MICRODENSITOMETRIC STUDIES ON NUCLEI OF TISSUE CULTURES FROM HUMAN EMBRYONAL KIDNEY, INFECTED WITH VIRUSES OF THE ECHO AND ADENO GROUPS

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Virus invasion processes are characterized by the formation of a new heterogeneous biological system, and are associated with a profound reorganization and biosynthetic manifestations of the host cell in the direction of securing virus reproduction. The processes developing at genetic level (3, 12), and the predomination of the virus genome over the cellular one by way of functional elimination or reorganization of the latter have an essential practical bearing on the creation of the new virus-cell system. Depending on the mechanism of virus reproduction realization, the above processes may run two different courses, namely: 1) inhibition or exclusion of the cellular genome, and accomplshment of the virus genetic program — in case of productive and abortive type of virus infection; 2) creation ,through incorporation of elements of the virus genome into the cellular one, of a new genetically heterogeneous system, unlimited in terms of time and reproductive capacities — in case of transformational type of the virus infection.

Functional elimination of the host genome in the productive virus — infection type is effected through virus-induced production of substances having the physical and chemical properties of histones — a natural repressor of the cellular genome transcriptional activity (5). Many changes which have been described at chromatin structure level in virus infected cells (1, 4), both with RNA and DNA viruses, may at least partially be explained by the morphogenetic role played by histones. On the other hand, an essential importance is being attributed to nuclear proteins insofar as determination of the chemical reactivity of chromatin is concerned. Differences have been noted in the hydrolysis curve of Feulneg's reaction in connection with the cell-tissue differentiation which, in turn, is related to an alteration in the heterochromatin-to-euchromatin, or DNA-to-histones ratios (2, 8, 11). In the field of quantitative cytomorphology, investigations on the genetic apparatus of the cell in virus infections are comparatively scarce, and mainly aimed at the study of changes in DNA mass (6, 7, 9, 10).

It is the purpose of this work to establish, on the basis of nonscanning microdensitometry, some strictly quantitative changes in the nuclear structure of the host cell in the early phases of infection with a number of DNA and RNA viruses.

Material and method

The study was conducted on primarily trypsinized tissue cultures from human embryonal kidney over lamellae. Infection was produced with several types ECHO (1, 9, 14, 19, 26) and ADENO (2, 4, 6, 9) viruses at contact adaptation 30 minutes, and 0.2 ml virus-containing liquid with titer ranging from 10^{-3} to 10^{6}. Within 24 hours of infection, the lamellae underwent fixation for 15 min in a mixture of saturated alcohol solution of sublimate, concentrated formalin and glacial acetic acid in a 80 : 15 : 5 ratio, and thereafter they were treated after the method of Feulgen as modified by I tikawa and Ogura (1954). To determine the hydrolysis curve, its duration varied in the range 10, 15, 20, 25, 30, 40 and 50 minutes. Microdensitometry was performed according to the plug method on the basis of arithmetic mean from three measurements in each nucleus. The extinction of ten nuclei was determined in each separate preparation. During differential densitometry using preparations hydrolyzed for 30 minutes, the permeability of 50 fields with diameter 1 micron from the object in each nucleus was determined in fifty nuclei of both control and infected tissue cultures. The obtained data for the individual nuclei were distributed in 10 per cent permeability ranges each. They were used to calculate the average transudation (T) and the entropy of the frequential distribution of transudation (H). The nuclear surface was calculated by means of planimetry of their photographic image. DNA mass (M) was equal to the product of surface by mean extinction which was obtained through mean permeability conversion. The study was conducted with a non-automatized OPTON microdensitometer, type MPM, at immersion magnification 100 x and monochromatis zation at 570 nm. All data underwent statistical elaboration, and the tables of Strelkov (1966) were employed for mean square deviation and confidentia-interval determination at incoincidence probability 0.05.

Results

The arithmetic mean data (Table 1) about the nuclei of infected celll relative to controls show that:

Table 1

Arithmetic mean data

Transudation	Control ADENO	X ADENO	Control ECHO	X ECHO
0—10				
10—20				
20—30				
30—40				
40—50		0,061 ± 0,001 0,058 ÷ 0,064		
50—60		0,209 ± 0,002 0,205 ÷ 0,213		0,092 ± 0,002 0,089 ÷ 0,095
60—70	0,183 ± 0,006 0,172 ÷ 0,194	0,314 ± 0,003 0,308 ÷ 0,320	0,195 ± 0,005 0,185 ÷ 0,205	0,275 ± 0,003 0,269 ÷ 0,281

contd

70—80	0,680 ± 0,005 0,670÷0,690	0,031 ± 0,003 0,325÷0,337	0,645 ± 0,006 0,633÷0,657	0,531 ± 0,003 0,525÷0,537
80—90	0,137 ± 0,002 0,132÷0,142	0,085 ± 0 001 0,083÷0,087	0,160 ± 0,003 0,154÷0,166	0,102 ± 0,002 0,098÷0,106
90—100				
X	0,3646 ± 0,002 0,3596÷0,3696	0,6173 ± 0,001 0,6153÷0,6193	0,3859 ± 0,003 0,3853÷0,3865	0,4887 ± 0,002 0,4857÷0,4917
T	74,56 ± 0, 081 71,40÷74,72	66,67 ± 0, 054 66,56÷66,77	74,65 ± 0.063 73,42÷75,88	71,41 ± 0,065 71,28÷71,54
S	548,46 ± 15,657 517,77÷579,15	593,56 ± 20,479 553,42÷633,71	383,42 ± 6,005 371,65÷398,16	474,32 ± 8, 109 458,43÷490,21
X	69,90 ± 0,263 66,06÷73,74	101,58 ± 3,551 97,61÷111,55	48,77 ± 0 ,784 47,23÷49,30	69,56 ± 1,154 67,30÷71,82

— the distribution of permeabilities is being characterized by the occurrence of rather lower transudations — in one quantum in ECHO-viruses and two quanta in ADENO-viruses;

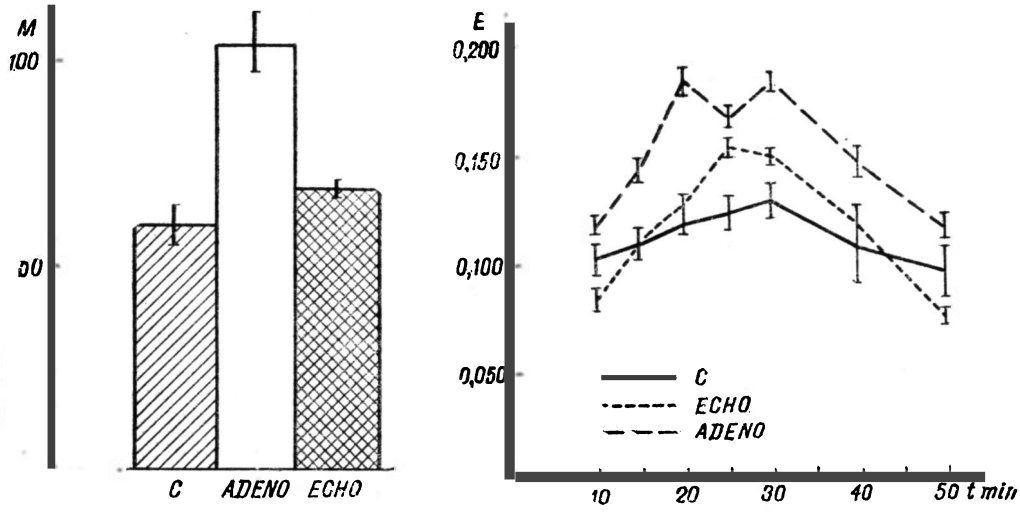

Fig. 1. Histogram of changes in DNA mass (the values are expressed in conditional units).

Fig. 2. DNA hydrolysis curves.

— the entropy of the frequential distribution of transudations, assumed as a measure of their variety, displays a significant increase because of their distribution in a greater number of quanta;

— the average transudation — assumed as a measure of mean compactization of chromatin — shows a statistically reliable reduction;

— the nuclear surface varies in rather wide range, and in ADENO-virus infected cells its increase is not statistically reliable;
— the mass of DNA in either experimental group discloses a statistically significand increase (Fig. 1).

Regarding the hydrolysis curves their shift in the zone of higher extinctions and the biapical character of the curve formed by nuclei infected with ADENO-viruses worthy of notice (Fig. 2).

Discussion

The results obtained point to the fact that in viral infection of cells from human embryonal kidney, using different types ECHO- and ADENO-viruses, essential changes occur in the quantitative indicators under study. The analysis of the primary data which served to build up the arithmetic means table shows that the individual types of a given group of viruses produce similar, monotype changes which is furthermore supported by the low values of mean square deviations.

The decrease of high and appearance of lower permeabilities which condition the reduction of the average transudation in the infected cells points to the presence of a heterochromatization which might be related to the virus-induced increase of histone-like nuclear proteins and respectively, to their morphogenetic spiralizing function in the desoxyribonucleine system.

DNA mass increase is statistically significant for each of the experimental groups in comparison with controls, and is beyond any doubt. As far as ADENO-viruses are concerned, our results comply with most of the literature data (7,10), and might be explained by the synthesis of virus DNA. The biapical character of the hydrolysis curve is in support of a similar interpretation. However, the elevated position of the first peak does not rule out an eventual increase of autochthonous DNA which might be the result of a prolongation of the synthetic and postsynthetic periods of the host cell. The solution of the problem thus outlined requires the application of tagged DNA precursors but anyway, a definitive answer could be hardly reached on the basis of the analytical possibilities of microdensitometry alone.

Interpretation of the established increase of DNA mass in case of infection with RNA-containing ECHO-viruses proves to be much more difficult. The analogy of the hydrolysis curve with that of the control group shows a homogeneity of DNA composition. It is presumable that in this particular case it is a matter of some extension of the synthetic and postsynthetic periods of the infected cells, or else that the genetic block is effected exactly in these two periods with the consequent rise in the number of cells with a hyperploy DNA quantity.

REFERENCES

1. Ацев, С. I конгрес по микробиологич, София, 20—27 ноември 1965. — 2. Зандриттер, В. В Введение в количественную цитохимию (В. Я. Бродский, Н. И. Поляков, ред.), Мир, Москва, 1969, 139. — 3. Рангелова, Ст. *Епидем., микробиол.*, грес на българските микробиолози, БАН, Со*З*ия, 1967, 711. — 5. Соловьев, В. Д., И. Г. Баландин. Клетка и вирус, Медицина, Москва, 1973.— 6. Atzev, S., J. Gagov. Comptes Rends. de l'Acad. bulg. Sci., 1969, 22, 193. — 7. Boyer, G. S.,

C. Leuchtenberger, H. S. Ginsberg. *J. Expti. Med.*, 1957, 105, 195. — 8. Caspersson, T. *Biochem. Z.*, 1932, 253, 97. — 9. Darnell, J. E., D. Baltimore, M. Girard, D. P. Summers, J. V. Maizel. In The molecular biology of viruses (J. S. Colter, ed.), Acad. Press, New York, 1976, 375. — 10. Leuchtenberger, C., R. Leuchtenberger. *Biochem. Pharmacol.*, 1960, 4, 128. — 11. Sibatani, A. *Nature*, 1950, 166, 355. — 12. Velicer, L. F., H. S. Ginsberg. *Proc. Natl. Acad. Sci.*, 1968, 56, 1264.

МИКРОДЕНСИТОМЕТРИЧЕСКИЕ ИССЛЕДОВАНИЯ ЯДЕР ТКАНЕВЫХ КУЛЬТУР ЧЕЛОВЕЧЕСКОЙ ЭМБРИОНАЛЬНОЙ ПОЧКИ, ЗАРАЖЕННЫХ ВИРУСАМИ ГРУПП ECHO И АДЕНО

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РЕЗЮМЕ

Исследована количественная денситометрическая характеристика ядер клеток человеческой эмбриональный почки, подвергнутых реакции Ге Іое на 24-ый час после их заражения вирусами группы ECHO (1, 9, 14, 19, 26) и группы АДЕНО (2, 4, 6, 9). Установлено, что в сравнении с контролями увеличивается ентропия частотного распределения проницаемостей, средняя проницаемость и масса ДНК.

Кривая гидролиза при инфекции вирусами группы АДЕНО имеет двугорбый характер. Эта особенность связывается с гетерогенностью ДНК в данном случае (аутохтонная и вирусная).