Influence of aluminium intoxication on experimental rats

Values estimated	Control group	Experimental group	Significance
No. of rats	8	8	
Blood glucose mg%	109.60 ± 4.25	103.30 ± 2.23	$P > 0.05$
Glycogen			
Liver g%	2.530 ± 0.085	0.309 ± 0.030	$P < 0.001$
Muscle g%	0.442 ± 0.028	0.271 ± 0.023	$P < 0.001$
Lactic acid			
Blood mg%	13.72 ± 0.76	13.55 ± 0.66	$P > 0.05$
Liver mg%	11.89 ± 0.75	15.36 ± 0.72	$P < 0.01$
Muscle mg%	32.36 ± 3.50	51.61 ± 2.80	$P < 0.001$
Pyruvic acid			
Blood mg%	0.265 ± 0.012	0.366 ± 0.019	$P < 0.001$
Liver mg%	0.296 ± 0.002	0.400 ± 0.021	$P < 0.001$
Coenzyme A liver	17.27 ± 1.03	5.05 ± 0.27	$P < 0.001$

tration in liver, while its level in muscle was decreased only slightly though still significantly ($P < 0.001$). The glucose and lactate level in blood did not change significantly. However, the level of lactic acid in liver and muscle was increased. The level of pyruvic acid increased too, mostly in liver. The coenzyme A values are expressed in mg of acetylated 4-aminoazo-benzene related to 100 g of fresh liver tissue. These levels are strongly decreased in the experimental group ($P < 0.001$).

These results seem to point to a disturbance of glycide metabolism accompanying increased supply of aluminium salts. The most pronounced changes were the decrease of glycogen concentration in liver and increase of pyruvic and lactic acids levels in the same tissue. These changes have perhaps a common cause, i.e. the disturbance in phosphorus metabolism provoked by excess doses of aluminium salts. Therefore it can be assumed that there is decreased glucose absorption from the gut. The results correlate well with the other experiments in which the incorporation of ^{32}P into various phosphate fractions under the influence of aluminium was studied. The incorporation of ^{32}P into phospholipids, RNA and DNA in experimental animals was found to be significantly lowered[3].

Zusammenfassung. Bei Intoxikation von Versuchsratten durch Aluminiumsalze wurden Störungen im Glyzid-Metabolismus festgestellt. Er ergab sich ein Glykogenrückfall in Leber und Muskelgewebe und eine Erhöhung des Brenztraubensäure- und Milchsäurespiegels. Es wird angenommen, dass die beobachteten Veränderungen mit Störungen des Phosphormetabolismus und der Phosphorilationsreaktionen durch die Aluminiumtoxikation zusammenhängen.

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Research Institute of Hygiene, Bratislava (Czechoslovakia), 21st March 1967.

The Mode of Timing of DNA Replication and of Mitosis in Cultured Animal Cells[1]

It has been postulated repeatedly, with little or no support from experimental evidence, that the DNA-synthesis period (*S*-period), the *G*2-period and the period of observable mitosis (*M*-period) of the mitotic cycle have each fixed durations, and that therefore, differences in generation time are due to expansion or contraction of the *G*1-period alone[2–5]. Evidence in favour of an alternative mode of timing of DNA replication and of mitosis has been obtained in carefully planned and executed experiments with 2 established (heteroploid) mouse cell strains, P815Y and L929, grown in fluid suspension at many different exponential rates in chemostat and cytogenerator[6–8]. Observations indicating that exposure of animal cells to exogenous thymidine alters the pre-established rate of DNA synthesis and subsequently modifies the normal chronology and duration of the cell-division-cycle, forced me to abandon the standard pro-

[1] This investigation was supported by the Office of Naval Research under contract No. Nonr-266(76) and by the Health Research Council of the City of New York under contract No. I-428, and was carried out at Columbia University (Department of Biochemistry).

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cedures used for the determination of S-period, $G1$-period and generation time, and to replace these methods by an indirect method of analysis of the mitotic cycle; a method which does not by itself affect or alter the parameters to be measured. The indirect method is based on a population model of asynchronous animal cell cultures[9] and on 3 mathematically formulated relationships, namely those expressing (1) duration of the $G2$-period (G_2) as a function of doubling time (τ^*) of cell cultures, (2) mitotic index (J_M) as a function of specific growth rate (k^*) of the cultures, and (3) DNA-synthetic index (i.e. instantaneous thymidine-H^3-cell labelling index, J_S) as a function of specific growth rate[9,10].

(I) *Theory.* On the basis of certain general assumptions[9,10] and specifically for the conditions of exponential culture growth (exponentially expanding and steady-state cell cultures) only, I have derived theoretical expressions for the 3 critical functions, (1) $\overline{G}_2 = f(\overline{\tau}^*)$, (2) $\overline{J}_M = f(\overline{k}^*)$, and (3) $\overline{J}_S = f(\overline{k}^*)$. The general forms of these expressions[11] are,

$$\overline{G}_2 = \begin{cases} (\overline{G}_2)_0 = \text{constant}, \\ \overline{C}_4\,\overline{\tau} = \overline{C}_4 \left(\dfrac{(\ln 2)}{\lambda_1}\right)\left(\dfrac{\overline{\tau}^*}{[(\ln 2)/\lambda_1] + \overline{\tau}^*}\right) \end{cases} \quad (1)$$

$$\overline{J}_M = \left(e^{(\overline{k}^* + \lambda_1)(\overline{M})} - 1\right)\left(\frac{\overline{k}^* + \lambda_2}{\overline{k}^* + \lambda_1 + \lambda_2}\right) \quad (2)$$

$$\overline{J}_S = \left(e^{(\overline{k}^* + \lambda_1)(\overline{G}_2 + \overline{M})}\right)\left(e^{(\overline{k}^* + \lambda_1)(\overline{S})} - 1\right)\left(\frac{\overline{k}^* + \lambda_2}{\overline{k}^* + \lambda_1 + \lambda_2}\right) \quad (3)$$

in which there is: $\overline{M}$ = duration of observable mitosis, in h, a constant ($\overline{M}_0$) or a variable (e.g. $\overline{M} = \overline{C}_4 \times \overline{\tau}$ = constant $\times\, \overline{\tau}$); $\overline{G}_2$ = duration of the $G2$-period in h, a constant ($(\overline{G}_2)_0$) or a variable (e.g. $\overline{G}_2 = \overline{C}_3 \times \overline{\tau}$ = constant $\times\, \overline{\tau}$); $\overline{S}$ = duration of the DNA-synthesis period in h, a constant ($\overline{S}_0$) or a variable (e.g. $\overline{S} = \overline{C}_2 \times \overline{\tau}$ = constant $\times\, \overline{\tau}$); $\overline{\tau} = \overline{G}_1 + \overline{S} + \overline{G}_2 + \overline{M}$ = generation time of proliferating or R-cells, in h ($\overline{G}_1$ = duration of the post-mitotic DNA-presynthetic phase); $\overline{k}^*$ = specific growth rate i.e. doubling rate of cell culture, per h = $(\ln 2)/\overline{\tau}^*$ ($\overline{\tau}^*$ = doubling time of cell culture in h); λ_1 = specific rate of spontaneous transitions of proliferating (or R-) cells into maintenance (or M'-) cells, per h, a constant; λ_2 = specific rate of spontaneous transitions of maintenance cells into senescent (or M''-) cells, per h, a constant; $\overline{k}^* + \lambda_1 = \overline{k} = (\ln 2)/\overline{\tau}$ = specific cell-birth rate, per h, a variable, $(\overline{k}^* + \lambda_2)/(\overline{k}^* + \lambda_1 + \lambda_2) = \overline{\phi}_1$ = fraction of proliferating (or R-) cells in the cultures (approximation valid for exponentially growing cultures of P815Y and L929 cells where the fraction of senescent cells is negligibly small), a variable.

Examination of these 4 theoretical functions has revealed their potential for discrimination, by comparison of their predicted forms with the experimental curves, between alternative modes of timing of DNA synthesis and mitosis in animal cells. Numerical analysis has shown that clear-cut distinction is feasable between the following alternative timing modes: (a) $\overline{G}_2$ = constant and $\overline{G}_2 \sim \overline{\tau}$ ($\sim$ = 'proportional'), on the basis of the function $\overline{G}_2 = f(\overline{\tau}^*)$; (b) $\overline{M}$ = constant and $\overline{M} \sim \overline{\tau}$, on the basis of the function $\overline{J}_M = f(\overline{k}^*)$; (c) $\overline{S}$ = constant and $\overline{S} \sim \overline{\tau}$, on the basis of the function $\overline{J}_S = f(\overline{k}^*)$. In addition to this I have shown that confirmation of results or supplementary information regarding the timing mode may be gained by examination of the correlation between mitotic index and concomitant DNA-synthetic index, $\overline{J}_M{}^{(i)} = f(\overline{J}_S{}^{(i)})$.

(II) *Experimental analysis.* Fluid-suspension cultures with stable exponential growth characteristics (exponentially expanding and stable steady-state cultures), of mouse ascites mast cells (clone P815Y) and of L929 mouse cells (clone NCTC929), were produced in the U-tube-batch-culture-system, the cytogenerator and the chemostat[12]. P815Y cultures were grown at doubling times ranging from $\overline{\tau}^* = 12$ h (specific growth rate, $\overline{k}^* = 0.0578$ h^{-1}) to $\overline{\tau}^* = \infty$ (infinity ($\overline{k}^* = 0$ h^{-1}), L929 cultures from $\overline{\tau}^* = 26$ h ($\overline{k}^* = 0.0267$ h^{-1}) to $\overline{\tau}^* = 124$ h ($\overline{k}^* = 0.0056$ h^{-1}). Measurements were made of the duration of the $G2$-period ($\overline{G}_2$), the DNA-synthetic index ($\overline{J}_S$) and the mitotic index ($\overline{J}_M$), either on whole cultures ($\overline{G}_2$, J_S) or on small samples thereof ($\overline{J}_M$, $\overline{J}_S$). $\overline{G}_2$ was determined directly from the time of appearance of the first wave of thymidine-H^3 labelled mitosis in TdR-H^3 exposed cultures. $\overline{J}_S$ was determined by TdR-H^3 pulse labelling of cells and autoradiography, and $\overline{J}_M$ by counting the number of mitosis in cell suspension samples pretreated with hypotonic saline[7].

(IIa) *The empirical form of $\overline{G}_2 = f(\overline{\tau}^*)$ in cell strain P815Y.* When $\overline{G}_2$ is a variable (case A) and is assumed to be directly proportional to generation time ($\overline{G}_2 = \overline{C}_3 \times \overline{\tau}$, 'proportional timing' mode), then the theory predicts (see 1) a linear relationship between $Y = 1/\overline{G}_2$ and $x = 1/\overline{\tau}^*$, $Y = a_1 + b_1 \times x$, with slope $b_1 = 1/\overline{C}_3 > 0$ and an ordinate-intercept $a_1 = \lambda_1/((\ln 2) \times \overline{C}_3) > 0$. If, on the other hand, $\overline{G}_2$ is a constant (case B), then the slope of the plot of y versus x must not deviate significantly from zero ($b_1 = 0$). Linear regression of the plot of the experimental values, $y_j = 1/\overline{G}_{2j}$, versus $x_j = 1/\overline{\tau}^*{}_j$ yielded a regression line with the slope $b_1 = 1/\overline{C}_3 = 5.278$ (standard deviation, $s(b_1) = \pm 0.723$) and an ordinate-intercept $a_1 = \lambda_1/(0.693 \times \overline{C}_3) = 0.383$ h^{-1} (see Figure 1). According to Student's t-test the slope b is highly significant ($P < 0.001$); thus $\overline{G}_2$ is not a constant. Furthermore, the F-test for equality of variances shows that the experimental plot is indeed a linear one ($F < F_{P=0.05}$).

Thus, $\overline{G}_2$ increases with increasing generation time homogenous-linearly (case A of timing), and hence, $\overline{G}_2$

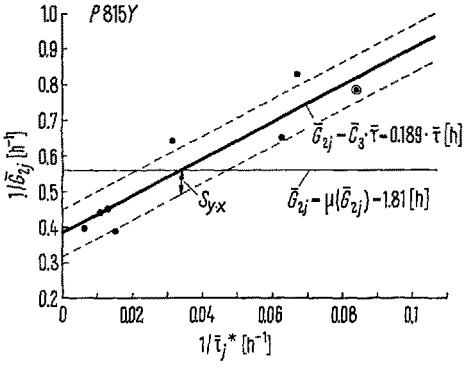

Fig. 1. Duration of the G2-period as a function of the doubling time of cultures. (a) Linear least-squares plot of $1/\overline{G}_{2j}$ versus $1/\overline{\tau}^*{}_j$, conforming to case (A) of timing of the G2-period ($\overline{G}_{2j} = \overline{C}_3 \times \overline{\tau}_j = 0.189 \times \overline{\tau}_j$ h). (b) Theoretical curve calculated on the assumption of case (B) of timing of the G2-period ($\overline{G}_{2j}$ = constant = $\mu(\overline{G}_{2j}) = 1.81$ h). ($\overline{G}_{2j}$ = mean value of $\overline{G}_2$ for a large sample of proliferating P815Y cells in a culture growing at a fixed doubling time $\overline{\tau}^*{}_j$; $s_{y.x}$ = standard error of estimate of $y = 1/\overline{G}_{2j}$ on $x = 1/\overline{\tau}^*{}_j$.)

[9] H. MOSER, Acta biotheor., in preparation for press.
[10] H. MOSER, Acta biotheor., in press.
[11] The 'bar' over any of the mathematical symbols, x, signifies 'value of x under conditions of exponential culture growth only'.
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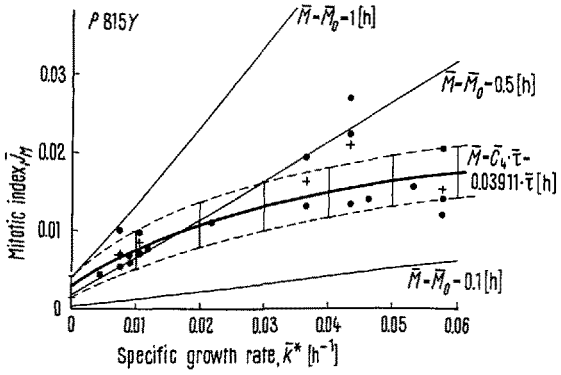

Fig. 2. Mitotic index, of asynchronous P815Y cultures, as a function of specific growth rate. (a) Least-squares plot of $\overline{J_M}$ versus $\overline{k^*}$ conforming to case (A) of timing of the mitotic period ($\overline{M} = \overline{C}_4 \times \overline{\tau} = 0.03911 \times \overline{\tau}$ h). (Bars indicate standard error of estimated $\overline{J_M}$; broken lines indicate the 99% confidence interval of $J_{M,est.}$) (b) Theoretical curves $\overline{J_M} = f(\overline{k^*})$ calculated on the basis of the assumption of case (B) of timing of the mitotic period ($\overline{M} = \overline{M}_0 =$ constant).

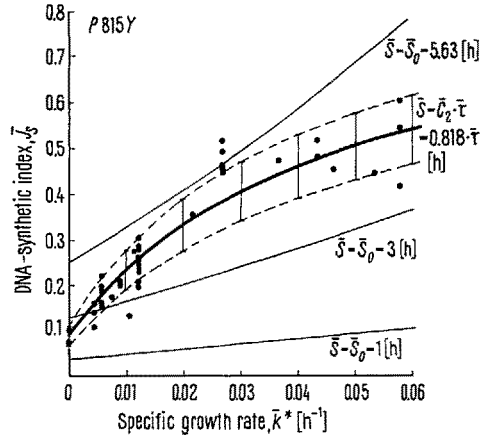

Fig. 3. DNA-synthetic index (instantaneous thymidine-H^3 cell labelling index), of asynchronous P815Y cultures, as a function of specific growth rate. (a) Least-squares plot of $\overline{J_S}$ versus $\overline{k^*}$ conforming to case (A) of timing of the S-period ($\overline{S} = \overline{C}_2 \times \overline{\tau} = 0.818 \times \overline{\tau}$ h). (Bars indicate the standard error of estimated $\overline{J_S}$; broken lines indicate the 99% confidence interval of $J_{S,est.}$) (b) Theoretical curves $\overline{J_S} = f(\overline{k^*})$ calculated on the basis of the assumption of case (B) of timing of the DNA-synthesis period ($\overline{S}$ = constant = $\overline{S}_0$).

composes always the same proportion of generation time, $\overline{C}_3 = \overline{G}_2/\overline{\tau} =$ constant. Averaging of the parameter estimates obtained from $\overline{G}_{2j} = f(\overline{\tau}^*_j)$ and its linearized form, $1/\overline{G}_{2j} = f(1/\overline{\tau}^*_j)$, yielded for the proportionality constant $\overline{C}_3$ and for the specific transition rate λ_1 in cell strain P815Y the values, $\overline{C}_3 = 0.1683 \pm 0.0262$ and $\lambda_1 = 0.0428 \pm 0.0086$ h^{-1}.

(II b, c) *The empirical forms of* $\overline{J_M} = f(\overline{k^*})$ *and* $\overline{J_S} = f(\overline{k^*})$ *in cell strain P815Y.* When $\overline{M}$, $\overline{G}_2$ and $\overline{S}$ are each variables (case A) and are assumed to be each directly proportional to generation time ($\overline{M} = \overline{C}_4 \times \overline{\tau}$, $\overline{G}_2 = \overline{C}_3 \times \tau$, $\overline{S} = \overline{C}_2 \times \overline{\tau}$), then the theory predicts for the functions $\overline{J_M} = f(\overline{k^*})$ and $\overline{J_S} = f(\overline{k^*})$ the particular forms,

$$\overline{J_M} = \overline{K}_M\,((\overline{k^*} + \lambda_2)/(\overline{k^*} + \lambda_1 + \lambda_2)) \qquad (4)$$

and

$$\overline{J_S} = \overline{K}_S\,((\overline{k^*} + \lambda_2)/(\overline{k^*} + \lambda_1 + \lambda_2)) \qquad (5)$$

in which

$$\overline{K}_M = e^{(\ln 2)\,\overline{C}_4} - 1$$

and

$$\overline{K}_S = e^{(\ln 2)\,(\overline{C}_3 + \overline{C}_4)}\,(e^{(\ln 2)\,\overline{C}_3} - 1)$$

are constants, with the ranges, $0 > \overline{K}_M < 1$ and $0 > \overline{K}_S < 1$. In this case both the mitotic index and the DNA-synthetic index are expected to increase, with increasing doubling rate of the cultures, from an initial value, $\overline{J_M}(0) = \overline{K}_M \times (\lambda_2/(\lambda_1 + \lambda_2)) > 0$ and $\overline{J_S}(0) = \overline{K}_S(\lambda_2/(\lambda_1 + \lambda_2)) > 0$ respectively, monotonically to an asymptotic maximum, $\overline{J_M}(\infty) = \overline{K}_M$ and $\overline{J_S}(\infty) = \overline{K}_S$ respectively. If on the other hand, $\overline{M}$ and $\overline{S}$ are assumed to be constants rather than variables (case B), $\overline{J_M}$ and $\overline{J_S}$ are expected to increase exponentially with increasing $\overline{k^*}$, at an ever increasing rate i.e. without reaching a finite maximum. Our data have made possible a clearcut distinction between these theoretical alternatives. The experimental plots of $\overline{J_M}$ and $\overline{J_S}$ versus $\overline{k^*}$ conform to equations 4 and 5 (see Figures 2 and 3). This was verified by rigorous statistical tests[7]. Parameter evaluation of (4) and (5) yielded for the constants $\overline{K}_M$ and $\overline{K}_S$ in cell strain P815Y the averaged least-squares estimates, $\overline{K}_M = 0.02747$ and $\overline{K}_S = 0.88142$ ($s(\overline{K}_M) = \pm 0.00326$; $s(\overline{K}_S) = \pm 0.10331$).

Conclusions. The indirect experimental evidence clearly indicates that the DNA-synthesis period, the G2-period and the mitosis period are not of fixed durations in P815Y cells, and that, therefore, expansion or contraction of generation time is not due to expansion or contraction of the $G1$-period alone. The data are consistent with the hypothesis that, under conditions of exponential cell proliferation (and probably only under such conditions), expansion or contraction of the generation time is due to concomitant expansions or contractions of each of the characteristic phases of the mitotic cycle. Since $\overline{G}_1$ (if present), $\overline{S}$, $\overline{G}_2$, and $\overline{M}$ are each homogeneous-linear functions of generation time, exponentially multiplying cells always spend the same fixed fractions of their generation time in DNA synthesis ($\overline{C}_2 = \overline{S}/\overline{\tau}$) and in mitosis ($\overline{C}_4 = \overline{M}/\overline{\tau}$), irrespective of their rate of proliferation ('proportional timing' mode). Because of this, alteration of (exponential) cell duplication rate ($\overline{k}$) within it's limits ($\ln 2/\lambda_1 \leqq \overline{k} \leqq \overline{k}_{max}$) does not modify the specific temporal order of characteristic events of the mitotic cycle,

$$\left\{ \begin{array}{llllll} E_1, & E_2, & E_3, & E_4, & & \\ \overline{C}_1 = & \overline{C}_1 + \overline{C}_2 = & \overline{C}_1 + \overline{C}_2 + \overline{C}_3 = & \overline{C}_1 + \overline{C}_2 + \overline{C}_3 + \overline{C}_4 = & & \\ \dfrac{\overline{G}_1}{\overline{\tau}} = & \dfrac{\overline{G}_1 + \overline{S}}{\overline{\tau}} = & \dfrac{\overline{G}_1 + \overline{S} + \overline{G}_2}{\overline{\tau}} = & \dfrac{\overline{G}_1 + \overline{S} + \overline{G} + \overline{M}}{\overline{\tau}} = & 1 = & \\ \text{constant} & \text{constant} & \text{constant} & \text{constant} & & \end{array} \right\}$$

(E_1 = 'onset of DNA synthesis'; E_2 = 'termination of DNA synthesis'; E_3 = 'onset of observable mitosis' i.e. chromosome separation; E_4 = 'completion of cell division').

Similar conclusions have been drawn from experiments conducted earlier with L929 mouse cells[7,8]. 'Proportional timing', of macromolecular events in cell duplication, is probably a characteristicon of animal cells in the exponential state of cell proliferation[13], applying to both normal and highly neoplastic cells[14,15].

Zusammenfassung. Eine indirekte Methode der Analyse des mitotischen Zell-Zyklus wurde an verschieden rasch, jedoch stets exponentiell wachsenden Suspensions-Kulturen von neoplastischen Maus-Mast-Zellen des Stammes P815Y und Kulturen von L929 Maus-Zellen geprüft. Die experimentellen Kurven (Dauer der *G2*-periode als Funktion der Kulturen-Verdopplungszeit, mitotischer Index als Funktion der spezifischen Wachstumsgeschwindigkeit der Kulturen, und DNA-synthetischer Index als Funktion der spezifischen Wachstumsgeschwindigkeit) sind mit der Hypothese im Einklang, nach der die Dauern der *G1*-, *S*-, *G2*- und der *M*-Periode homogen-lineare Funktionen der Generationsdauer respektive der Gesamtdauer des Zell-Zyklus sind. Demzufolge sind unter Bedingungen streng exponentiellen Kulturenwachstums die DNA-Synthese-Periode, die *G2*-Periode und die Mitose-Periode nicht von konstanter Dauer, wie üblicherweise angenommen wird. Die experimentell erzwungene Verlängerung oder Verkürzung der Generationsdauer der sich in der Kultur teilenden Zellen (*R*-Zellen) wird somit nicht allein durch die *G1*-Periode bestimmt, sondern durch gleichzeitige und prozentual gleichmässige Expansion oder Kontraktion aller vier charakteristischen Phasen des Zell-Zyklus.

H. Moser[16]

Station de Zoologie expérimentale de l'Université, 1200 Genève (Switzerland), 1 March 1967.

[13] In the experiments with P815Y cells growth rate was in all likelyhood limited (controlled) by the O_2 concentration in the culture medium. The possibility that the timing mode is altered under different conditions of growth control, therefore, cannot be excluded at present.

[14] Acknowledgments: the author deeply appreciates the technical help of Dr. S. Graff and Mr. F. Lum in setting up apparatus.

[15] This paper has been dedicated to my former teacher Prof. Ad. Portmann on the occasion of his 70th birthday.

[16] Recipient of Career Scientist Award of the Health Research Council of the City of New York under contract No. I-428. Present address: Station de Zoologie Expérimentale, Université de Genève, 154 Route de Malagnou, Chêne-Bougeries, Genève (Switzerland).

Atypic Chronology of the Mitotic Cycle of Neoplastic Mouse Mast Cells[1]

The life cycle of actively proliferating mammalian cells is characterized by the existence of an extensive G1-period and by a G2-period which extends from 10–20% of the generation time. Typical examples are diploid hamster cells[2], human HeLa cells[3] and mouse L929 cells[4]. My studies with P815Y cells[5–7] revealed, for the first time in a mammalian cell strain, atypical behaviour of the mitotic cycle. Conclusive evidence has been obtained that mouse ascites mast cells, with an exponential state of proliferation, lack a detectable G1-period in their cell-division-cycle. Calculation of the invariant fractions of generation time spent in observable mitosis ($\overline{C}_4 = \overline{M}/\overline{\tau}$, $\overline{\tau}$ = duration of the mitotic cycle i.e. generation time, $\overline{M}$ = duration of observable mitosis) and in DNA synthesis ($\overline{C}_2 = \overline{S}/\overline{\tau}$, $\overline{S}$ = duration of the DNA synthesis period)[8], by solving the exponential equations,

$$e^{(\ln 2)\,\overline{C}_4} - 1 = \overline{K}_M = 0.02747 \qquad (1)$$

and

$$e^{(\ln 2)\,(\overline{C}_3 + \overline{C}_4)}\left(e^{(\ln 2)\,\overline{C}_4} - 1\right) = \overline{K}_S = 0.88142, \qquad (2)$$

in which $\overline{C}_3 = \overline{G}_2/\overline{\tau} = 0.16821$ ($\overline{G}_2$ = duration of the *G2*-period), yielded $\overline{C}_4 = 0.03911$ and $\overline{C}_2 = 0.81838$. From this I de rivedfor $\overline{C}_1 = \overline{G}_1/\overline{\tau} = 1 - (\overline{C}_2 + \overline{C}_3 + \overline{C}_4)$ ($\overline{G}_1$ = duration of the *G1*-period) the value $\overline{C}_1 = -0.0257$ with a standard deviation of $s(\overline{C}_1) = \pm 0.07704$, which is, according to Student's *t*-test not significantly different from $\overline{C}_1 = 0$[9]. Since $\overline{C}_3 = \overline{G}_2/\overline{\tau} = 0.16821$ is probably an overestimate (for reasons of methodology[5,6]), the specific chronology of the mitotic cycle of exponentially multiplying P815Y cells, expressed in % of generation time, is most likely the following[9]:

$$100\left(\frac{\overline{G}_1}{\overline{\tau}}, \frac{\overline{S}}{\overline{\tau}}, \frac{\overline{G}_2}{\overline{\tau}}, \frac{\overline{M}}{\overline{\tau}}\right) = (0\%,\ 81.84\%,\ 14.25\%.\ 3.91\%).$$

Thus, in P815Y cells, DNA synthesis commences within minutes after cell division, probably in a single chromosome pair and in a specific nucleotide sequence. Replication of chromosomal DNA is terminated when the cell has passed through approximately 82% of its total life span, probably also at a specific site in a specific chromosome pair[10]. Since P815Y cells do have a *G2*-period ($\overline{C}_3 > 0$), and since the durations of the *G2*-period and of mitosis are of a magnitude which is typical for mammalian cells, the DNA-synthesis period is expanded entirely at the expense of the *G1*-period.

[1] This investigation was supported by the Office of Naval Research under contract No. Nonr-266(76) and by the Health Research Council of the City of New York under contract No. I-428, and carried out at Columbia University (Department of Biochemistry).

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[8] H. Moser, Experientia *23*, 913 (1967).

[9] During transient growth periods of the cultures (lag-phase, approach to steady-state etc.) cell proliferation is non-exponential. Under such conditions the chronology of the cell-division-cycle is expected to deviate from it's uniform exponential characteristics. It is possible therefore, that under such conditions P815Y cells may manifest G1-periods of variable durations.

[10] The replication periods of specific chromosome pairs are not identical and overlap each other[2]. As a rule chromosomes which begin replication early in the S-period complete duplication late, and chromosomes which start late complete DNA-synthesis early (Y. Kikuchi and A. Sandberg, J. natn. Cancer Inst. *32*, 1109 (1964)). The duration of the S-period is, therefore, determined by at least 1 chromosome pair, the chromosome pair with the most extensive replication period. Preliminary observations indicate that this ordered asynchrony of chromosomal replication also applies to P815Y cells.