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.

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[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.

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

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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).

[2] J. H. Taylor, J. biophys. biochem. Cytol. *7*, 455 (1960).

[3] T. T. Puck and J. Steffen, Biophys. J. *3*, 379 (1963).

[4] C. P. Stanners and J. E. Till, Biochim. biophys. Acta *37*, 406 (1960).

[5] H. Moser, Acta biotheor., in press.

[6] H. Moser, S. Graff and F. Lum, Acta biotheor., in press.

[7] H. Moser, S. Graff and O. Kastner, Acta biotheor., in press.

[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.

The fact that atypical behaviour of the mitotic cycle, in animal cells, has been previously observed only among embryonic cell varieties[11,12], and now also in an adult mammalian cell derived from an extremely virulent neoplasm, is interesting and suggestive. Metabolic similarities between embryonic cells and neoplastic cells have long been recognized and related to an increased capacity for proliferation of both of these categories of cells. It now appears that these metabolic similarities also manifest themselves in specific deviations from the characteristic timing pattern of the normal, adult, animal cell cycle. If it can be confirmed, by further experimentation, that malignant cell transformation indeed alters the normal cell-division-cycle of adult mammalian cells by relaxing, to a variable degree, the controls which suppress DNA synthesis in normal adult cells during the early part of the interphase ($G1$-period), then a new quantitative assessment of the degree of malignancy is feasible, an assessment based on the evaluation of the parameters $\overline{C}_1 = \overline{G}_1/\overline{\tau}$ and $\overline{C}_2 = \overline{S}/\overline{\tau}$.

The observation that DNA synthesis commences, in certain rapidly proliferating cells such as neoplastic mouse mast cells and embryonic cells, immediately, that is within minutes after cell division, is of considerable interest also in regard to the problem of growth control. The observation that the cell reproduction cycle is interrupted – by inhibition of RNA and protein synthesis, for example – most easily and dramatically in early stages of the interphase[13,14], had led to the belief that the biochemical activities which form the basis of the $G1$-period establish the requisite conditions for cell division and for mitosis[13]. Our findings (absence of $G1$), in P815Y cells, indicate that specific biochemical activities of newly duplicated cells, such as the synthesis of specific RNA's or proteins during the early part of interphase, are not an essential requirement for the induction of a new cycle of DNA synthesis and mitosis. They also suggest the possibility that the primary and decisive factors involved in the propulsion of the cell reproduction cycle begin to operate at some stage during mitosis, rather than after cell division[15,16].

Zusammenfassung. Im mitotischen Zyklus vollständig asynchroner, exponentiell proliferierender neoplastischer Mausmastzellen des Stamms P815Y ist die $G1$-Phase vollständig abwesend. DNA-Synthese (S-Phase) beansprucht 84%, $G2$-Phase 14% und Mitose 4% der vollen Dauer des Zell-Zyklus.

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

[11] M. E. Gaulden, Genetics *41*, 645 (1956).
[12] R. T. Hinegardner, B. Rao and D. E. Feldman, Expl Cell Res. *36*, 53 (1964).
[13] D. M. Prescott, Natn. Cancer Inst. Monogr. *14*, 57 (1964).
[14] L. Lieberman, J. biol. Chem. *238*, 3955 (1963).
[15] The author deeply appreciates the technical help of Dr. S. Graff and Mr. F. Lum in setting up apparatus.
[16] This paper has been dedicated to my former teacher Prof. A. Portmann on the occasion of his 70th birthday.
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Integumental Tyrosinase Activity in Reptiles

Reptiles form a critical vertebrate class in evolutionary studies as they are basically terrestrial and show the developmental (amniote) characteristics of the higher vertebrates, but retain the primitive inability to independently maintain a constant body temperature. In an evolutionary study[1], it appeared that the amniotes, in contrast to the anamniotes, showed low integumental tyrosinase activity levels as well as the limitation of the subcellular localization of enzyme activity to the particulate fraction after ultracentrifugation. As a result, representative species of the 3 major orders of the class Reptilia, the Chelonia (turtles), Crocodilia (crocodiles) and Squamata (suborder Serpentes, snakes and suborder Sauria, lizards) were investigated in regard to the various aspects of their integumental tyrosinase activity levels, including the anatomic and subcellular tyrosinase distribution and the extent of tyrosine carboxyl incorporation into melanin.

Methods and materials. All reptiles used (Table I) were decapitated and the dorsal and ventral skin areas were immediately removed and frozen (– 27 °C). In *Opheodrys* the lateral skin was also studied. The enzyme in *Trionyx* integument bound to the carapace and plastron was compared with that in the remaining portions of the skin. The enzyme preparation, radiometric assay procedures and

Table I. Species utilized in the study of integumentary tyrosinase activity in reptiles

Species	No. of animals used[a]	Boby weight (g)[b]
Trionyx ferox (Florida softshell turtle)	3	11 (10–12)
Caiman sclerops (Spectacled caiman)	3	235 (209–257)
Opheodrys aestivus (Vine snake)	3	17 (14–22)
Anolis carolinensis (American 'chameleon')	10 (2)	3.6 (2.7–4.1)

[a] The number in parenthesis represents the total number of pooled samples. Otherwise the number of enzyme preparations is equal to the number of animals used. [b] Mean weight (weight range).

[1] Y. M. Chen and W. Chavin, Adv. Biol. Skin *8*, 253 (1967).