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 (*G*1-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 *G*1-period establish the requisite conditions for cell division and for mitosis[13]. Our findings (absence of *G*1), 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 *G*1-Phase vollständig abwesend. DNA-Synthese (*S*-Phase) beansprucht 84%, *G*2-Phase 14% und Mitose 4% der vollen Dauer des Zell-Zyklus.

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[15] The author deeply appreciates the technical help of Dr. S. Graff and Mr. F. Lum in setting up apparatus.
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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).

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Table II. Integumental tyrosinase activity in reptiles

Species	T.U.[a]/mg skin			Specific activity[b]			Tyrosine carboxyl group incorporation %[c]		
	H	P	S	H	P	S	H	P	S
Trionyx ferox									
Dorsal	153 ± 5	150 ± 3	0	17 ± 0	23 ± 1	0	15.6 ± 0.7	15.9 ± 0.9	0
Ventral	46 ± 1	44 ± 2	0	5 ± 0	6 ± 0	0	14.8 ± 0.3	15.4 ± 0.4	0
Carapace	24 ± 1	25 ± 1	0	6 ± 0	10 ± 1	0	21.4 ± 0.5	22.3 ± 0.6	0
Plastron	10 ± 1	10 ± 1	0	9 ± 0	14 ± 1	0	16.7 ± 1.1	17.3 ± 0.7	0
Caiman sclerops									
Dorsal	235 ± 11	224 ± 11	0	17 ± 0	29 ± 1	0	31.9 ± 0.7	33.3 ± 1.1	0
Ventral	112 ± 3	109 ± 4	0	9 ± 0	21 ± 1	0	36.4 ± 0.8	37.5 ± 0.4	0
Opheodrys aestivus									
Dorsal	367 ± 7	102 ± 4	272 ± 7	39 ± 2	16 ± 1	86 ± 3	31.5 ± 0.7	40.0 ± 0.7	27.5 ± 0.5
Lateral	99 ± 5	48 ± 2	48 ± 2	3 ± 0	2 ± 0	7 ± 0	37.9 ± 0.6	42.9 ± 1.1	35.7 ± 0.6
Ventral	139 ± 2	51 ± 2	92 ± 2	6 ± 0	3 ± 0	12 ± 0	34.2 ± 0.3	26.7 ± 0.7	37.0 ± 0.6
Anolis carolinensis									
Dorsal	262	265	0	16	19	0	28.1	28.1	0
Ventral	180	183	0	8	10	0	15.8	15.8	0

[a] 1 T.U. (tyrosinase unit) is defined as the amount of tyrosinase activity required to convert 1 picomole of L-tyrosine to melanin under the conditions of the described assay during a 16 h incubation period at 30 °C. [b] Specific activity is defined as the number of T.U./μg protein nitrogen. [c] Expressed in % of total L-tyrosine converted.

substrates utilized have been described[1,2]. The activities of the enzyme preparations were dopa dependent, completely inhibited by sodium diethyldithiocarbamate (6 mM) and stable for at least 2 weeks at 0–4 °C. The methods of protein analysis and statistical evaluation also have been reported[2]. Data are presented in the form $\bar{x} \pm \sigma_{\bar{x}}$.

Results and discussion. The tyrosinase activities (Table II) in reptiles were, in descending order, *Opheodrys*, *Anolis*, *Caiman* and *Trionyx* in the dorsal skin, and *Anolis*, *Opheodrys*, *Caiman* and *Trionyx* in the ventral skin. Tyrosinase activity in reptilian skin was relatively low when compared to the amphibia[3] but was higher than that present in the normal integument of the more advanced amniotes (birds, mammals)[1].

The anatomic distribution of tyrosinase activity demonstrated the presence of higher enzymic activity levels in the dorsal skin than in the ventral skin. The activity in the dorsal skin was 3.3, 2.6, 2.1 and 1.5 times higher than that in the ventral integument of *Trionyx*, *Opheodrys*, *Caiman* and *Anolis*, respectively. Interestingly, the lateral skin of *Opheodrys* contained less tyrosinase activity than either the dorsal or ventral skin. In *Trionyx*, the integument covering the carapace contained 16% of the tyrosinase activity found in the dorsal skin and that covering the plastron contained 22% of the tyrosinase activity found in the ventral skin. The tyrosinase activity of the carapace area was 2.4 times higher than that of the plastron area. These findings suggest that the enzymic activity when expressed per unit mass may not reveal the real activity, for the specific activity data demonstrate the differences between these areas to be considerably smaller (Table II).

Among the reptiles studied, tyrosinase activity occurred only in the soluble fraction of *Opheodrys*. In this species the major portion of the enzymic activity (74, 50 and 66% of the total in the dorsal, lateral and ventral skin, respectively) was in the soluble fraction. As the tyrosinase activity in all other normal adult amniotes[1] studied is associated with the particulate fraction, the snake appears to have left the main evolutionary pathway[1] in regard to the subcellular distribution of tyrosinase activity.

The specific activity of the particulate fraction appeared to be higher than that in the homogenate in all species but the snake. This may have resulted from the removal of the soluble fraction proteins from the homogenate. In *Opheodrys*, a consistent increase in enzymic activity is present after homogenate fractionation, strongly suggesting the presence of a tyrosinase inhibitor(s) as reported previously in other vertebrate classes[1,4,5].

The tyrosine incorporation with the carboxyl group[6] in reptiles ranged from 14.8 to 42.9% of the total L-tyrosine converted. The extent of incorporation of tyrosine carboxyl groups among the Reptilia studied falls in the range of vertebrates generally[1,7].

Zusammenfassung. Bei Reptilien ist die Tyrosinaseaktivität am höchsten in der Rückenhaut. Mit Ausnahme der Schlangenhaut wird sie nach Ultrazentrifugation im Sediment gefunden. Der Einbau der Carboxylgruppe des Tyrosins in Melanin verläuft ähnlich wie bei andern Vertebraten. In der Haut finden sich vermutlich Tyrosinase-Inhibitoren.

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