Acta Radiologica Oncology 21 (1982) Fasc. 1

FROM THE DEPARTMENT OF MEDICAL RADIATION BIOLOGY, KAROLINSKA INSTITUTET, S-10401 STOCKHOLM, SWEDEN.

PROLIFERATION KINETICS AND SPECIFIC BEHAVIOUR OF G_2 CELLS OF THE Bp8 MOUSE ASCITES SARCOMA IN VIVO

S. CAO, S. SKOG, A. BRAHME and B. TRIBUKAIT

Ascites forms of tumours have been widely used in experimental oncology. The advantage in the use of ascites tumour cells is the access to a nearly pure cell material well suitable for the evaluation of the total number of cells, and for quantitative cytochemical and biochemical measurements. These advantages were the reason for the choice of an ascites tumour in cellular investigations on radiation effects in this laboratory.

The Bp8 mouse ascites sarcoma used was known to be easy to grow in a reproducible way. This tumour can also be grown in vivo as a solid tumour and in vitro in a suspension. Furthermore, stem cell properties by means of the in vitro colony growth technique can be evaluated in this tumour.

The growth characteristics of the non-irradiated tumour are now presented. Besides the quantitative changes of the cell number during the growth, which have been previously evaluated in many other types of ascites tumour, special attention was paid to the quantitative changes of the cell number in the various parts of the cell cycle. In addition, using these quantitative data, the flow rate of the cells through the compartments of the cell cycle could be evaluated. The possibility of treating data in this way does not seem to have been used previously in ascites tumour.

Material and Methods

Experimental animals and the tumour. Male NMRI mice, aged about 3 months with a body weight of 20 to 25 g were used. Water and standard food were given ad libitum. Dependent on the cell concentration of the ascites 0.15 to 0.2 ml of the undiluted ascites fluid was transferred intraperitoneally with a fixed number of 18×10^6 ascites cells from a donor carrying the tumour for 10 days. Dependent on the number of animals transplanted, ascites from 3 to 6 donors was pooled. The cell number was calculated by counting in a Bürker hemocytometer. After the transplantation, tumour growth was obtained in 100 per cent of the mice. The animals generally died from day 13 after inoculation and no animals survived after day 17.

Volume of the ascites, total number of tumour and normal cells. The volume of the ascites and the numbers of tumour cells were measured indirectly by using an isotope dilution technique (PATT et coll. 1953). 0.2 ml of ^{125}I-labelled human albumin containing about 200 000 cpm after appropriate dilution with Tris EDTA buffer solution, spec.act. 1.85 MBq/ml (50 μCi/ml) was injected intraperitoneally. Ten min after the injection, giving activity values at a plateau level, the animals were killed by cervical dislocation, the ascites removed and the activity of 0.025 ml ascites measured after dilution to 1 ml in an automatic gamma spectrometer (Intertechnique CG 30). The ascites volume was calculated according to: Ascites volume (ml)=(injected cpm−background/ascites cpm−background)−inj. volume.

Accepted for publication 9 February 1982.

Table 1

Growth characteristics of the mouse Bp8 ascites sarcoma. Mean values ± SEM of 6 to 10 animals

Days after inoculation	Volume of ascites (ml)	Total No. of cells ($\times 10^6$)	Normal cells (per cent)	Dead cells (per cent)	Tumour cells (per cent)			
					G_1	S	G_2	M
0	0.15	18.0	10.8	0.4	34.6	45.2	18.1	2.1
1	0.5+0.02	69.8+1.7	13.7±1.8	0.6+0.08	20.9±0.8	50.0±0.6	27.2±0.9	1.9±0.007
2	1.4±0.1	169.9±13.2	19.3±2.0	0.7±0.09	27.6±2.2	58.3+1.0	11.7±1.5	2.5+0.05
3	2.1±0.1	313.6±9.4	9.2±0.7	0.4±0.07	26.3±1.0	59.1+0.7	12.1±0.8	2.4+0.07
4	3.2±0.1	558.3±16.6	13.8±1.5	0.3±0.04	28.4±1.0	58.8+1.0	10.6±0.4	2.2+0.04
5	5.7±0.3	908.3±54.0	9.8±0.8	0.3±0.05	31.0±1.0	55.5+0.8	11.6±0.5	2.0+0.07
6	6.7±0.4	1070.1±81.8	9.5±1.1	0.6±0.09	31.2±1.1	54.3+1.1	12.7±0.8	1.8+0.1
7	12.1±1.4	1592.0±132.8	12.1±1.2	0.4±0.04	29.3±1.1	54.8+0.9	14.0±0.8	1.9+0.06
8	13.1±0.7	1553.9±46.6	10.2±1.2	0.5±0.09	37.9±2.0	46.1+0.9	14.1±1.9	1.9+0.05
9	13.2±1.2	1417.4±141.0	9.6±0.8	0.6±0.04	36.9±2.0	46.4+1.3	14.7±1.1	1.9+0.05
10	11.5±0.8	1406.4±51.4	10.8±1.0	0.4±0.07	34.6±1.9	45.2+1.5	18.1±1.7	2.1+0.1
11	13.2±0.7	1240.0±106.2	10.1±1.1	0.7±0.08	37.1±2.4	42.4±1.8	18.4±2.6	2.2±0.2
12	12.0±0.7	1338.4±40.1	11.5±1.2	0.5±0.08	33.5±2.2	45.0±2.7	19.7±1.8	1.9±0.07
13	12.2±0.9	1296.3±119.7	12.5±1.6	3.1±0.6	34.4±2.4	48.1±1.3	16.0±1.4	1.6±0.1
14	12.6±0.6	1122.7±56.3	8.5±1.5	2.5±0.5	30.6±1.8	48.6±1.5	19.4±0.4	1.4±0.1

After estimating the total number of nucleated cells in 0.025 ml ascites by counting in a Bürker hemocytometer the total number of cells was estimated from the known ascites volume.

From cellular DNA histograms, the proportion of normal and tumour cells was estimated and the total number of normal and tumour cells calculated.

Mean relative cell volume was calculated from the packed cell concentration (International Hemacrit Centrifuge, 11000 rpm, 5 min) divided by the cell concentration in the ascites.

Mitotic index. Smears were prepared and fixed for 15 min in ethanol-acetone 3:1. Thereafter the cells were treated with 99.5 per cent ethanol for 5 min followed by 90, 70 and 50 per cent for 2 min each. After washing in running water for 15 min the specimens were stained by Hams haematoxylin solution for 15 to 20 min. After a 5 s treatment in hydrochloric acid spirit (990 ml 70% ethanol + 10 ml HCl conc.) the cells were washed in water for 20 min and stained in Eosin solution (1.5 g in 100 ml 70% ethanol) for 3 to 5 min. Finally, they were treated in 70 per cent ethanol for 5 s, 95 per cent ethanol for 1 min and 99.5 per cent ethanol for 5 min. The mitotic index in 1000 cells was counted in each preparation.

Dead cells were determined by the dye exclusion test with Lissamine Green B. Before counting the cells in a Bürker hemocytometer 0.025 ml 5 per cent Lissamine Green B were added to 1 ml of the diluted ascites suspension.

Flow cytofluorometry. The cellular DNA content was measured using the rapid-flow cytofluorometric

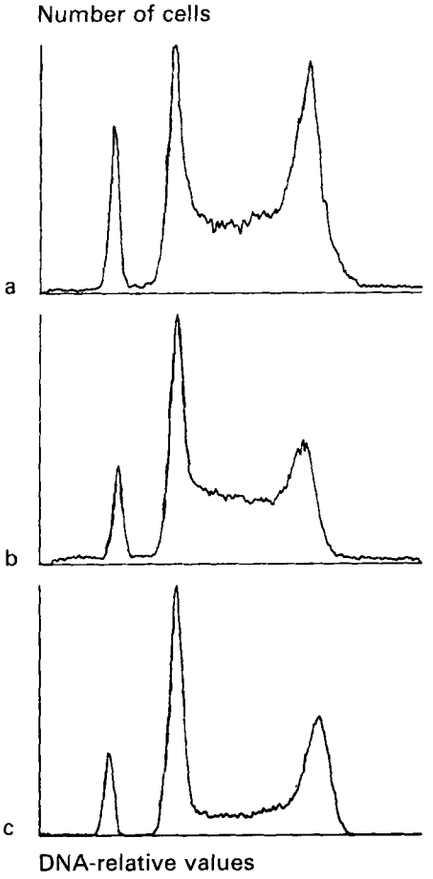

Fig. 1. Cellular DNA histograms of Bp8 ascites sarcoma on day 1 (a), day 3 (b) and day 10 (c) after inoculation. The calculated proportions of cells in G_1, S-phase and G_2+M are: 22.4, 48.1 and 29.5 per cent for day 1, 21.9, 61.8 and 16.3 per cent for day 3 and 36.3, 42.1 and 21.6 per cent for day 10. The peaks to the left represent normal diploid cells. The total number of cells measured was about 100000.

Table 1 (*cont.*)

Cells/ml ($\times 10^6$)	Packed cell volume (per cent)	Relative cell volume ($\times 10^{-6}$)
120.7	28.6	23.9
131.7±5.7	34.3±0.7	26.3±1.1
124.9±11.5	36.3±1.4	30.0±1.7
150.3±20.2	40.8±0.7	27.9±1.4
174.1±7.5	45.8±1.4	26.5±0.9
156.5±7.5	40.3±2.1	26.4±2.0
145.8±5.5	36.5±1.4	25.2±1.0
136.1±7.1	32.6±1.9	24.3±1.6
110.9±2.6	27.0±0.6	24.5±1.0
106.4±5.0	27.3±1.0	25.9±0.8
120.7±5.9	28.6±0.9	23.9±0.9
99.2±5.4	28.1±1.4	28.6±1.3
109.3±12.2	27.2±1.4	26.2±2.9
105.8±5.1	28.3±2.0	26.9±1.7
89.4±1.3	28.2±1.0	31.5±0.8

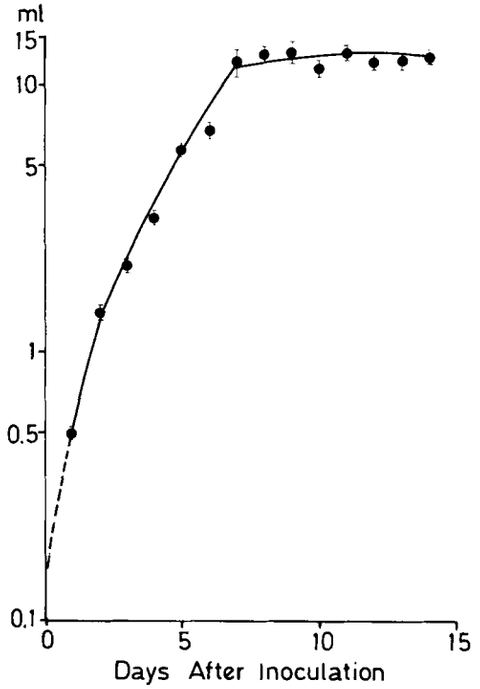

Fig. 2. Ascites volume (ml) of the Bp8 ascites sarcoma after inoculation of 18×10^6 cells. Mean values ±1 SEM of 8 to 10 animals. The line is drawn by hand.

method as described previously (TRIBUKAIT et coll. 1975). In brief, the cells were washed in Tris-EDTA buffer solution and fixed in 96 per cent ice-cold ethanol. The fixed cells were washed in Tris-EDTA buffer together with 1 mg/ml RNase, in order to remove all RNA. Suspensions of single cell nuclei were obtained by pepsin treatment. After washing in the buffer, the nuclei were stained using 2.5×10^{-5} mol/l ethidium bromide in Tris-EDTA buffer of high molality.

The risk of unspecific binding of ethidium bromide is further reduced when this high molality is used. The DNA contents of the cell nuclei were analysed using a rapid-flow cytofluorometer ICP 11 (Phywe, W. Germany) with a flow rate of up to 1 000 cells/s. The excitation and emission wavelengths were 455 to 490 nm and 590 to 630 nm, respectively. The output was sorted using a 256 multichannel analyser. After correction for background the proportion of cells in the different phases of the cell cycle (G_1, S and G_2+M) was determined from the areas of the histograms assuming a Gaussian function of the G_1 and G_2+M maxima and attributing the remaining part of the DNA histogram to the cells of the S-phase. Since the tumour cells contain about 75 per cent more DNA than normal cells, the proportion of these normal cells has also been calculated from the histograms.

Results

The primary data, including ascites volume, cell number, the proportion of normal and dead cells, the percentage of tumour cells in the various parts of the cell cycle, cell concentration, packed cell volume and the relative cell volume are given in Table 1. The percentage of cells in the different parts of the cell cycle were calculated from the cellular DNA distribution histograms and the mitotic index. Fig. 1 shows examples for DNA histograms, taken on day 1, 3 and 10, respectively. The first maxima belong to normal cells while the second and third maxima are the G_1 and G_2+M, respectively, of the tumour cell population. The cells of the S-phase were located in between the second and third maxima. Twenty-four hours after inoculation of cells from donor animals of day 10 (Fig. 1 c), an increase in the proportion of G_2+M cells and also of S-phase cells is obvious (Fig. 1 a) as well as a further increase in the proportion of S-phase cells on day 3 (Fig. 1 b).

The volume of ascites reached plateau values of about 12 ml on day 7. The increase in the ascites volume was most rapid during the first 2 days and followed by a nearly exponential increase until day 7 with a doubling time of about 38 hours (Fig. 2).

The total number of cells generally paralleled the increase in ascites volume and reached a maximum

value on day 7 of about 1400×10^6 cells (Fig. 3). Some deviations from the exponential growth can, however, be observed. Thus the cell concentration varied during growth reaching a maximum concentration on day 4 with 175×10^6/ml. During the 7th to 14th day period the cell number decreased significantly. The relative cell volume showed maximum and minimum values on day 2 and 10, respectively. The proportion of normal cells amounted to about 10 per cent during the whole observation period. The proportion of dead cells was about 0.5 per cent but increased significantly during the last two days. The mitotic index was around 2 per cent even during the time after day 7 where no increase in the cell number was present. The proportion of cells in the various parts of the cell cycle also changed only slightly in spite of the great changes of the cell production. G_2 values were obtained by subtracting the percentage of mitotic cells from the combined G_2+M values of the DNA histograms. In general, some decreasing S-phase values and increasing G_2-values were observed. Only a temporary change in the cell distribution with a decrease of the G_1 cell compartment and an increase in the G_2 cell compartment between day 0 and day 1 was observed.

From the data given in Table 1 the total number of tumour cells together with the total number of cells in the various parts of the cell cycle including the total number of normal cells were calculated (Fig. 3). It is obvious that the changes in cell numbers in the various cell compartments are non-exponential during the growth period.

Comparing the behaviour of the cells in the various parts of the cell cycle some differences should be noticed. The 30 per cent decrease of the total tumour cell number in between day 7 and day 14 is only due to a decrease of cells in G_1, S and M. G_2 cells remain constant at a steady level of 200×10^6 cells. Normal cells seem to behave in this respect just like G_1, S and mitotic tumour cells though normal cells are much more variable over the whole growth period compared with tumour cells. The rapid increase in G_2 cells during the first 24 hours from 3 to 16×10^6 cells followed by 24 hours with unchanged values may further indicate specific regulation factors acting on G_2 cells compared with cells from the other parts of the cell cycle.

In order to find a suitable type of mathematical function for the total number of cells, which can be fitted to the experimental data, a simple model was developed. In the model used it is assumed that to a

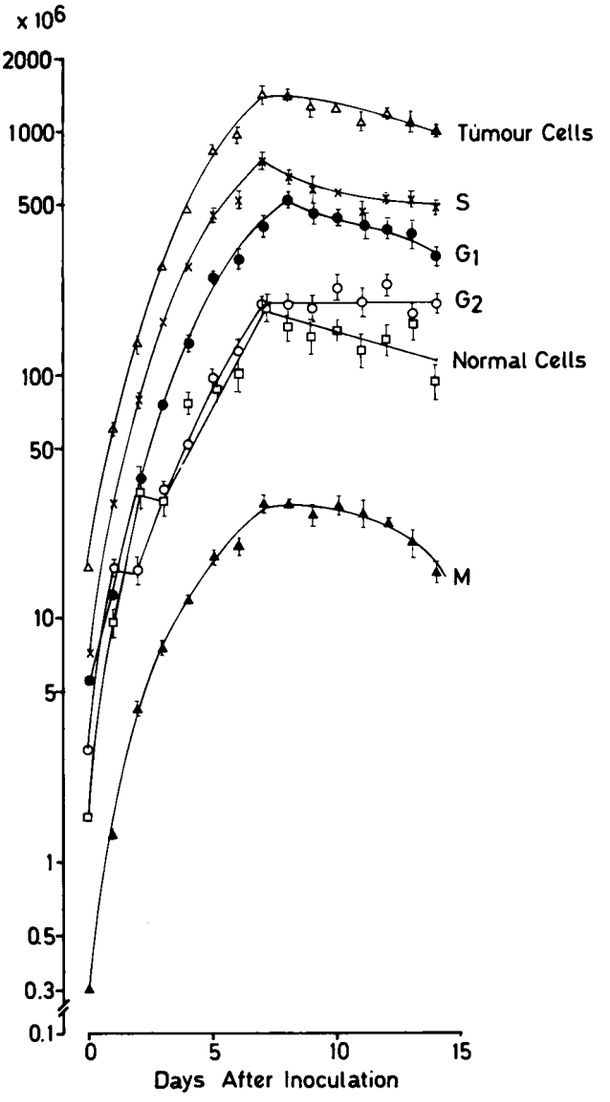

Fig. 3. Growth curves of the total number of Bp8 ascites sarcoma cells, the total numbers of G_1, S-phase, G_2 and mitotic cells as well as normal cells after inoculation of 18×10^6 cells.

first approximation the growth rate of the population is proportional to the total number of cells. As a perturbation of the resultant pure exponential growth it was assumed that some proliferation inhibiting substance is produced by the cells (or the host animal) in proportion to the number of cells at each time and that this substance has a certain exponential decay rate in the system. The following integro-differential equation may thus be set up for the total number of cells:

$$\frac{dN(t)}{dt} = \alpha N(t) - \beta \int_0^t N(\tau)\, e^{-\nu(t-\tau)}\, d\tau \qquad (1)$$

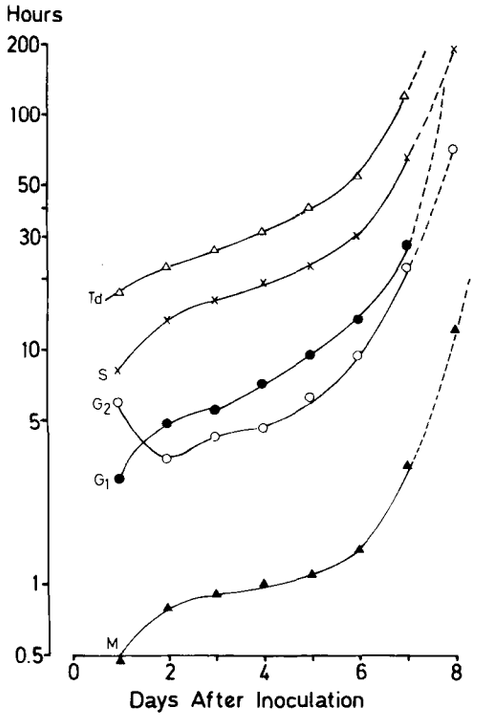

Fig. 4. Calculated duration (hours) of the cell cycle time (T_d) and of the G_1, S-phase G_2 and mitosis of the cell cycle during the growth up to 8 days after inoculation of 18×10^6 Bp8 ascites sarcoma cells.

where the last term expresses the concentration at the time t of the substance due to the total production, including a correction for its decay. The exponential decay constant is denoted ν. If eq. (1) is differentiated twice and the remaining integral can be neglected ($\nu^3\beta$ small) it can be written:

$$\frac{\partial^4 N}{\partial t^4}-\alpha\frac{\partial^3 N}{\partial t^3}+\beta\frac{\partial^2 N}{\partial t^2}-\nu\beta\frac{\partial N}{\partial t}+\nu^2\beta N=0 \quad (2)$$

This is a homogeneous linear differential equation with constant coefficients which, depending on the values of α, β and ν, will give different types of time dependences to $N(t)$. One probable form of the solution to eq. (2) is:

$$N(t)=e^{At}\cos(B+Ct)(D+Et) \quad (3)$$

where the constants A, B, C, D and E depend on the initial value of $N(t)$ and the parameters α, β and ν. The cosine term may be interpreted as describing the deviation from exponential cell growth when the production of the inhibiting substance becomes important.

Recently an exact solution to eq. (1) was found by using Laplace transformation. The exact solution becomes:

$$N(t)=N(0)\,e^{\frac{\alpha+\nu}{2}t}\left\{\cos t\sqrt{\beta-\left(\frac{\alpha-\nu}{2}\right)^2}+\frac{\sin t\sqrt{\beta-\left(\frac{\alpha-\nu}{2}\right)^2}}{\sqrt{\frac{4\beta}{(\alpha-\nu)^2}-1}}\right\} \quad (3^*)$$

This solution is identical to the approximate solution, eq. (3), for the case when $E\equiv0$, but has the advantage that A, B and C are expressed in the initial coefficients α, β and ν. Fortunately due to the small value of E used it is unimportant whether the exact or approximate solution is used in the present application.

A set of parameter values which fits the experimental data for the total number of cells over the first week within a few per cents is: $A=0.57$ d^{-1}, $B=1.262$, $C=0.302d^{-1}$, $D=63\times10^6$ and $E=-3.4\times10^6 d^{-1}$.

At each given point on the curve it is possible to calculate the cell doubling time using eq. (3) for the total number of cells.

$$T_d(t_0)=\frac{ln\,2N(t_0)-ln\,N(t_0)}{\left(\frac{d\,ln\,N(t)}{dt}\right)_{t_0}}=\frac{ln\,2\times n(t_0)}{\left(\frac{dN}{dt}\right)_{t_0}} \quad (4)$$

Using eq. (4) for $N(t)$ the doubling time becomes simply

$$T_d(t)=\frac{ln2}{A-C\tan(B+Ct)+\frac{1}{D/E+t}} \quad (5)$$

It is interesting to observe that the quasi-exponential growth observed can be described quite well over a limited interval of time by an equation of type (3).

As can be seen from Table 2 in which the total number of tumour cells was calculated for the growth period day 1 to day 7 according to eq. (3), good agreement with the experimental data is obtained. The cell doubling time (T_d) calculated using eq. (5) shows an increase from 17.6 h on day 1 to 120.3 h on day 7. The course of the daily changes of T_d is also presented in Fig. 4.

In order to obtain the duration of the different parts of the cell cycle, two different methods were used. Both methods are based on the cell doubling time, or the increase in the total number of cells

Table 2

Calculated cell cycle parameters of the Bp8 ascites sarcoma

Days after inoculation	No. of tumour cells ($\times 10^6$)		Cell doubling time (T_d) (hours)	Relative cell flow rate (per cent/h)				Phase duration (hours)*			
	Experimental	Calculated		G_1	S	G_2	M	G_1		S	
								1	2	1	2
1	60.1	60.4	17.6	35.7	12.1	16.7	209.3	2.78	2.80	8.27	8.24
2	137.0	139.0	22.4	20.6	7.5	28.3	120.8	4.80	4.86	13.20	13.30
3	284.5	273.6	26.9	18.2	6.2	23.0	108.1	5.50	5.49	16.10	16.03
4	481.5	482.3	32.1	14.1	5.2	21.7	99.0	7.10	7.10	19.30	19.20
5	820.5	770.9	39.6	10.4	4.4	16.1	87.6	9.50	9.63	22.70	22.62
6	971.0	1110.0	54.5	7.5	3.3	10.8	71.1	13.30	13.33	30.40	30.21
7	1400.1	1396.0	120.3	3.6	1.5	4.5	30.5	27.60	27.47	67.0	66.67

* The duration of the phases of the cell cycle was calculated according to methods 1 and 2 (cf. text).

during a specific time period calculated according to eqs (6) to (9), and the distribution of cells in the various parts of the cell cycle, known from the DNA histograms and the mitotic index.

For method 1 an exponential age distribution of cells in the cell cycle was assumed and the duration of the various parts of the cell cycle was calculated according to the following eqs given by NACHTWEY & CAMERON (1968):

$$t_M = T/ln\,2\,ln\left(\frac{M}{N}+1\right) = k\,ln\left(\frac{M}{N}+1\right) \quad (6)$$

$$t_{G_2} = k\,ln\left(\frac{G_2}{M+N}+1\right) \quad (7)$$

$$t_s = k\,ln\left(\frac{S}{G_2+M+N}+1\right) \quad (8)$$

$$t_{G_1} = k\,ln\left(\frac{1}{1-(G_1/2N)}\right) \quad (9)$$

where T=generation time, N=total number of cells observed, ln=natural logarithm ($\log_e$), M, G_2, S, or G_1=number of cells in mitosis, G_2, S, or G_1, and the constant, $k=T/(ln\,2)$ or $T/0.693$.

For method 2 the flow rate of the cells proceeding each phase of the cell cycle per time unit was calculated. For this purpose, the increase in the total number of cells during the short time period of 0.1 h was calculated. This increase in the total number of cells corresponds to the number of cells which have divided. Since every mitotic cell gives rise to two G_1 cells, the inflow of new G_1 cells from the mitotic compartment is known. From the inflow of cells into G_1 and the number of cells found in the different compartments, the outflow is consequently known. The mean value of the inflow and the outflow per time unit is called mean cell flow rate, the mean flow rate per time unit divided by the mean number of cells in the different phases is called the relative flow rate. The inverses of the relative flow rates are equal to the duration of the phases. The sum of the phase durations is equal to the cell cycle time, T_c. The values from these calculations are summarized in Table 2. Figs 4 and 5 show the cell cycle phase durations as calculated according to method 2 and the changes in the relative flow rate. No essential differences were found in the phase durations using these two methods. The S-phase duration was longer than all the other phases together. The duration of the cell cycle phases or the relative cell flow rate increased discontinuously, and changed most markedly between day 1 and 2, and day 6 and 7 with the exception of G_2 which decreased in the duration between day 1 and 2. Reaching the plateau phase on day 7, with no increase in the total number of cells, the number of G_1 cells increased significantly from 400×10^6 to 500×10^6 cells between day 7 and 8. Thus at least 50×10^6 cells must have passed mitosis. During this time about the same number of cells had left from the S-phase. Assuming that half of these cells were lost from the S-phase and the other half had proceeded to G_2, from which part as many cells as in the S-phase were also lost, durations for the S-phase on day 8 of 190 h, for G_2 of 80 h and for mitosis of 12 h were calculated, while for G_1 the duration tended to infinity. These values are also shown in Fig. 4 going over into an infinite prolongation of all cell cycle phases during the following period. Between day 8 and 14 the daily cell decrease is about 5 per cent exclusively due to decrease of the cells from the S-phase, G_1 and mitosis.

Table 2 (*cont.*)

G_2		M		Cell cycle time (T_c) (hours)
1	2	1	2	
6.0	5.98	0.54	0.48	17.5
3.40	3.54	1.0	0.83	22.5
4.30	4.36	1.0	0.92	26.8
4.50	4.61	1.10	1.01	31.9
6.20	6.21	1.20	1.14	39.5
9.10	9.26	1.60	1.41	54.2
22.0	22.37	3.70	3.27	119.8

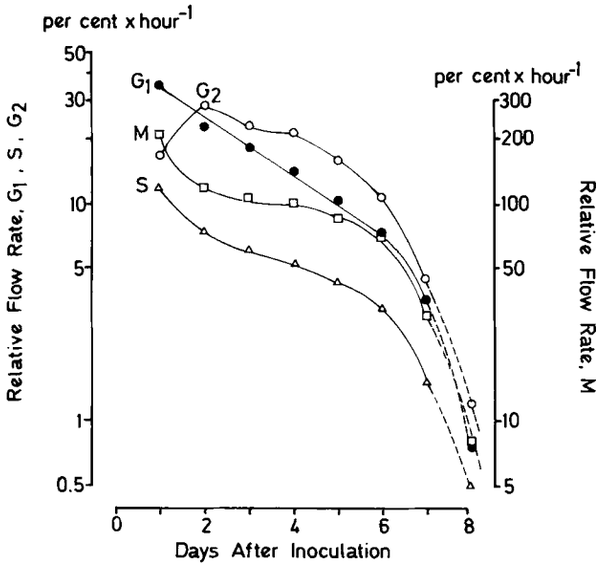

Fig. 5. Relative flow rate expressed as the percentage of cells per hour through G_1, S-phase, G_2 and mitosis up to 8 days after inoculation of 18×10^6 Bp8 sarcoma cells.

Discussion

The present Bp8 ascites sarcoma was originally developed as a solid tumour by Dr Craigir, of the Imperial Cancer Research Fund Laboratories, London, in 1943 by a benzpyrene implant in a C_3HB mouse, subsequently persuaded to grow as an ascites tumour. According to cellular DNA analysis this sarcoma has a chromosome number of about 70 and can therefore easily be discriminated by DNA analysis from the cells with normal DNA content.

The growth characteristics of this sarcoma are in agreement with those found in a number of ascites tumours, i.e. a more or less initial exponential increase in the number of cells during early growth followed by a progressive decline in the growth with a plateau of the cell number (KLEIN & RÉVÉSZ 1953, PATT & STRAUBE 1956, LALA & PATT 1966).

Cell kinetic investigations using the percentage of labelled mitoses technique and the continuous labelling technique using ^{3}H-thymidine injection generally revealed an increase in the duration of the various phases of the cell cycle with the age of the tumour and unchanged or decreased growth fraction and an unchanged, low or increased cell loss (PEEL & FLETCHER 1969, FRINDEL et coll. 1969, DOMBERNOWSKY & HARTMANN 1972, SCHIFFER et coll. 1973, DOMBERNOWSKY et coll. 1973, for summary cf. also STEEL 1977).

In the present work special attention was paid to the quantitative changes of the various parts of cell cycle during growth and the possibility of using these data for calculating the flow rate of the cells through the cell cycle.

The decrease in growth rate of ascites tumour cells with time, leading to a plateau state of the cells, has been explained in different ways, such as due to accumulation of toxic metabolites, O_2-deficiency or the competition of the cells for nutrition, an increase in the cell loss due to cell death or migration, but also as the result of an auto-regulation similar to the homeostatic growth regulation of normal tissues (BICHEL 1971, STEEL).

For estimation of the doubling time a mathematical model was adopted generally based on exponential cell increase but influenced by growth inhibitory factor(s) proportional to the cell number and with an exponential decay rate. This model describes well the changes in the experimental data up to day 7 but is not able to describe the decline of growth during an extended plateau phase. Other models of growth in which the cube root of the cell number increases linearly with time as proposed by KLEIN & RÉVÉSZ or the Gompertzian model may also be adapted to the present cell system. The biologic basis for the cube root growth is the idea that the cell growth rate is proportional to the supply of some critical nutrient and therefore to the surface of the tumour.

Two methods have been applied for calculation of the cell kinetic data and they give identical values during the growth period up to day 7. In method 1 a continuous cell flow from one compartment of the cell cycle to the next one was assumed based on an exponential distribution of cells in the cell cycle. Though the entire growth course was in fact not

exponential, during a limited time period of 24 h an exponential increase can be used to describe the growth with an acceptable degree of accuracy. In method 2 the cell numbers in the different parts of the cell cycle were calculated individually and the flow rate from one compartment to the following one calculated. Both methods are based on the same assumptions, a growth fraction of 1.0 and no cell loss. In another experimental series the cell loss in Bp8 ascites sarcoma was analysed directly by total body measurement of the activity after prelabelling the tumour cells with ^{125}I deoxyuridine. A rather constant decrease in the activity of about 8 per cent per day between day 0 and 4 and about 5 per cent between day 4 and 11 was found (KANEKO & TRIBUKAIT, to be published). Since a growth fraction of less than 1.0 and cell loss cause a corresponding decrease in the cell cycle time—for example a growth fraction of 0.5 or a cell loss of half of the cells results in a shortening of the cell cycle time to half the value—the true cell cycle time must be shorter, in the order of 10 per cent compared with the estimated times.

The conceptual principal of both methods for calculation of the cell flow rate is the same and is based on the observed increase in the cell number. The advantage of method 2 is the possibility of estimating cell flow even if the inflow of cells into a compartment and the outflow of cells from this compartment is changed by such factors as blocking events.

The plateau phase of the cell growth is characterized by a nearly unchanged composition of the cells in the cell cycle including mitotic cells but with a continuous decrease in the total cell number of about 5 per cent per day. This type can be observed when cell loss and cell production are nearly balanced, e.g. the cell loss factor is about 100 per cent. Since no increase of cell loss is indicated by an increase in the number of dead cells up to 12 days and direct experiments with radioactive prelabelled cells showed a 5 per cent cell loss per day the presented data indicate a non-specific prolongation of all parts of the cell cycle. A closer evaluation of the total number of different cells in the cell cycle, however, reveals the most interesting observation of the constancy of the total number of G_2 cells while all the other cells decreased. Assuming the concept of a regulated cell system for ascites tumour cells, the constant number of G_2 cells may indicate that the regulation process is specifically linked to the G_2 stage of the cell cycle. Experiments with disturbed cell proliferation may give further support to this hypothesis.

In conclusion, the measurements of the changes in the total cell number together with the proportions of the number of cells in the different phases of the cell cycle show that further possibilities exist for investigating cell proliferation kinetics.

SUMMARY

Growth characteristics of the Bp8 ascites sarcoma were investigated by means of evaluation of total cell number, the cellular DNA content, the mitotic index and the percentage of dead cells. Following intraperitoneal inoculation of 18×10^6 cells into NMRI mice a plateau level of about $1\,400\times10^6$ cells was reached after 7 days followed by a daily decline of 5 per cent up to day 14, the terminal stage of life. Based on the increase of the cell number and the distribution of cells in the cell cycle the flow rate through and the duration of the various parts of the cell cycle were calculated. Generally, the durations of all phases of the cell cycle including mitosis increased with age, most markedly after day 8 when the durations tended to become infinite. At the plateau phase the total number of G_1, S-phase and mitotic cells decreased significantly while G_2 cells remained at a constant level. This finding may be of interest in view of the concept of ascites tumour cells behaving as a regulated cell system.

ACKNOWLEDGEMENT

The investigation was supported by the Cancer Society of Stockholm.

Request for reprints: Professor B. Tribukait, Department of Medical Radiation Biology, Karolinska Institutet, P.O. Box 60204, S-104 01 Stockholm, Sweden.

REFERENCES

BICHEL P.: Autoregulation of ascites tumour growth by inhibition of the G_1 and G_2 phase. Europ. J. Cancer 7 (1971), 349.

DOMBERNOWSKY P. and HARTMANN N. R.: Analysis of variations in the cell population kinetics with tumor age in the L 1210 ascites tumor. Cancer Res. 32 (1972), 2452.

— BICHEL P. and HARTMANN N. R.: Cytokinetic analysis of the JB-1 ascites tumour at different stages of growth. Cell Tissue Kinet. 6 (1973), 347.

FRINDEL E., VALLERON A. J., VASSORT F. and TUBIANA M.: Proliferation kinetics of an experimental ascites tumour of the mouse. Cell Tissue Kinet. 2 (1969), 51.

KANEKO I. and TRIBUKAIT B.: To be published in Acta radiol. Oncology.

KLEIN G. and RÉVÉSZ L.: Quantitative studies on the multiplication of neoplastic cells in vivo. I. Growth curves of the Ehrlich and MC1M ascites tumors. J. nat. Cancer Inst. 14 (1953), 229.

LALA P. K. and PATT H. M.: Cytokinetic analysis of tumor growth. Proc. nat. Acad. Sci. U.S. 56 (1966), 1735.

NACHTWEY D. S. and CAMERON I. L.: Cell cycle analysis. *In:* Methods in cell physiology. Vol. III, p. 213. Academic Press, New York, London 1968.

PATT H. M. and STRAUBE R. L.: Measurement and nature of ascites tumor growth. Ann. N.Y. Acad. Sci. 63 (1956), 728.

— BLACKFORD M. E. and DRALLMEIER J. L.: Growth characteristics of the Krebs ascites tumor. Proc. Soc. exp. Biol. (N.Y.) 83 (1953), 520.

PEEL S. and FLETCHER P. A.: Changes occurring during the growth of Ehrlich ascites cells in vivo. Europ. J. Cancer 5 (1969), 581.

SCHIFFER L. M., NELSON J. S. R., DILETTUSO B., MIGLIORATO D. and RANDOLPH W.: Cytokinetics of the S-180 ascites tumor system. Cell Tissue Kinet. 6 (1973), 165.

STEEL G. G.: Growth kinetics of tumours. Clarendon Press, Oxford 1977.

TRIBUKAIT B., MOBERGER G. and ZETTERBERG A.: Methodological aspects on rapid-flow cytofluorometry for DNA analysis of human urinary bladder cells. *In:* First International Symposium on Pulse Cytophotometry (Nijmegen 1974). European Press, Medikon, Ghent 1975.