QUANTITATIVE CHANGES IN DENSITOMETRIC AND GEOMETRIC PARAMETERS OF THE CELL NUCLEUS IN TISSUE CULTURES FROM HUMAN EMBRYONAL KIDNEY INFECTED WITH INFLUENZA $A_{2/75}$ VIRUS AND PARAINFLUENZA TYPE I VIRUS

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In earlier works (4, 13) we developed a quantitative method for structural characteristics assessment of nuclear chromatin based on frequency distribution entropy of optical permeabilities, mean permeability, nuclear area and DNA mass. Applied to a variety of experimental models insofar as character of cellular differentiation (7, 8, 9, 10), functional state (1, 11, 12) or pathological changes in the cell (5, 6) are concerned, the listed above parameters have enabled the working out of a number of integral assays, endowed with a rather high informative value. In a previous work (3) we were successful in demonstrating that in the cell nuclei from human embryonal kidney tissue cultures, infected with viruses of the ECHO and ADENO groups, characteristic changes take place in the quantitative structural pattern of the nuclear chromatin, preceding the cytopathogenic effect (CPE) specific for this particular group. Of a greater interest from biomedical point of view are the viral infections where CPE demonstrative in morphological respect has not been recorded, at least thus far. The latter problem is related more particularly to analytical possibilities in the viral cytopathology of widely diffused viral diceases with a periodic character and a tendency for pandemic widespreading, such as periodic influenza epicemic outbursts.

In the study submitted an attempt is made at outlining the quantitative changes in certain parameters of the cell nucleus in tissue cultures iniected with influenza $A_{2/75}$ virus and parainfluenza types 1 virus.

Material and Methods

The influenza virus strain used was isolated on chick embryos, and adapted to cell culture from human embryonal kidney (HEK). Infection with influenza and parainfluenza viruses was effected with 0.2 ml virus -containing fluid over lamellae. At 24 hours, the lamellae were removed and dried, and thereafter fixed with a saturated sublimate solution in alcohol with addition of 15 per cent concentrated formol and 5 per cent glacial acetic acid, and finally treated after the method of Feulgen. Measurements were performed using non-automated cytophotometric apparatus at monochromatic light 570 nm and explorative field with diameter one micron in the object's plane. In each nucleus the permeability in 50 points was measured, and from the data obtained calculations were

made of mean permeability (T), entropy of the frequental distribution of permeabilities (H), and through mean permeability conversion using special tables, the mean extinction indispensable for DNA mass calculation (M) was determined. The latter was registered in conditional units as a product of the extinction by the nucleus area (S). The area was obtained through planimetry on plotting paper is a negative photographic material projection from nuclei photographed in advance. All data underwent statistical elaboration using the tables of Strelkov (1966).

Results and Discussion

The obtained results such as arithmetical means with indication of the mean square deviation, and the limits of the confidential interval at probability of incoincidence 0.05, are presented in Table 1. It can be seen that:

Table 1

Permeability	Control	Influenza $A_{2/_{75}}$	Parainfluenza type 1
0— 10			
10— 20			
20— 30			
30— 40			
40— 50			0,043 ± 0,002 0,039 ÷ 0,047
50— 60			0,312 ± 0,005 0,302 ÷ 0,322
60— 70	0,178 ± 0,002 0,173 ÷ 0,183	0,075 ± 0,002 0,071 ÷ 0,079	0,574 ± 0,007 0,560 ÷ 0,588
70— 80	0,699 ± 0,003 0,692 ÷ 0,706	0,777 ± 0,004 0,773 ÷ 0,781	0,071 ± 0,002 0,067 ÷ 0,075
80— 90	0,123 ± 0,002 0,118 ÷ 0,128	0,148 ± 0,004 0,146 ÷ 0,155	
90—100			
$\bar{H}$	0,3504 ± 0,004 0,3495 ÷ 0,3513	0,2848 ± 0,007 0,2833 ÷ 0 2848	0,4215 ± 0,005 0,4205 ÷ 0,4225
$\bar{T}$	74,43 ± 0,069 74,20 ÷ 74,56	75,72 ± 0,094 75,63 ÷ 75,81	61,48 ± 0,201 61,09 ÷ 61,87
$\bar{S}$	354,92 ± 8,960 337,36 ÷ 372,48	702,80 ± 22,354 658,99 ÷ 746,01	506,18 ± 21,819 463,42 ÷ 548,94
$\bar{M}$	45,54 ± 1,166 43,25 ÷ 47,83	85,01 ± 2,681 79,75 ÷ 90,26	106,60 ± 4,611 97,56 ÷ 115,74

In cells iniected with parainfluenza type-1 virus densitometric changes manifested with a heterochromatization through the appearance of lower optical permeabilities, accordingly with a decrease in mean permeability, are

established. A general shift of optical permeabilities' frequential distribution towards the lower range ones is recorded. This conditions also the comparatively slighter increase in the entropy of frequential distribution of permeabilities, irrespective of its significance and statistical reliability relative to controls. An increase in the nuclear area and DNA mass is likewise noted. According to values they are similar to the changes in the listed parameters found by us in a previous study of virus-infected cells using viruses of the ECHO and ADENO group (3). The interpretation of the latter phenomenon which has been consistently observed in virus-infected cells from HEK, regardless of whether or not it was a matter of infection with DNA-or RNA-containing viruses, raises a number of problems. While in DNA viruses it is logical to attribute such an increase to the presence of a newly synthesized viral DNA, in RNA viruses such an interpretation is impossible. In all likelihood the mechanisms involved are two — firstly, one might consider a derangement in cell reproduction processes where either the duration of the synthetic period is increased, or a greater number of cells are retained in the postsynthetic period which, in turn, augments the DNA-pool values; secondly, it is possible that the increase in DNA has a virtual character, conditioned by changes in the physico-chemical structure of nucleoproteins. Having in mind that in the typical CPE, as a rule it is a matter of genetically repressive mechanisms in terms of the host genome by histone-like substances (2), the possibility that the increase in DNA values is being at least partially conditioned by the heavy chromatin densification, rendering it more resistant against overhydrolysis than the hydrochloric acid used for the purpose, is by no means ruled out.

Changes at nuclear level in cell elements infected with influenza $A_2/_{75}$ virus are of a distinctly different type. Regarding the frequential distribution of permeability, one is impressed by the discrete although statistically reliable tendency of high permeabilities to increase, i. e. euchromatization. The entropy of the optical permeabilities' frequential distribution is somewhat reduced owing to the stronger ammassment of permeabilities within two quanta (70-80 per cent and 80-90 per cent). The mean permeability is close to that of the control. DNA mass values display a moderate increase, lower than that in parainfluenza, and on the whole, entering within the framework of the already established diapason of increase among virus-infected cells (3). The most characteristic changes of all indicators under study are displayed by the area of nuclei which is strongly increased (up to two times), as compared to the control. In this respect the changes found are clearly distinguished from those in virus-infected cells hitherto observed. The interpretation of the latter phenomenon may be seeked in a transitory, unspecific in its essence but very strongly pronounced reaction of the cell nucleus, characteristic of this particular type of viral infection. Although it is possible that this reaction has the same nature as in other types of viral invasions, the degree of its expression or its quantitative characteristic might be accepted as pathognomonic for the influenza infection. It is presumable that such a swelling of the nucleus is supposed to maintain the noxogenic factor at subthreshold pathogenic doses, and is realized by way of heavy hydration of the nucleus. The solution of the problem posed requires comprehensive and specific researches.

In conclusion, it must be stated that the differential-densitometric analysis applied enables the detection of definite changes in the structure of the nucleus also in a virus infection type, qualified as acytopathogenic. This, on

the one hand, emphasizes the high analytic value of the quantitative cytologic analysis, and more particularly, of the cell nucleus differential densitometry, and on the other, justifies the widened scope of study in this field with a view to adopt quantitative cytologic methods and to broaden the possibilities of virus cytodiagnostics.

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КОЛИЧЕСТВЕННЫЕ ИЗМЕНЕНИЯ В ДЕНЗИТОМЕТРИЧЕСКИХ И ГЕОМЕТРИЧЕСКИХ ПАРАМЕТРАХ КЛЕТОЧНОГО ЯДРА В ТКАНЕВЫХ КУЛЬТУРАХ ИЗ ЭМБРИОНАЛЬНОЙ ПОЧКИ ЧЕЛОВЕКА, ИНФЕЦИРОВАННЫХ ГРИППОЗНЫМ ВИРУСОМ $A_2/_{75}$ И ПАРАГРИППОЗНЫМ ВИРОСОМ — ТИП$_1$

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РЕЗЮМЕ

На пластинках из тканевых культур эмбриональной почки человека, инфецированных посредством контактной адаптации 0,2 мл содержащей вирус жидкостью (гриппозный вирус $A_2/_{75}$ и парагриппозный вирус тип I) и обработанных по методу Fenlgen были произведены измерения по 50 пунктов ядра посредством многозондовой цитофотометрии при монохроматическом освещении 570 nm и эксплоративном поле с диаметром I микрон в плоскости объекта.

Были вычислены средняя проницаемость (Т), энтропия частотного распределения проницаемости (Н) и масса ДНК (М), а также произведение площади ядер и экстинкции. Результаты статистически обработаны, для чего использовались таблицы Стрелкова (1966).

Установлена гетерохроматизация, увеличение площади ядер и массы ДНК клеток, инфецированных парагрипозным вирусом тип I и умеренное увеличение массы ДНК с исключительно большим увеличением площади ядер при гриппе $A_2/_{75}$.