On Cell Cycle of Ascites Tumor

Eicho Hirai, Katsuhiro Kawashima, Ichiro Yonome and Tatsuo Tobe
Department of Radiology, School of Medicine Gunma University
(Director Prof. Tatsuo Tobe)

腹水腫瘍の増殖と細胞周期

群馬大学医学部放射線医学教室（主任　戸部龍夫教授）
平井栄長，川島勝弘，鰀目一郎，戸部龍夫

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cell cycle の各 phase の期間，従つてまた cell cycle time が変動するという事実については多くの報告がある．

cell cycle の各 phase の期間を算出するに当つて，一般には，それらの期間が一定値をとるものと仮定している．しかし，Barrett はいかなる細胞集団においても，ある程度の変動は避けられないという考えのもとに各々の期間を，皮下移植されたラットの乳癌について統計的分析をなし，実験から得られたデータとよく一致すると報告している．Mendelsohn は C3H マウス乳癌について，3種類の統計的モデルを想定して，generation time の分析を算出している．

我々は，すでに報告した吉田腹水肉腫およびその後観察した AH-13 腹水肝癌のcell cycle phase について Barrett と同様な統計的分析を，Monte Carlo 法により，電子計算機 OKITAC-5090を用いて行なつたが，実験データとかなり良い一致をみた．

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There are many reports on the duration of each phase of the cell cycle and on variation in cell cycle time[1)~11)].

However, as pointed out by Barrett[12)], durations of the phases are generally computed on the hypothesis that they have constant values. He considers that some variability is inevitable in any population of cells. And so he statistically investigated the duration of each phase in subcutaneously transplanted mammary cancer in rats, and found that the thus obtained results agreed well with the experimentally acquired data. Mendelsohn[13)] established 3 statitical models of mammary cancer of C3H mouse, and computed the distribution of the generation time (hereafter referred to as T). Authors attempted after Barrett, statistical analysis of the cell cycle phases in Yoshida sarcoma and AH-13 ascites tumor which had often be used in our experiments. The methods and results will be described below.

Experimental Methods:

i) In the previous experiments[14)], 10^6 cells of Yoshida sarcoma (hereafter referred to as Y.S.) were intraperitoneally transplanted into Wister rats, and on 4 days thereafter, T was computed by autoradiography using 3H-TdR. The results were published in the first report[14)]. In this text the above data were used as material of statistical analysis.

ii) On 2 days after intraperitoneal transplantation of 10^6 cells of AH-13 hepatic ascites cancer (hereafter referred to as AH-13) into Donryu rats, T was computed by autoradiography using 3H-TdR of 100 μCi (specific activity, 2.5 Ci/m M), and the data were used for statistical analysis.

Statistical procedure:

The data were dealt with statistically by a Monte Carlo method using an electronic computer. In the program, the time scale was divided into intervals corresponding to 1 hour. The program proceeds as follows.

1. Average m and standard deviation σ are given to each phase.

2. The average and the standard deviation for the log-normal distribution are computed from m and σ for each phase.

3. According to the log-normal distribution, probability of each phase being found in time period between t_{i-1} and t_i is computed.

4. According to the obtained probability, integer out of 0–999 is assigned to t_i for each phase, that is, when integer N_i Corresponding to the time period between t_{i-1} and t_i is generated by a computer, time period of a phase should be registered as t_i.

5. One of the uniform random numbers of 0–999 is generated, and the duration of G_2 phase, T_{G_2}, is firstly determined by t- corresponding to the random number. Then, T_{G_2} is obtained by the formula $T_{G_1} = t_{G_2}$-0.5.

6. Random number is generated to determine the duration of S phase, t_s. Then $T_{G_{2+s}} = (t_{G_2}\text{-}0.5) + (t_s\text{-}0.5)$.

7. 1 is added to each of the channels corresponding to the integers of T_i which satisfy $T_{G_2} < T_i \leqq T_{G_{2+s}}$.

8. Random number is generated to determine the duration of of G_1 phase, t_{G_1}. Subsequently is obtained formula $T_{G_{2+s+G_1}} = t_{G_2} + t_s + t_{G_1} - 1.5$.

9. Random number is generated to determine again the duration of G_2 phase, t_{G_2}. Then $T_{G_2} + s + G_1 + G_2 = t_{G_2} + t_s + t_{G_1} + t'_{G_2} - 2.0$.

The same procedures are repeated until the total 40 hours is produced.

10. The above procedures are repeated usually 1,000 times.

11. In the end, the numerals added to all the channels are read out. Therefore, the sum was obtained of the number of cells now in mitosis that had been in S in any particular hour. The used computer was OKITAC-5090.

Results

1. At 4 days after intraperitoneal transplantation of 10^6 cells of Y.S. into Wister rats, T and the duration of each phase were determined autoradiographically. The measurement gave 19, 0.35, 12.4, 5.5, and 0.75 hours for T and durations of G_1, S, G_2 and M phases,respectively, whereas the statistical computation gave 18.9, 1.9, 11. 0 and 5.9 hours for T, G_1, S and G_2 + M, respectively. There is good agreement between the two series of the values (Fig. 1).

II. At 2 days after intraperitoneal transplantation of 10^1 cells of AH-13 into Donryu rats, autoradiographical determination of T and durations of G_1, S, G_2 and M gave 13.0, 0.9, 8.0, 3.5 and 0.6 hours, respectively. On the other hand, the statistical computation gave 13.8, 2.9, 7.5 and 3.5 hours for T,

Fig 1. The Mitotic Cycle of Y.S (4days fter transplantation)

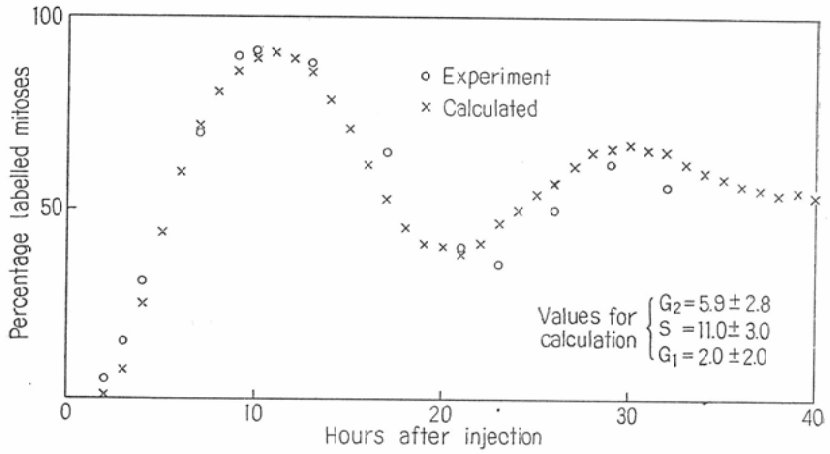
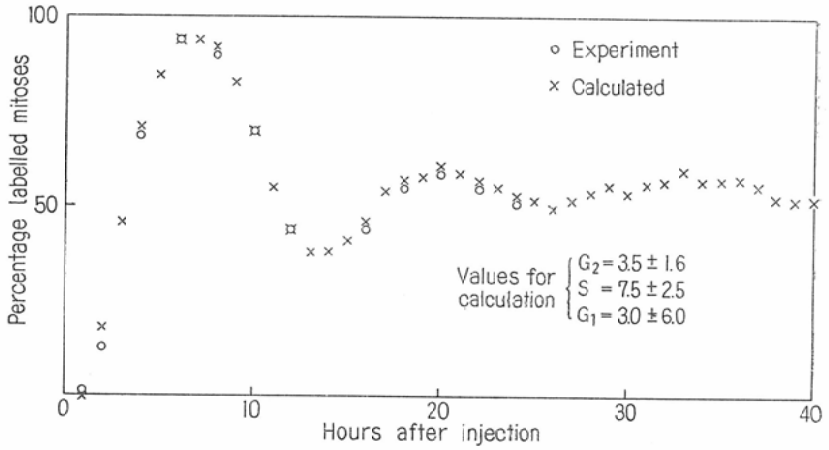


Fig 2. The Mitotic Cycle of AH-13 (2days after transplantation)



G_1, S, and G_2 + M, respectively. There is good agreement between the two series of values (Fig. 2).

Discussion

Many reports have been made by the use of 3H-TdR on the duration of each phase of growth of a population of cells. But the majority of them determined it on the assumption that it is constant in all the cells. Kozuka and More[15], who observed HeLa cells during culture cinematographically and autoradiographically, found that, the duration of the same phase varied from cell to cell. Barrett assumed that in any population of cells the duration of each phase would show statistical variation, and on this assumption he theoretically computed percentage labeled mitoses and frequency distribution of cycle time of Marshall tumor cells. He reported that the thus computed values were in good agreement with the experimentally obtained values. In the present experiments authors autoradiographically determined T and the duration of each phase of growth of Y.S. and AH-13, and found that the values obtained agreed well with the results of computation which was performed on the basis of assumption that the duration of each phase would have log-normal distribution. It was subsequently assumed that at least in trans-

planted tumor cells, the duration of each phase would not be the same in all of them, but show statistical variation.

Summary

T and durations of phases G_1, S, G_2 and M of Y.S. and AH-13 were autoradiographically determined by using 3H-TdR, and 19, 0.35, 12.4, 5.5 and 0.75 hours were obtained for Y.S., and 13, 0.9, 8.0, 3.5 and 0.6 hours for AH-13, respectively. Furthermore it was found out that when T and the durations of the phases were statistically computed by giving a definite average and a standard deviation to each on the assumption that each has log-normal distribution, the results were in sufficiently good agreement with the experimentally determined values.

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