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# ELECTROCONDUCTIVITY CHANGES DURING THE MITOTIC CYCLE IN EHRLICH ASCITES TUMOUR CELLS

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SUMMARY

Ehrlich ascites tumour cells were separated according to their size by a velocity sedimentation technique in a linear stabilizing viscosity gradient. Fractions obtained were found to be considerably enriched in cells in the $G_1$, S and $G_2$ periods. The content of viable cells in all the fractions was not less than 95 %, while that of the non-tumour cells was about 13 %.

It was shown that the specific electroconductivity of the cells was high in the $G_1$ period, decreased by a factor of 2–2.5 in the S period and increased again in the $G_2$ period. Some possible reasons for the observed changes in cell membrane permeability and their role in cell metabolism are discussed.

## INTRODUCTION

It has been noted that cell division cycles can be initiated by such different agents as surface-active agents[1], agglutinins[2] and enzymes[3] or mechanical damage of the cell surface[4], isolation of individual cells from tissues[5], *etc.* All these effects have this in common: they disturb the stationary ion and water distribution between the cell and its environment[6]. If the initiation of cell division and ion–water distribution are mutually related, one would, by analogy, expect the ion homeostasis to suffer some periodic changes in the cells dividing autonomously.

The disturbances in the cell homeostasis could be due to changes in the diffusional properties of the cell surface membrane and/or to changes in the intensity of the active transport. In this paper we examine the changes in the electrical parameters of the plasma membrane during the mitotic cycle in Ehrlich ascites tumour cells.

## MATERIALS AND METHODS

### *Preparation of cell suspensions*

Hyperdiploid Ehrlich ascites tumour cells ($50 \cdot 10^6$) were inoculated intraperitoneally into white mongrel mice. Ascites cells were harvested 7–8 days after inoculation when the number of cells reached $500 \cdot 10^6$–$600 \cdot 10^6$ cells per mouse. Haemorrhagic tumours and tumours containing more than 3 % dead cells (determined by the eosin

test) were discarded. Cells were washed twice with Morgan, Morton and Parker's Medium 199[7] and resuspended ($12 \cdot 10^6$ cells per ml) in approximately 60 ml of the medium.

*Synchronization procedure*

Cells were synchronized by sedimentation in a 5–45 % linear gradient of bovine serum in Medium 199 at unit gravity. The gradient was generated in cylindrical dividing funnels 40 mm in diameter. The height of the gradient column was about 100–110 mm. Cell suspension was carefully layered onto the gradient. The number of cells per funnel was not allowed to exceed $130 \cdot 10^6$, otherwise it resulted in cell streaming rather than regular sedimentation and hence to deterioration of cell separation. Cells were separated for 2 h at 20° and five fractions were collected. Fraction volumes collected were (in ml): 50–60, 25, 20–25, 15–20 and 15 for Fractions 1, 2, 3, 4 and 5, respectively. The cells with the highest speed of sedimentation were collected in Fraction 1, and the slowest cells in Fraction 5. The control cells which were incubated in Medium 199 containing 20 % of bovine serum for 2 h, and those collected after sedimentation, were washed with Medium 199 and resuspended in this medium to approximately 20 % volume concentration of cells.

DNA synthesis in cells was followed by tracer techniques. Mice were inoculated intraperitoneally with 20 $\mu$C per mouse of [$^3$H]thymidine (20C/mmole; British Drug Houses). 1 h after inoculation the tumour was removed, tumour cells were separated as described above and precipitated on paper filters, $1 \cdot 10^6$ cells on each. The completeness of precipitation was specially checked. The filters were fixed with methanol and incorporated radioactivity was determined in a liquid scintillation counter. The specific activity in counts per min was calculated per cell.

*Cell counts*

Cell counts, in unfractioned suspensions and in fractions from viscosity gradients, were made using haemocytometers. Dead cell content was determined by the eosin test. Counts of the non-tumour cells were made on methanol-fixed Giemsa-Romanovsky stained smears. Volume concentration of cells was determined, using a haematocrit centrifuge TH2 (Janetzki, Leipzig), by centrifugation of suspension samples at $12000 \times g$ for 4 min. Six to eight haematocrits were used to measure the volume concentration of cells in each sample; this value was measured with an accuracy greater than 2 %. No corrections for the intercellular volume were introduced since, under the conditions used, the height of the cell columns remained constant after further rotation or increase of rotation speed.

*Electroconductivity of cell suspensions*

The electroconductivities of cell suspensions were measured by the bridge technique at 9 kHz. This frequency is far below the relaxation frequency of the cell because the resistance of the surface membrane of ascites tumour cells is about 1 $\Omega \cdot cm^2$ (ref. 8). At such a low active resistance the contribution of the capacitive component, which is not more than 1 $\mu F/cm^2$ for all cells[9], in the surface membrane admittance does not exceed 6–7 % at 9 kHz. Therefore the resistivity measured for the cell is hardly affected by the reactance of the membrane.

The conductivity chamber contained two parallel platinum disc electrodes

covered with platinum black. The electrode surface was 2 $cm^2$. The volume of the conductivity chamber was 0.67 ml. The temperature of the chamber was kept constant in a water ultrathermostat. The resistive and capacitive components of the current were compensated by parallel connected sets of resistors and capacitors. The equilibrium point of the bridge was checked using a resonance zero indicator. The absence of noticeable electrode polarisation under these conditions[10] was demonstrated by the fact that the electrical conductance of the cell medium changed by not more than 0.2 % for a frequency change from 5 to 20 kHz. The resistivity of cells ($r_2$) was calculated by MAXWELL's formula[11]:

$$\frac{1 - r_1/r}{2 + r_1/r} = \rho \frac{1 - r_1/r_2}{2 + r_1/r_2} \quad (1)$$

where $r$ is cell suspension resistivity, $r_1$ is suspension medium resistivity, and $\varrho$ is volume concentration of cells in suspension. The resistivity of Medium 199 changed 6–7 $\Omega$ (64–71 $\Omega \cdot$cm) from batch to batch. The resistance measurements were carried out with great precision (0.1 %) in every test. After the measurement of the resistivity of a suspension cells were sedimented and the resistivity of a supernatant was measured. This value was usually almost equal to the resistivity of the original medium; in these cases it was taken as $r_1$. The values for $r_2 \pm$ S.E. (see Tables III and IV) were calculated from Eqn. 1 by substituting $\varrho \pm$ S.E. obtained from measurement.

Preliminary experiments had shown that $r_2$ calculated from Eqn. 1 was found to be independent of volume concentration in the range 5–30 %.

## RESULTS

12 experiments, in which ascites tumour cells were separated by their volumes, were carried out, giving fairly consistent results.

*Cell synchronisation in the stabilizing gradient.*

In Table I the results from three experiments of cell separation in the stabilizing gradient are presented. The data obtained show that sedimentation of cells in the stabilizing gradient led to the separation of cells according to their volumes. The results obtained are fairly constant: (1) $V_1/V_4 = 1.95$–$2.3$; $V_2/V_4 = 1.7$; $V_3/V_4 =$

TABLE I

SEPARATION OF EHRLICH ASCITES TUMOUR CELLS

Cell suspension was diluted to $13 \cdot 10^6$ cell per ml with Medium 199 and layered onto a gradient of bovine serum in the same medium. After 2 h sedimentation five fractions were collected. $V_k$ is the mean volume of tumour cells, $V_m$ is the calculated average volume of cells after separation. $V_i$ is the relative volume and $\Delta_i$ is the relative portion of cells in each fraction (where $i =$ Fraction 1, 2, 3 and 4).

| *Expt. No.* | *Tumour age (days)* | $V_k$ ($\mu m^3$) | $V_m$ ($\mu m^3$) | $V_1:V_2:V_3:V_4$ | $\Delta_1:\Delta_2:\Delta_3:\Delta_4$ (%) |
|---|---|---|---|---|---|
| 1 | 7 | 1800 | 1810 | 2.16:1.69:1.08:1 | 6:21:56:17 |
| 2 | 8 | 2400 | 2450 | 2.35:1.65:0.90:1 | 7:28:44:19 |
| 3 | 8 | 2500 | 2350 | 1.95:1.71:1.18:1 | 6:14:21:58 |

TABLE II

[$^3$H]THYMIDINE LABEL DISTRIBUTION AMONG THE FRACTIONS

20 $\mu$C per mouse of [$^3$H]thymidine were injected intraperitoneally, 1 h before the ascites tumour was removed. Cells from each fraction and from the control sample were precipitated on paper filters ($1 \cdot 10^6$ cells per filter), fixed with methanol and counted in a liquid scintillation counter. Specific activities ($n_i$) are given as means $\pm$ S.E.

| *Fraction No.:* | *1* | *2* | *3* | *4* | *Control* | *Calculated mean* |
|---|---|---|---|---|---|---|
| Mean cell volume ($\mu m^3$) | 3640 | 2700 | 1730 | 1600 | 1800 | 1810 |
| $\Delta_i$ (%) | 6 | 21 | 56 | 17 | — | — |
| $n_i \pm$ S.E. (counts/min per cell) | $5.65 \pm 0.05$ | $9.10 \pm 1.04$ | $5.90 \pm 0.28$ | $3.80 \pm 0.64$ | $5.90 \pm 0.68$ | 6.17 |

0.9–1.2. (2) The portion of the largest cells ($\Delta_1$) was about 6–7 %, the portion of cells collected in Fraction 2($\Delta_2$) was about 20 %, the remaining portions were collected in Fractions 3 and 4, but distribution of cells between these fractions is not constant.

The data regarding Fraction 5 are not presented as this fraction was highly contaminated with lymphocytes and erythrocytes. The portion of non-tumour cells in Fractions 1–4 was fairly constant and did not exceed 13–16 %. The percentage of dead tumour and non-tumour cells in these fractions and the control samples was also constant and did not exceed 6–8 %. This means that the separation procedure, under the conditions mentioned, does not damage cells.

The results from one of the experiments, where [$^3$H]thymidine label was introduced, are presented in Table II. Specific activities for cells from Fractions 2 and 4 were always significantly different ($P < 0.01$) in contrast with those of Fractions 1 and 2.

Mean specific activity ($n_{calc}$) was calculated from the equation:

$$n_{calc} = \frac{\sum_{i=1}^{4} n_i \Delta_i}{\sum_{i=1}^{4} \Delta_i} \qquad (2)$$

These values were reproducible. The specific activity of the cells collected in Fraction 5 did not differ significantly from the background activity. Such a pattern of the specific activity distribution among fractions is in agreement with the suggestion that cell size strongly depends upon cell position in the mitotic cycle, *i.e.* that Fractions 1,2 and 3–4 are enriched with cells which are in the $G_2$, S and $G_1$ periods, respectively.

*The electroconductivity of cells*

The results from a typical experiment, presented in Table III, show that $r_2$ from Fraction 2 was higher than that of cells from Fractions 1 and 4. $r_2$ of the control (non-separated) cells is approximately equal to the average resistivity of the separated cells.

TABLE III

RESISTIVITY OF CELLS IN DIFFERENT FRACTIONS

$r$ and $r_1$ were measured at 20° and the resistivity of cells ($r_2 \pm$ S.E.) was calculated from Eqn. 1 (see MATERIALS AND METHODS).

| *Fraction No.:* | *1* | *2* | *3* | *4* | *Control* |
|---|---|---|---|---|---|
| Mean cell volume ($\mu m^3$) | 4700 | 3300 | 1800 | 2000 | 2450 |
| $r_2$ ($\Omega \cdot$ cm) | 460 ± 10 | 860 ± 60 | 650 ± 30 | 360 ± 15 | 600 ± 75 |
| $n_i$ (counts/min per cell) | 7.2 ± 1.1 | 7.6 ± 0.7 | 4.4 ± 0.2 | 1.7 ± 0.3 | 5.4 ± 0.1 |
| $\Delta_i$ (%) | 7 | 28 | 44 | 19 | — |

Cell conductivity data are summarized in Table IV. It should be noted that in all the experiments the electroconductivity of the cells from Fraction 4 was approximately twice as high as that from Fractions 2 and 3. The electroconductivity of cells from Fraction 1 was higher than that from Fractions 2 and 3. Such a persistent conductivity decrease could be noted even when the [$^3$H]thymidine label in Fraction 1 was not significantly different from the label in Fraction 2, but when mean cell volumes in these fractions were significantly different. This suggests that conductivity begins to increase not in the $G_2$ period, but a little earlier, at the end of the S phase.

TABLE IV

THE COMPARATIVE ELECTROCONDUCTIVITIES OF CELLS AFTER SEPARATION

Electroconductivities are given as ($1/r_2$) ± S.E. relative to the lowest observed in an experiment.

| *Expt. No.* | *Comparative electroconductivities of cells* | | | |
|---|---|---|---|---|
| *Fraction No.:* | *1* | *2* | *3* | *4* |
| 1 | 1.5 ± 0.02 | 1.0 ± 0.04 | 1.5 ± 0.08 | 1.8 ± 0.04 |
| 2 | 1.9 ± 0.04 | 1.0 ± 0.08 | 1.3 ± 0.07 | 2.4 ± 0.1 |
| 3 | 2.1 ± 0.04 | 1.0 ± 0.03 | 1.0 ± 0.07 | 1.5 ± 0.06 |
| 4 | 1.2 ± 0.02 | 1.0 ± 0.06 | | 1.7 ± 0.01 |
| 5 | 2.4 ± 0.07 | 1.0 ± 0.09 | | 2.6 ± 0.09 |
| 6 | 1.0 ± 0.1 | 1.05 ± 0.05 | | 2.5 ± 0.03 |

## DISCUSSION

A very detailed study of the cell cycle kinetics for the Ehrlich ascites tumour was carried out by TANNOCK[12]. According to him the mean cell cycle time ($T_c$) for the hyperdiploid strain of the Ehrlich ascites tumour on the 6–8th day after transplantation is about 44 h. The S period ($T_S$) takes about half of this; then the $G_1$ and $G_2$ phases ($T_{G_1}$ and $T_{G_2}$) are 0.4 $T_S$ and 0.1 $T_S$, respectively.

If we assume that: (1) before separation the cell population was completely asynchronous, *i.e.* $\Delta_S/\Sigma\Delta_i = T_S/T_c$; and (2) on application of the sedimentation procedure $G_1$ and $G_2$ cells completely separated and thereafter were confined to Fractions 4 and 1, respectively (this follows from the fact that $V_1 \simeq 2V_4$), then, if the ratio

$T_s/T_c$, $\Delta_i$ and $n_i$ are known, the approximate portion of S cells in each fraction, $M_s^i$ (%) can be calculated from the equation:

$$M_s^i = \frac{T_s}{T_c} \frac{n_i \cdot 100}{n_{calc}} = \frac{T_s}{T_c} \frac{n_i \Sigma \Delta_i}{\Sigma n_i \Delta_i} \cdot 100 \qquad (3)$$

The results of the calculation described above are presented in Table V.

TABLE V

DISTRIBUTION OF CELLS IN FRACTIONS AFTER SEPARATION

$M_s^i$ was calculated from Eqn. 3. With the assumptions mentioned in the text, the percentage of $G_1$ and $G_2$ cells in each fraction was obtained from the percentage of S cells (100%). Ranges given in the table for the cell distribution were obtained from the three experiments.

| | *Fraction No.* | | | |
|---|---|---|---|---|
| | *1* | *2* | *3* | *4* |
| S (%) | 37–60 | 73–78 | 40–45 | 17–31 |
| $G_1$ (%) | — | 22–27 | 50–60 | 69–83 |
| $G_2$ (%) | 40–63 | | — | — |
| $V_i$ | 2.1 | 1.7 | 1.15 | 1 |
| $\Delta_i$ (%) | 7 | 25 | 50 | 18 |

McDONALD AND MILLER[13] have recently succeeded in the synchronization of mouse L cells by a velocity sedimentation technique in a linear gradient of foetal calf serum in phosphate-buffered saline. Though the cell cycle parameters of L cells differed markedly from those of Ehrlich ascites tumour cells, the results which we obtained with the Ehrlich ascites were in good agreement with the results reported by McDONALD AND MILLER[13]. They also showed that L cells could not be synchronized by sedimentation in a density gradient, as during the mitotic cycle there were no regular changes in the average cell density. This should mean that increase in the cell volume is accompanied with an increase in the cell mass. Our results seem to suggest that the cell volume changes in the first half of the cell cycle are rather slow, which somewhat impairs cell separation in Fractions 3 and 4. Our data are consistent with the observations that the increase in the mean cell volume and cell mass follows the exponential law during the $G_1$ and S periods of the cell cycle[14–17].

Contamination of Fraction 4 with [$^3$H]thymidine-labelled cells and of Fraction 2 with $G_1$ cells can be also due to the individual mass variations in cells entering DNA-synthesis phase[18]. Contamination of Fraction 1 with S cells can be explained by a slow-down in the increase of the cell mass in $G_2$ cells.

Cell distribution among fractions (Table V) and the results on the specific electroconductivity of cells in these fractions (Table IV) allow us to conclude that the minimum cell electroconductivity is observed during a certain part of the S period Cell resistivity mainly depends on the resistivity of the cell surface membrane. Electroconductivity of the cell membrane is the measure of passive permeability of cells for ions. Hence the changes in the cell electroconductivity are indicative of changes in the passive permeability of cells. It should be noted that electric resistance of the membrane changes much more sharply than does $r_2$ since: (1) the fractions are contaminated with cells in other phases, (2) there is a certain contribution to $r_2$

provided by the electric resistance of cytoplasm which can be considered to be roughly constant throughout the mitotic cycle.

JUNG AND ROTHSTEIN[16] observed fluctuations in the cation content and an increase in the individual fluxes of sodium and potassium at the beginning and end of the cell cycle of the mouse leukaemic lymphoblasts. Our results can, to a certain degree, explain these fluctuations by changes of the passive barrier properties in the cell membrane.

It was recently shown that, as observed in fertilized loach eggs, the changes in the electric resistivity of the cell membrane can be correlated with division bursts[19]. The reasons for such changes in the membrane properties during the mitotic cycle are unknown. Yet it can be noted that the periods of high permeability correspond fairly well to the periods of the synthesis of new cell surface components. BOSMANN AND WINSTON[15] showed that the synthesis of lipids and glycolipids, to be incorporated into the cell membrane of the synchronized mouse leukaemic lymphoblasts, occurs in $G_2$, M and at the beginning of the $G_1$ period. According to GERNER *et al.*[20] the biosynthesis of the cell surface membrane (incorporation of the labelled precursors of protein, sugars and lipids into the cell membrane) occurs in the $G_1$ period of the cell cycle in KB cells. Such a correlation between the phase of the cell cycle and the synthesis of the surface membrane was also reported for cells of different origin[21,22]. Thus a close relationship exists between the membrane synthesis, changes in the membrane permeability and initiation of cell division in autonomously dividing cells.

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