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

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The cell size is variable in normal tissues. There are also phenomena called hypertrophy and atrophy of cells in normal and morbid tissues. Variabilities in cell size are remarkable especially in tumor tissues. It is believable that cell size depends on the cellular environment and characteristics of the cell. It is desirable to know the biological activities of cells from their size.

With this purpose, the factors related to the mean cell size of a cell population, and the relationship between size and multiplication of individual cells in a cell population was investigated on HeLa strain by the cell culture methods.

I. STUDY ON THE RELATIONSHIP BETWEEN CELL SIZE AND NUTRITIONAL ENVIRONMENT

Variability in cell size has often been found in tumor cells *in vivo*. Although a change in the size of living tumor cells has not been clearly demonstrated, the biological significance of cell size is very interesting.

Some workers[3)5)6)] found no change in the average cell volume during tumor growth, while others[4)7)8)] found decrease in the average cell volume during growth of ascites tumors. There are also reports on the occurrence of enlargement of cells in an early stage of transplantation into animals.[1)5)]

Concerning the average cell volume, current knowledge is very complicated. To reveal the intrinsic nature of cell size, simplification of cellular environment is desired. Using the cell culture method, a factor which may be related to cell size was clarified in this study.

MATERIAL AND METHODS

The growth medium employed here consisted of; (a) 0.4% lactalbumin hydrolysate in Earle's solution containing 0.45% glucose, 100 units of penicillin per 1 ml of saline, 1 mg streptmycin per 1 ml, and 0.02 mg phenol red per 1 ml, (LE solution) and, (b) bovine serum inactivated by incubation at 56° C for 30 minutes.

The cells used in this study were HeLa strain. Stock cultures were maintained in a medium which consisted of 10% bovine serum and 90% LE solution.

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For the experiment, cell suspensions were prepared from stock monolayer cultures by incubation for 10 minutes with 0.04% disodium versenate in Ca-Mg-free phosphate buffer saline. After centrifugation the cells were resuspended in LE solution, pipetted carefully to disperse cell clumps, enumerated with a Thoma's hemocytometer, and photographed.

The cell suspension was inoculated into an appropriate number of bottles of $6\times3\times3$ cm. Each bottle contained 2.5×10^5 cells. The bottles were divided into 3 groups, which contained 20%, 10% and 5% bovine serum in medium, respectively. Half of the bottles in each group contained a cover glass of 12 $\times5$ mm. The cultures were kept in an incubator at 37° C. Renewal of medium was made every 24 hours. Every 2 days, a culture in each group was treated with versenate. After centrifugation the cells were resuspended in LE solution, enumerated and photographed microscopically.

Experiment was performed for 8 days after inoculation of cells.

The cell diameter of 500 cells was measured at randam by projection of photographed slides.

For measurement of mitotic index and diameter of mitotic cells, culture with a cover glass of each group was employed every 2 days. The cells on a cover glass were fixed with Zenker's formol and stained by hematoxylin and eosin. Diameter of 100 metaphasic cells was measured by a micrometer and the mitotic index was also estimated at the same time.

The reason why metaphasic cells were selected as representative of mitotic cells was that metaphasic cells were the most sperical during growth cycle of cells and their diameter scarcely changed by fixation and staining. Metaphasic cells might be compared with living cells which become spherical in form by versenate treatment.

RESULTS

Logarythmic multiplications of HeLa cells cultured in media of 3 grades of serum concentration are illustrated in Chart 1. The difference in multiplication rate of the 3 groups is most prominent on the 2nd day after inoculation. The multiplication rates, thereafter, are approximately parallel in all 3 groups. On the 8th day, a decrease in multiplication rate in the 20% serum group was observed.

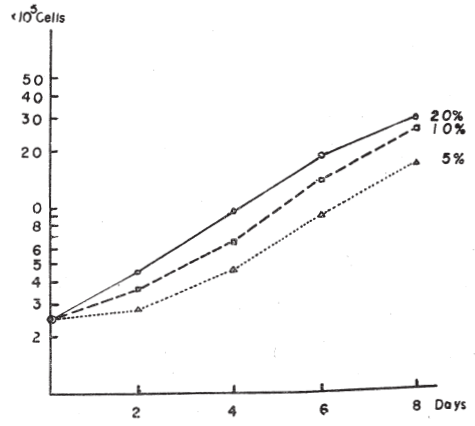

CHART 1. Multiplication curve of HeLa cells in medium of 20%, 10% and 5% bovine serum.

Chart 2 shows the distributions of diameter of 500 cells of stock culture used in this study, and of the 500 cells cultured in a medium containing 20%, 10%, and 5% serum respectively. The distribution curve of stock culture does not show a

normal distribution and has two peaks. The mean diameter of cells was 14.2±4.1 μ. Concerning the 20%, 10%, and 5% serum containing groups, remarkable enlargement of the mean diameter and obvious widening of range of varieties in cell diameter were recognized on the 2nd day after inoculation in all groups. There was a tendency for larger mean diameters to be observed in the cell population cultured in the medium of higher serum concentration. Decrease in average cell diameter occurred on the 4th day after inoculation except in the case of the 20% serum group. The variation in cell size also decreased in every groups with culture age.

The average diameter of 100 metaphasic cells in each group is presented in Table 1. The average diameter and the standard deviation quite agree with the descending slope of each distributive curve of cell diameter.

The average diameter of metaphsic cells decreased with the culture age. The standard deviation also decreased with culture age.

Mitotic index of 5 000 cells is shown on Table 2. Generally, a higher mitotic index was observed in the group cultured in medium of higher serum concen-

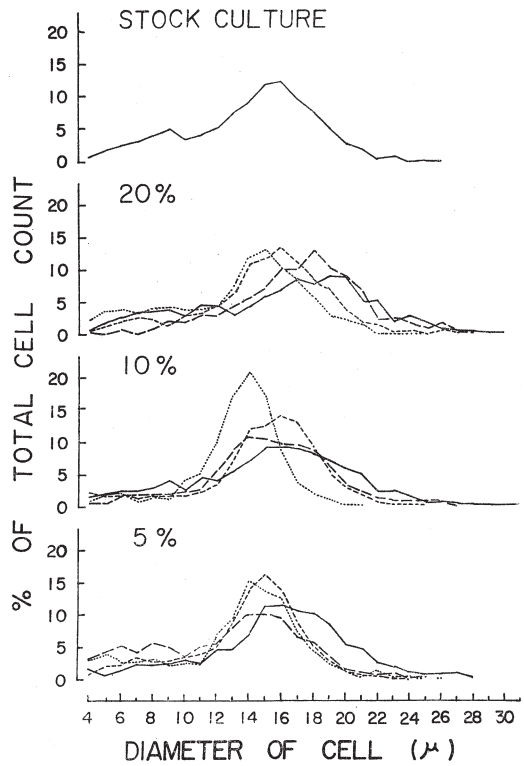

CHART 2. Changes in distributions of diameter according to serum concentration of culture medium and culture age.

Stock Culture; Cell diameter was measured at the time when cell suspension from stock culture was inoculated. 20%, 10% and 5% indicate serum concentration in culture medium.

——— shows 2nd day culture after inoculation.

– – – – shows 4th day culture after inoculation.

– – – – – shows 6th day culture after inoculation.

····· shows 8th day culture after inoculation.

TABLE 1. The Mean Diameter of 100 Metaphasic Cells

Culture days	Mean diameter of metaphasic cells		
	20% serum (μ)	10% serum (μ)	5% serum (μ)
2	21.1±4.6	21.8±4.3	20.0±4.9
4	22.3±3.2	20.5±5.0	19.8±3.9
6	19.2±4.4	18.8±3.3	18.6±3.7
8	17.8±2.9	17.5±2.9	17.1±3.0

TABLE 2. Mitotic Index and Frequency of Irregular Mitosis

Culture days	Mitotic index			Frequency of irregular mitosis		
	*20%	*10%	*5%	*20%	*10%	*5%
2	4.18	3.86	2.70	9.1	9.8	8.8
4	4.62	4.20	4.04	6.1	6.2	9.9
6	4.82	4.28	4.32	1.6	1.4	2.8
8	3.84	4.60	4.56	2.1	3.5	2.6

* Means per cent of serum in cultured medium. Mitotic Index was calculated from observation of 5000 cells. Frequency of irregular mitosis indicates per cent of irregular mitosis in total mitosis.

tration. This relation is most prominent in all 3 groups on the 2nd day after inoculation. After the 4th day, it becomes vague. On the 8th day, the mitotic index of the 20% serum group became low. The proportion of anomalistic mitosis also decreased with culture age in each group.

DISCUSSION

Klein and Révész,[3] and Lucke and Berwick[5] using the hematocrit technique, found no change in the average cell volume during growth of Ehrlich ascites tumor from 3 to 16 days. Peacock, Williams and Mengoli[6] also reported no significant change in cell volume of Krebs-2 ascites carcinoma during tumor growth by using the Coulter electronic cell counter.

On the other hand, decrease in the average cell volume during growth of ascites tumor has been reported by Rice and Shelton[8] for L1210 lymphoid leukemia and by Révész and Klein for the DBA, EL4 and 6C3HED lymphoias.[7]

This study also showed decrease in the average cell volume during the culture age. There, however, was found enlargement of the cells in the early stage after inoculation. Goldie and Felix[1] for Sarcoma 37, and Lucke and Berwick[5] for Ehrlich ascites tumor observed considerable increment of cell size during the first day or two after inoculation into animals.

There might be many interpretations for the decreases in average cell diameter with culture age following the enlargement in the early stage after inoculation.

It is conceivable that the initial enlargement of cells resulted from inhibition of multiplication by versenate treatment or other factors which might occur at inoculation. If this is true, it can not be interpreted that enlargement of cell size occurred more evidently in a group of higher serum concentration, for its multiplication rate was higher than the group of lower serum concentration, and enlargement of cell size was recognized more obviously on the 4th day than the 2nd day in the 20% group.

Rice and Shelton[8] described a decrease in average tumor-cell volume with increasing tumor age was believed to be associated with accumulation of metabolic wastes in ascitic fluid, anoxia, and limitation of nutrient supply that develops as the number of tumor cells increases in the peritoneal cavity.

Chart 3 illustrates the correlation among diameter of cells, cell count and serum content in culture medium.

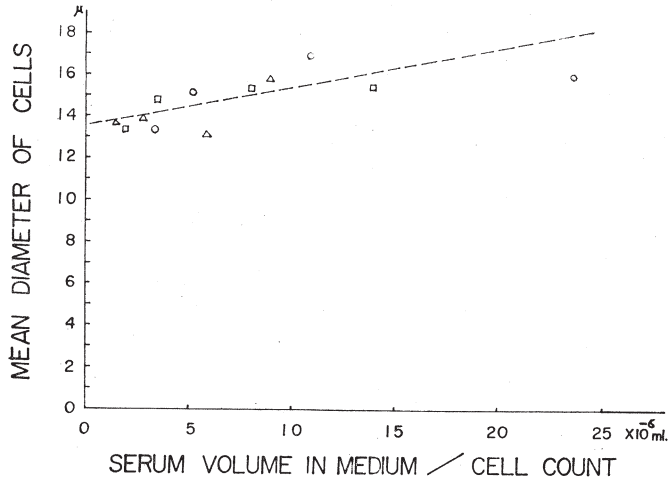

CHART 3. Correlation between mean diameter of cells and Bovine serum content per a cell.

○ indicates cells cultured in medium containing 20% serum.
□ indicates cells cultured in medium containing 10% serum.
△ indicates cells cultured in medium containing 5% serum.
--- shows regressive line.

$$\text{Mean Diameter of Cells } (\mu) = 1.877 \times 10^{6} \frac{\text{Serum Volume (ml)}}{\text{Cell Count}} + 13.5$$

$$(r = |0.6797| > 0.553, \quad \text{d.f.} = 11, \quad p = 0.05)$$

From this correlation, it is possible that the average diameter of cells may increase untill the cell number reaches a limit which depends on serum content and is represented as above formula, and that average diameter of cells may decrease with the multiplication after cell number reaches this limit, as shown the formula.

The enlargemnet of cell size on the 2nd and 4th days of 20% serum group may be interpreted from this correlation.

The correlation was, hitherto, discussed from the view point of serum content. In general, it is possible to replace serum content with the nutritional supply.

Decreases in average cell volume during growth of tumor *in vivo* may be related to the relative decreases in nutritional supply to the increasing cell number.

In the case of no change in the average cell volume during growth of tumor, reported by Klein and Révész,[5] Lucke and Berwick[5] and Peacock *et al.*,[6] the nutritional supply may be carried smoothly and the nutritional level may be maintained constant proportional with the increase in cell number.

The cell size thus changes according to nutritional environment and cell number so that the pattern of distribution of cell size may require more caution

to be defined, though Morgan,[2] Lucke and Berwick[5] and Peacock *et al.*[6] presented the pattern of distribution of cell size in several strains of tumor and cultured cells.

Decrease in multiplication rate and mitotic index on the 8th day of the 20% serum group may have resulted from the cell number reaching the limit which might be related to nutritional factors. Regarding this problem, Waymouth[9] reported on strain L cells by using the hematocrit method that daily renewal of nutrient clearly resulted in continution of growth after the culture without renewal of medium had reached a stationary state.

Concerning the increment of variety in cell diameter on the 2nd day in all groups, it may be related in part to mitotic inhibition resulting from injuries which might be caused by treatment of removal of cells from stock culture. Mitotic inhibition may occur then, because the mitotic index showed lower values on the 2nd day in all groups, especially in the group of lower serum concentration.

On the other hand, it is also possible that increase in variety of cell size depends mainly on the rapid change of nutritional environment. When nutritional environment changes suddenly, the pattern of the distribution curve of cell size with its mean value will change to a new distribution pattern which depends on a new nutritional equilibrium. At the same time, there will appear an irregular distribution curve.

It is supposed that a cell population has a normal like distribution of cell size in a stable nutritional environment. If change of nutritional environment occurs gradually, the distribution of cell size will shift to a new equilibrium, keeping a form resembling a normal distribution.

SUMMARY

Diameter of 500 cells of the HeLa strain cultured in media containing 20%, 10%, and 5% serum was measured. Multiplication rate, mitotic index and diameter of 100 metaphasic cells in each medium were also estimated.

Distribution of cell diameter varied with culture age and serum concentration in each medium. Ranges of variability of cell diameter and frequency of irregular mitosis also varied with culture age.

A correlation between the mean diameter of cell number and serum content was recognized in this study.

Distribution of diameter of metaphasic cells agreed with the descending slope of the distribution curve of cell diameter.

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