CELL SIZE WITH SPECIAL REFERENCE TO MULTIPLICATION OF HELA STRAIN *IN VITRO*

II. MEASUREMENT OF MITOTIC AND INTERMITOTIC DURATION WITH SPECIAL REFERENCE TO SIZE OF NUCLEUS

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The mitotic duration of various tissues has been measured by many investigators and the length of interphase has also been estimated by indirect methods by a number of workers. No one has measured directly the intermitotic duration of tumor cells, though Fell and Hughes[1] measured it in a film record of 17 cells of mouse spleen culture.

On HeLa cells, a strain of human epithelium cultured from a cervical carcinoma, Puck *et al.*[7] and Yamada and Takano[8] estimated indirectly the generation time.

It is very interesting to know whether cells with polypolar division have different intermitotic duration from cells with normal bipolar division, and what the fate of cells after the polypolar division. Moreover, it is desirable to clarify the correlation between mitotic duration and intermitotic of individual cell.

With these purposes in mind, observations were made on the interphase and mitotic duration of HeLa cells.

MATERIAL AND METHODS

Stock cultures of strain HeLa were maintained by stationary test tube culture. The cells in the test tube were removed with 0.45% trypsin, and washed three times in nutrient medium by centrifugation. After suspending the cells in medium, 1.5 ml of the cell suspension containing about 15 000 cells was inoculated into a flat sided tube of $70 \times 13 \times 3$ mm. After 24 hours incubation, the tube was set upside down on a microscope that was kept at 37° C.

The nutrient medium consisted of 0.1% yeast extract dehydrate and 0.5% lactalbumin hydrolysate in Earle's balanced salt solution, to which 20% bovine serum was added. Earle's solution was modified to contain 0.45% glucose and 20 mg phenol red per 1 000 ml of medium. The nutrient medium was adjusted to pH 7.8 with addition of 1.4% $NaHCO_3$. The medium of the culture was renewed every 24 hours. The pH of the cultured medium dropped to 6.4–6.8 at that time. Observation was made on the cells in one field under the microscope

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from the 3rd day to the 7th after inoculation of cells. The data of the cells which migrated out of sight, broke down spontaneously or did not show mitosis were omitted. The duration of interphase and mitotic phases was measured by direct observation. For measuring the diameter of the nucleus, the cells cultured were photographed every one hour and the geometrical mean of the maximum and minimum diameters of the nucleus was calculated from the photograph within one hour before mitosis, because within this period the nuclear size was the most stable through the process of interphase.

Definition of the terms used in this study—The definition of mitotic phase has differed according to the investigator. The following definition was used here for convenience.

Interphase; Period from the beginning of the disintegration of the nuclear membrane to the completion of arrangement of chromosomes in the equatrial plane.

Metaphase; Period from the end of prophase to the start of separating movement of chromosomes.

Anaphase; Period from the start of the separation of chromosomes to the beginning of cytoplasmic cleavage.

Telophase; Period from beginning of cleavage to the completion of cytoplasmic division.

Generation time; Time from the beginning of interphase to the end of telophase.

RESULTS

Both the intermitotic and mitotic durations of 46 cells derived from 16 cells were measured. Some cells were observed for 3 generations. At the same time 9 cells with polypolar division were observed.

Generation time and mitotic pattern—Chart 1 shows the mitotic pattern and generation time. Some cell lines had short and comparatively equal lengths of generation time and others long generation time. In cells of the former there usually occurred normal bipolar mitosis. In cells of the latter there often occurred abnormal mitosis. The longest generation time measured in this study was more than 81 hours and 55 minutes. There might, however, exist longer generation times in HeLa cells, for a few cells which seemed to be certainly alive did not show any mitosis during more than 100 hours. The variation in generation time of individual daughter cells was usually limited to within 2 hours, with the exception of a rare case, where it was more than 14 hours. The cells which showed polypolar division except tripolar division, often formed one or two multinucleate cells which had 2 to 4 nuclei. The daughter cells of the cells with polypolar mitosis frequently broke down spontaneously. Although the temporal furrowing of nucleus was sometimes observed, amitotic division was not recognized. In one case, all the daughter cells derived from a tripolar mitosis, showed normal bipolar division.

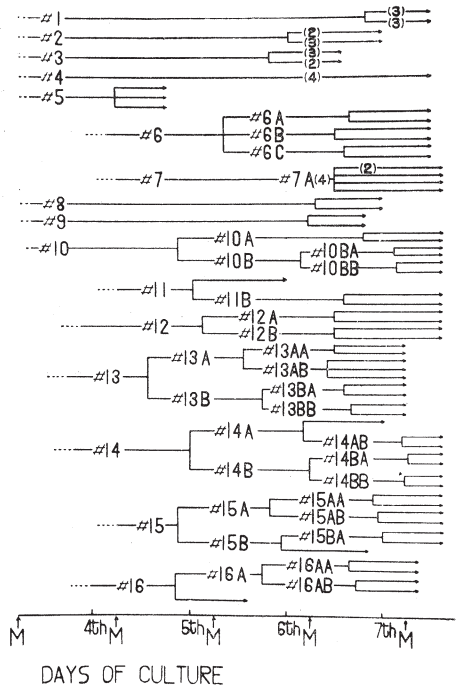

CHART 1. Mitotic pattern and generation time of individual cells

↑ M indicates the time of renewal of culture medium.

Number in parentheses indicates the number of nuclei of a multinucleate cell.

Solid line shows the generation time of individual cell observed directly.

Dotted line indicates that the cell has entered the interphase already before start of observation.

→ indicates that observation was discontinued on the way of interphase.

Nuclear size, polarity and number of nucleoli—The nuclear size of HeLa cells was from 15 to 32 micra in diameter just before mitosis, as shown on Table 1. As a rule, cells with the nucleus larger than 28 micra in diameter were found to divide polypolarly. Concerning the cells that divided bipolarly, the diameter of nucleus was usually from 16 to 25 micra. The nucleus had 1 to 7 nucleoli. Number of nucleoli was not related to nuclear size, polarity and intermitotic duration. The number of nucleoli of the daughter cells varied, even if they were divided from the same cells, as reported by Lewis.[4]

Duration of interphase—The intermitotic duration on which the generation time depended mainly also showed variable lengths. There was, however, a tendency for the larger nucleate cell to show longer intermitotic duration, independent of the polarity of mitosis. This correlation between nuclear size and intermitotic duration was de-

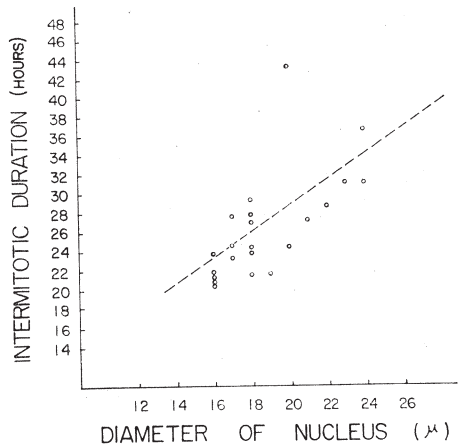

CHART 2. Correlation between inter mitotic duration and diameter of nucleus.

TABLE 1. Nuclear Size and Duration of Intermitotic and Mitotic Phases

Cell	Diameter of nucleus	Interphase	Prophase	Metaphase	Anaphase	Telophase
	Microns	Hours Min.	Min.	Min.	Min.	Min.
# 8	32	X+68.00	·····20·····		·····20	10
# 1**	31	X+78.59	·····148·····			28
# 4**	30	X+28.10	80	·····27·····		
# 7**	30	X+37.22	·····88·····			
# 5*	29	X+18.40	30	20	44	6
# 2**	28	X+59.13	27	23	126	13
# 3**	28	X+52.37	·····336·····			
# 6*	27	X+27.57	·····67·····			
# 12	25	X+20.34	32	56	6	5
# 9	24	X+66.35	17	14	14	6
# 11	24	X+18.02	36	24	5	6
# 11 B	24	36.49	10	20	6	12
# 12 B	24	31.17	·····59·····			3
# 6 C	23	29.43-Pro.	··	36	5	5
# 12 A	23	31.07	18	36	29	4
# 6 A	22	28.43	30	15	93	27
# 6 B	21	27.08	30	17	28	5
# 10	21	X+34.10	19	11	14	7
# 15 B	20	24.30	27	3	3	6
# 10 A	19	43.24	·····168·····			24
# 15 A	19	21.41	5	32	12	3
# 10 B	18	29.24	28	20	5	3
# 10 BA	18	23.57	·····26·····		3	2
# 10 BB	18	24.22	22	6	4	3
# 14 A	18	27.03	9	20	11	5
# 14 B	18	27.55	9	14	17	7
# 16 A	18	21.36	14	19	7	4
# 13	17	X+19.45-Pro.	··	12	7	15
# 13 A	17	23.17	11	7	11	4
# 13 B	17	27.48	12	·····22·····		4
# 14 AB	17	23.52-Pro.	··	19	10	9
# 15 BA	17	24.37	23	13	10	6
# 13 AA	16	21.49	10	11	7	6
# 13 AB*	16	20.57	8	16	9	3
# 13 BB	16	20.44	45	15	24	2
# 14	16	X+26.55	·····98·····			5
# 14 BA	16	24.00-Pro.	··	22	11	2
# 14 BB	16	23.05-Pro.	··	8	6	11
# 15	16	X+ 8.40	8	·····37·····		9
# 15 AA	16	23.09-Pro.	··	2	39	2
# 15 AB	16	23.49	5	58	11	5
# 16	16	X+21.54-Pro.	··	28	27	5
# 16 AA	16	20.27	7	25	7	3
# 16 AB	16	21.36-Pro.	··	13	6	15
# 11 BA	15	19.15-Pro.	··	22	6	2
# 7 A***	14 12 12 11	13.55	25	25	·····7·····	

*=tripolar division

**=multipolar division

***=multinucleate cell.

X+=time was not observed, because the cell had entered the interphase already before start of observation.

-Pro. means that data of interphase including the duration of prophase.

monstrated in Chart 2, and was estimated as significant.

$$(r=0.6062>0.526, \quad df.=21, \quad p=0.01)$$

The regression was as follows;

$$I=1.30N+2.24 \qquad (A)$$

I = Intermitotic duration, hours.
N = Diameter of nucleus within one hour before mitosis, micra.

The mean intermitotic duration of normal bipolar division was 26.8 ± 5.6 hours.

Duration of mitotic phases—Duration of mitotic phases varied widely. But among the daughter cells, the variation of the total mitotic duration was usually slight as that of intermitotic duration. It seemed that there were some tendencies in the distributions of mitotic durations, as follows;

prophase ; from 10 to 30 minutes
metaphase ; from 15 to 25 minutes
anaphase ; from 5 to 15 minutes
telophase ; from 2 to 10 minutes

A significant correlation was not obtained in the duration of each phase and other factors of individual cells as Moorehead and Hsu's result.[6] The mean value of total mitotic time was 65 ± 38 minutes in normal bipolar division. As a rule, multipolar mitosis which was found usually in large nucleate cells, took a longer mitotic time. Between the total mitotic duration and the nuclear size, there was a significant correlation as shown in Chart 3.

$$(r=0.4529>0.418, \quad df.=35, \quad p=0.01)$$

Its regression was as follows;

$$M=5.48N-31.04 \qquad (B)$$

M = Mitotic duration, minutes.
N = Diameter of nucleus, micra.

Concerning the correlation between intermitotic duration and mitotic, Chart 4 shows a significant correlation.

$$(r=0.6178>0.526, \quad df.=21, \quad p=0.01)$$

The regression was as follows;

$$M=4.61I-59.59 \qquad (C)$$

M = Mitotic duration, minutes.
I = Intermitotic duration, hours.

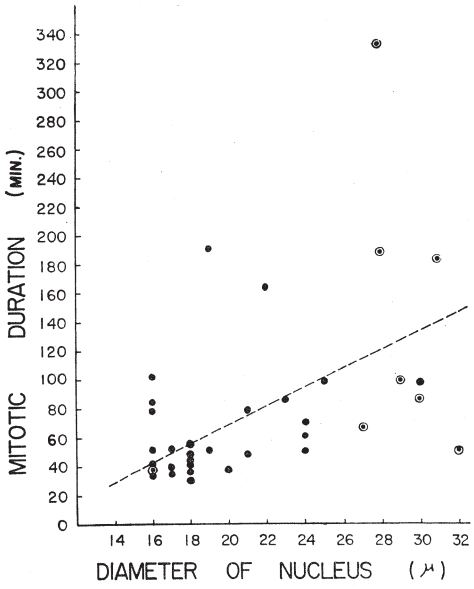

CHART 3. Correlation between mitotic duration and diameter of nucleus.
● Shows bipolar division
◉ Shows multipolar division

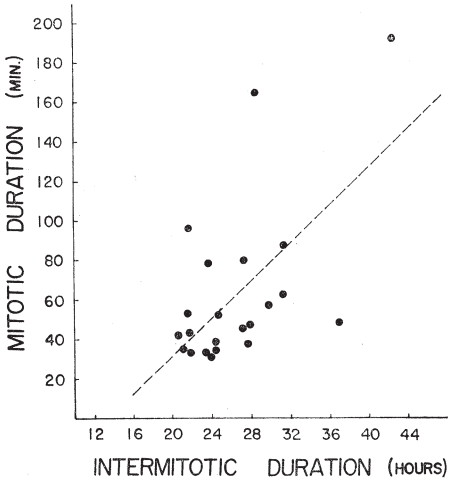

CHART 4. Correlation between mitotic duration and intermitotic one.

DISCUSSION

Between the intermitotic duration and nuclear size, a clear correlation was recognized as shown in Chart 2. It was presumably due to the nucleus growing relatively regularly with the duration of interphase, though the nuclear size varied already soon after mitosis and might depend on the nuclear size of the mother cell.

Makino and Nakahara[5] reported on 4 cells of ascites sarcoma of rats that the duration of the mitotic phases was longer in the large sized cells than in the small sized ones. Such a relation was also obtained between nuclear size and mitotic duration, though the reason why such a correlation existed there could not be ascertained.

Hirono[2] and Lewis M. R., and Lewis W. H.,[3] described that tripolar cells required more time for mitosis than bipolar ones. In this study, multipolar division showed usually longer mitotic and intermitotic duration. It is possible that cells which divide multipolarly require longer mitotic duration and larger amount of energy for initiation of mitosis than cells which divide bipolarly due to complexities in the chromosomes and other mitotic apparatus, and therefore need longer intermitotic duration for accumulation of a larger energy to initiate mitosis.

The meaning of the correlation between mitotic duration and intermitotic is not clear. It may be considered that there is no direct relationship, but

results merely from two correlations between mitotic duration and nuclear size (A), and between intermitotic duration and nuclear size (B).

SUMMARY

1) The intermitotic and mitotic durations of 46 cells derived from 15 cells of HeLa strain were observed. Among them, 9 cells with polypolar division were also observed.

2) Both the intermitotic durations and the mitotic revealed a wide range of distribution.

3) The cells in the same cell line, especially the daughter cells had usually similar intermitotic and mitotic duration times.

4) In normal bipolar division, the means of intermitotic and mitotic duration were 26.8±5.6 hours and 65±38 minutes respectively.

5) It was supposed that there exists a parallel relationship between nuclear size, duration of interphase and mitotic duration of each cell.

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