Growth of Ascites Tumor and Cell Cycle

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腹水腫瘍の増殖と細胞周期

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腫瘍の増殖は一般に exponential growth と考えられているが，日常観察し得る腫瘍に於てその増殖は exponetial growth のみでは説明し難い．我々は吉田腹水肉腫（Y.S）及び AH.13 を Rat の腹腔に移植し経日的に細胞の計数により Doubling time（d.t）を測定し又 auto radiography（^{3}H-TdR 使用）法にて移植後，前，中，後期の generation time（g.t）を測定した．移植後細胞は直ちに増殖を開始せず或る期間の lag phase を経て増殖する事が判明した．この事は特に one cell 移植で明らかである．移植後，前，中，後期の g,t 及び d,t を測定すると次第に延長する．従つて腫瘍増殖の exponentiality は認められない事を示唆している．併し腫瘍早期から，g,t は延長していくのでなく或る増殖期間は exponential growth と考えられその後は necrosis，免疫，栄養等の因子が関与 linear growth となりそれ以後は増殖率は低下すると考えられる．即ち我々の実験から腫瘍の増殖は logistic growth と考えられる．

Introduction

Generation time (g.t.) and doubling time (d.t.) of a population of cells are of course different from each other as can be clearly seen in their definitions. If all the cells in the population from their growth fractions, and if no cells fall out of the population, the two will be identical. Authors obtained growth curves of transplanted rat ascites tumors (Yoshida sarcoma (Y.S.) and AH 13), and periodically determined g.t. during the growth by the autoradiographic method using 3H-TdR, and in this way investigated the pattern of growth of the transplanted tumor.

Experimental materials and Methods

1. Growth curve

1) Into Wister rats, 10^0, 10^3, 10^5, 10^6, 10^7 and 10^8 cells of Y.S., and into Donryn rats, 10^0, 10^3, 10^6 cells of AH-13 were intraperitoneally transplanted, and the animals were periodically sacrificed by decapitation. All the intraperitoneal tumor cells, which were collected by washing the peritoneal cavity several times with saline solution, were counted. And averages each for 3 animals were computed.

2) After intraperitoneal transplantation of 10^7 Y.S. cells into Wister rats, all the intraperitoneal tumor cells were enumerated as periodically as described above, and the obtained values (which were averages for each 3 animals.) were plotted to draw the growth curve. From this curve d.t.'s immediately at 1.5 and

4 days after the transplantation were determined.

ii. Growth at early stage

1) After intraperitoneal transplantation of 10^7 cells of AH-13 into Donryu rats, all the intraperitoneal tumor cells were counted at 1/4, 1/2, 1, 2, 3, days by the same procedure as described above. The values which were plotted were averages for each 3 animals.

2) At 12 hours after intraperitoneal transplantation of 10^3, 10^5 and 10^7 all labeled cells of AH-13 into Donryu rats, labeling index (l.i.) was computed in each case in order to see the pattern of growth of the tumor cell in the early stage after the transplantation. The cell labeling was performed by the procedure Baserga et al used[1]. It is as follows: At 3 days after intraperitoneal transplantation of 10^6 cells of AH-13 into Donryu rats, that is, when the cells increased to about 10^7, the animals were injected with 30 μCi of 3H-TdR (specific activity 2.5 Ci/mM) every 2 hours, 7 times in total, and at 2 hours after the final injection, 10^3, 10^5 and 10^7 of the intraperitoneal tumor cells (l.i. 97%) were transplanted into other Donryu rats. Then immediately and 12 hours after the transplantation, l.i. were computed autoradiographically.

3) Immediately and at 1.5 and 4 days after intraperitoneal transplantation of 10^7 cells of Y.S. and at 2 days after similar transplantation of AH-13, g.t.'s were determined and compared with d.t.'s of Y.S. in the experiment I, 2). The values were averages for each 3 animals.

Results

I. Growth curve

1) Since there were about 10^6 reactive cells in the peritoneal cavity of the normal Wister rat, those exceeding 10^6 are considered to be the tumor cells. So 10^6 was the original point for counting the tumor cells. Days of attaining 10^6 differed depending on the number of transplanted cells. When 10^0, 10^3 and 10^5 cells were transplanted, 10^6 was attained at 4, 6 and 10 days after the transplantation, respectively, and at 5—6 days, the cells increased to 10^9, that is, the saturation number, leading the animal to death. The greater the number of the transplanted cells, the blunter the slope of the growth curve, and the longer the d.t. (Fig. 1, 2)

Fig. 1. Growth curve of Y.S.

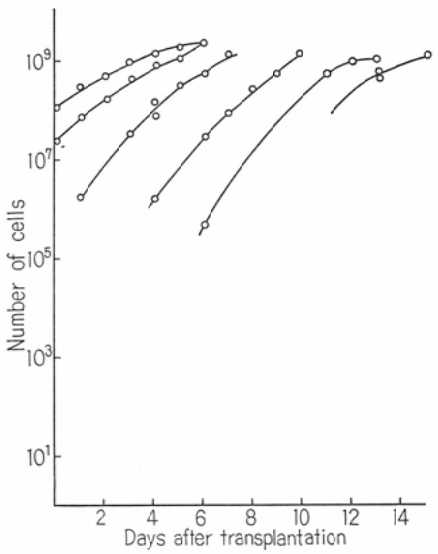
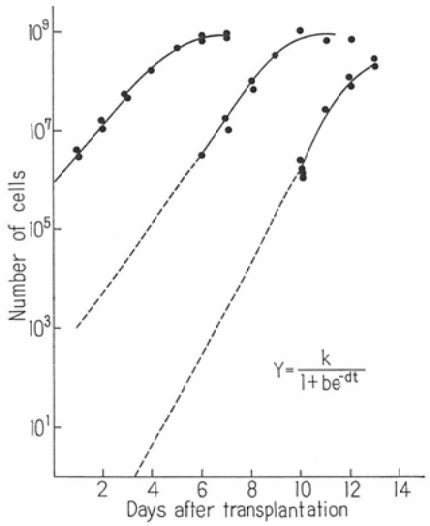


Fig. 2. Growth curve of AH-13 tumor cells.



2) After intraperitoneal transplantation of 10^7 Y.S. cells into rats, the total intraperitoneal tumor cells were calculated periodically to draw growth curve. The d.t.'s measured on the curve were 13, 15 and 22 hours, immediately and at 1.5 and 4 days after the transplantation, respectively. Namely, d.t. was prolonged with the lapse of day. It can be said that the growth rate of tumor is not constant, but decreases with growth (Fig. 3).

II. 1) At 6 hours after intraperitoneal transplantation of 10^7 cells of AH-13 into Donryu rats, the total intraperitoneal cells, collected by washing the cavity, counted below 10^7 at 12 hours they returned to the original 10^7. Thereafter, the cells increased gradually until they attained the saturation point of 10^9, which brought the death of the animal. If we graphically extrapolate the curve to obtain the starting point it reaches 10^6, the curve appears as if the growth started from 10^6 cells. (Fig. 4).

Fig. 3. Growth curve of Y.S.

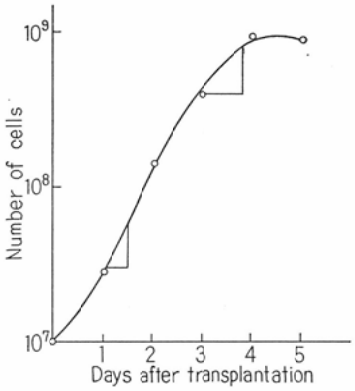


Fig. 4. Growth pattern of tumor cells of early stage after transplantation

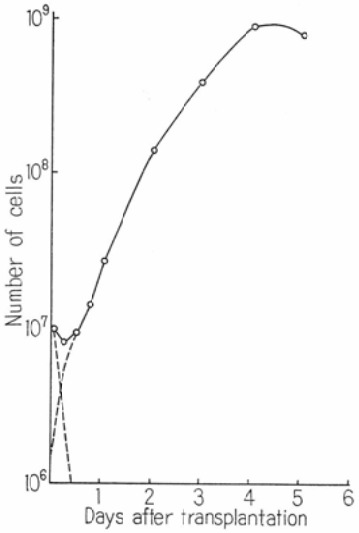


Table 1. Examination of labeled AH-13 cells

Number of trasnplanted cells with are already labeld	The rafio of labeled cells to non labeled cells* + lab. c	
	just after transplantion	12 hrs after transplantation
10^3	1/10000	2/10000
10^5	9/1000	15/1000
10^7	42/100	8/100

* non labeled cells mean peritoneal exudate cells

2) In order to reexamine the early pattern of the growth as observed in the experiment II, 1) 10^3, 10^5 and 10^7 cells (all labeled) of AH-13 were intraperitoneally transplanted into Donryu rats, and l.i. was determined immediately and at 12 hours after the transplantation. In the case of 10^3 cells, the values at two time points were 1/10,000 and 2/10,000 and in the case of 10^5, they were 9/1,000 and 15/1,000 and in the case of 10^7, 42/100 and 8/100 (Table 1).

III. Amount of 10^7 Y.S. cells was intraperitoneally transplanted into rats. Immediately and at 1.5 and 4 days thereafter g.t.'s were autoradiographically determined, 13, 16 and 19 hours were obtained, respectively (Fig. 5). These are in approximate corespondence with 13, 15 and 22 hours, computed from the growth curve (Table 2).

At 2 days after transplantation of AH-13, both g.t. and d.t. were in 12—13 hours, showing good agreement. (Fig. 6)

Fig. 5. Generation Times

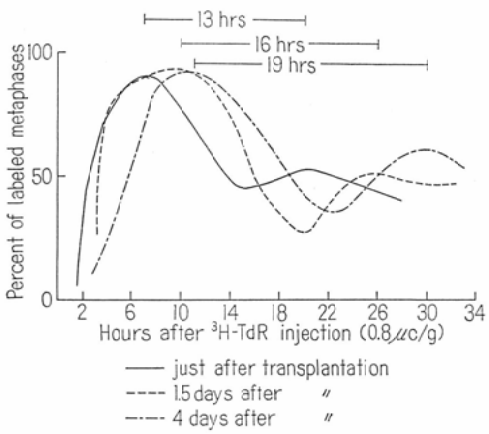


Fig. 6. Cell cycle of AH-13 (2 days after trans plantation)

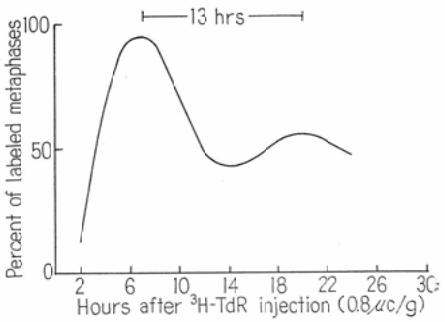


Table 2. Generation time and Doubling time (Y.S)

	Elapsed time after transplantation (days)		
	0	1.5	4
Generation time	13 hr	16 hr	19 hr
Doubling time	13 hr	15 hr	22 hr

Discussion

What was especially concerned with in the present experiments was growth of tumor prior to the logarithmic phase, that is, early after the transplantation. And it was found out that g.t. early after the transplantation was nearly equal to that in the logarithmic phase. On the other hand, the number of the cells tended to decrease until 6 hours after the transplantation as shown in Fig. 4. This was verified in 3H-TdR uptake experiments— after transplantation of ascites tumor cells into rats, the animals were intraperitoneally injected with 3H-TdR from time to time to label 97% of the tumor cells, which then were again transferred to healthy rats, and l.i.'s immediately and 12 hours thereafter were compared with each other. It was expected in this case, that l.i. at 12 hours might be twice as large as that immediately after the transplantation. As shown in Table 1, the result was nearly as anticipated when the number of the transplanted cells was relatively small. But when 10^7 cell were transplanted, l.i. at 12 hours became conversely smaller —about 0.2 times that obtained just after transplantation. In this supplementary experiment, nearly 100% labeled cells were transplanted, but nevertheless l.i. was $1/10^4$ of the transplanted cells when these were 10^3. This appears contradictory at the first glance. But, as above mentioned, we assumed that prior to the transplantation, there might be 10^6 cells in the intraperitoneal cavity, and the assumption was

warranted by our repeated experiments in this series of study. If this number is taken into consideration, l.i. becomes $1/10^3$, which is 10 times greater than the measured value $1/10^4$. The same manner can be applied to the case of 10^5 cells transplantation. It will be after all, seen that out of the transplanted cells, 9/10 may have the fate of destruction without having any chance of growth, and 1/10 may start multiplication immediately after transplantation. The above mentioned contradiction can be explained if we can assume in this way. However, the results obtained by transplantation of 10^7 cells could not permit the application of the above calculation.

This may indicate that when a large amount of cells are transplanted the host assumes different behavior to them. The possibility of confusion resulting from labeling of mono-nuclear leucocytes is considered negligible, since labeled cells represent only several per cents of the total[2]. These points will be investigated further in our later studies. At any rate it is plausible in the transplanted early stage that not all the transplanted cells but a fraction of them will undergo growth nearly at the same rate as in the logarithmic phase. Assumedly on account of this, we could not demonstrate in the present experiments that ascites cells grow by the Gompertz's formula as reported by Laird[3], that is, the earlier after the transplantation, the shorter is g.t.

When a smaller number of cells, say only one cell as an extreme example is successfully transplanted, can we say that its 1/10 will start growth? If one wants, one can interpret 1/10 as the probability of the chance of growth, that is to say that the growth will take place in one out of ten attempts of one cell transplantation. But this will not be the case either, as the actual percentage of successful growth must be much higher. In effect, Isaka[4] reported 85% success in his one cell transplantations. There is another estimation of still higher plausibility that after one cell transplantation it may be kept in the dormant stage for a definite period of time. This point is now under experiment, and the result will be reported in the near future.

In the later stage, that is, the period beginning at 3—4 days after transplantation, d.t. increased monotonically until the cells attained the saturation point of about 10^9, leading the animal to death at about 7 days after transplantation. As for the cause of this saturation was can consider, as generally accepted, space effect of the peritoneal cavity, deficiency of oxygen, nutrient and others resulting from circulatory disturbance caused by tumor infiltration, appearance of immunity against the tumor and migration of tumor cells. At 4 days after transplantation, g.t. and d.t. were 19 and 22 hours, respectively. Both were prolonged compared with those in the early and the middle stage after transplantation, d.t. to a greater extent than g.t.. This indicates that in the later stage, destruction or death of the cell takes place more vigorously.

It was found out that the main cause which produced increase of g.t. was not the prolongation of G_1 but of S and G_2 phases. S became longer in the later stage. It is, however, unthinkable that S would become shorter in proportion as it occurs earlier after transplantation. Presumably the degree of shortening would remain same in the early as in the middle stage. In determining the duration of the S phase of a population of cells it is necessary to choose an adequate number of cells to be transplanted, and adequate days after transplantation. The duration thus obtained can be recorded as the shortest one.

Summary

Cells of Y.S. and of AH-13 were intraperitoneally transplanted into rats, and their g.t.'s and d.t.'s

were obtained. Changes in the both values during the growths of the tumors were pursued.

The result is as follows:

The g.t. was about 12—13 hours in the early and the middle stage of growth, but was progressively prolonged in the later stage. The d.t. nearly corresponded to g.t. in the middle stage, but in the early stage, the number of the cells rather diminished, while in the later stage d.t. was prolonged, and to a rather greater extent than g.t.. The main cause which elicited prolongation of g.t. was not prolongation of G_1 phase, but of S and G_2 phases.

References

1) Baserga, R. & et al.: Cancer Res., 20, 910–917, 1960.
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4) Isaka, H.: Proceedings of the Japanese Cancer Association The 25th Annual Meeting, 131–132, 1966.