота выступления двуядерных клеток увеличивается от 1% у новорожденных хомячков до 20% у взрослых хомячков (рис. 1). Меньший объем одноядерных клеток паренхимы печени выступает у новорожденных хомячков и составляет 1355 ± 46,2 μ^3, в первый месяц жизни удваивается,

а у старших особей (несколько месяцев) наблюдается дальнейшее незначительное увеличение объема клеток (табл. 1). Средний объем двуядерных клеток у взрослых особей составляет 4375 $\pm$ 124 μ^3, т. е. на 45% больше объема одноядерных клеток печени хомячков этого возраста. Ядро клетки печени в течение всей жизни хомячка обнаруживает только небольшие изменения среднего объема (табл. 2). В результате отношение объема ядра к объему клетки изменяется от 10% в первые дни жизни хомячка примерно до 5% у взрослых особей (табл. 3).

Констатировано также, что ядра клеток паренхимы печени золотистого хомячка чаще всего содержат одно ядрышко (71,83%), относительно часто два ядрышка (24,44%), а иногда три ядрышка (3,73%). Среднее количество ядрышек в одном ядре в период жизни хомячка не изменяется, зато большой объем ядрышек в ядре обнаруживается в первые дни после рождения (2,9 $\pm$ 0,17 μ^3 у хомячков, живущих 1 день). Через несколько недель объем ядрышек уменьшается в 2 раза (рис. 4). Это вызывает изменение нуклеоле-нуклеусового показателя от 2,3% в первый день после рождения до 1,2% у взрослых хомячков (рис. 5).