

Integumental Tyrosinase Activity in Amphibians

During the course of a study of the evolutionary aspects of skin tyrosinase activity<sup>1</sup>, 2 amphibian species (*Rana pipiens* and *Rana catesbiana*) were found to possess the highest tyrosinase activity among the normal vertebrates studied and, in addition, were characterized by an unusual anatomic distribution of the enzyme. In order to characterize the nature of these features in other amphibian species, the present study has extended the original observations. The findings show that some urodeles possess the highest integumental tyrosinase activity levels among vertebrates.

**Methods and materials.** The Amphibia used (Table I) were decapitated, the dorsal and ventral skin areas immediately removed, trimmed and frozen (− 27 °C) for several weeks. At the time of assay, the frozen skin was sliced on dry ice and ground with chilled 0.1 M phosphate buffer (1:40) pH 6.8 as previously described<sup>2</sup>. The assay procedures, substrates, methods of protein analysis, and statistical evaluation have been described<sup>1,2</sup>. Data are presented in the form,  $\bar{x} \pm \sigma_{\bar{x}}$  wherever possible. The activities of the enzyme preparations are DOPA dependent, inhibited by 6 mM sodium diethyldithiocarbamate and are stable for at least 2 weeks at 0–4 °C. Hydrolysis (3 N HCl, 90 °C, 8 h) of the incubates did not significantly reduce the radioactivity, confirming the previously described findings<sup>2</sup> in other vertebrates.

<sup>1</sup> Y. M. CHEN and W. CHAVIN, Adv. Biol. Skin 8, 253 (1967).  
<sup>2</sup> Y. M. CHEN and W. CHAVIN, Analyt. Biochem. 13, 234 (1965).

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

Species	No. of animals used	Sex	Body weight (g), range
(A) Urodela			
<i>Amphiuma means</i> (Congo eel)	2	♂ + ♀	314♂, 645♀
<i>Necturus maculosus</i> (Mudpuppy)	3	1♂ + 2♀	94–149
<i>Ambystoma tigrinum</i> (Tiger salamander)	3	"	31–41
<i>Diemictylus viridescens</i> (Newt)	3 (1)*	"	2.0–2.3
(B) Anura			
<i>Rana pipiens</i> (Leopard frog)	3	♂	45–53
<i>Rana catesbiana</i> (Bull frog)	3	2♂ + 2♀	260–314
<i>Bufo marinus</i> (Giant toad)	3	♂	163–173

\* No. in parenthesis indicates the number of enzyme preparations used. Otherwise, the number of preparations is equal to the number of animals. " Data not available.

Table II. Comparison of in vitro tyrosinase activity\* in amphibian skin

Species	T.U./mg skin				Specific activity			
	H <sup>b</sup>	P	S	P/S	H	P	S	P/S
<i>Amphiuma means</i>								
Dorsal	18,275	4,641	13,474	0.34	1,793	698	3,749	0.19
Ventral	14,300	3,305	10,975	0.30	1,345	501	2,858	0.18
<i>Necturus maculosus</i>								
Dorsal	240 ± 21	167 ± 8	65 ± 3	2.56	22 ± 1	23 ± 2	19 ± 2	1.21
Ventral	126 ± 6	88 ± 3	32 ± 1	2.75	12 ± 0	13 ± 1	9 ± 0	1.44
<i>Ambystoma tigrinum</i>								
Dorsal	2,115 ± 76	1,680 ± 10	439 ± 8	3.82	84 ± 4	105 ± 5	46 ± 3	2.28
Ventral	16,646 ± 231	9,639 ± 10	6,956 ± 31	1.38	626 ± 14	526 ± 6	842 ± 9	0.62
<i>Diemictylus viridescens</i>								
Dorsal	16,238	15,062	2,720	5.53	780	1,584	249	6.36
Ventral	9,085	5,892	3,213	1.83	464	585	345	1.70
<i>Rana pipiens</i>								
Dorsal	1,112 ± 60	866 ± 27	250 ± 3	3.46	47 ± 2	61 ± 2	25 ± 1	2.44
Ventral	1,510 ± 13	1,097 ± 67	492 ± 5	2.22	52 ± 2	47 ± 1	66 ± 2	0.71
<i>Rana catesbiana</i>								
Dorsal	847 ± 49	396 ± 8	471 ± 5	0.84	108 ± 8	84 ± 3	155 ± 3	0.54
Ventral	1,114 ± 20	513 ± 6	592 ± 5	0.86	137 ± 3	94 ± 2	219 ± 4	0.43
<i>Bufo marinus</i>								
Dorsal	1,400 ± 36	357 ± 22	1,061 ± 12	0.33	226 ± 13	112 ± 10	348 ± 18	0.32
Ventral	1,632 ± 17	595 ± 18	1,054 ± 26	0.56	229 ± 6	146 ± 6	351 ± 5	0.42

\* 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. Specific activity is defined as the number of T.U./μg protein nitrogen in the enzyme preparation. <sup>b</sup> H, homogenate; P, particulate fraction; S, soluble fraction.

Table III. In vitro tyrosine carboxyl group incorporation into melanin by amphibian skin tyrosinase

Species	Skin region	Tyrosine carboxyl group incorporation (%) <sup>a</sup>		
		H <sup>b</sup>	P	S
<i>Amphiuma means</i>	Dorsal	22.6	25.5	21.9
	Ventral	19.3	24.1	17.0
<i>Necturus maculosus</i>	Dorsal	14.9 ± 0.5	15.3 ± 0.4	15.8 ± 0.3
	Ventral	13.5 ± 0.3	13.5 ± 0.1	15.8 ± 0.3
<i>Ambystoma tigrinum</i>	Dorsal	10.0 ± 0.3	9.7 ± 0.3	8.5 ± 0.5
	Ventral	3.6 ± 0.1	3.6 ± 0.1	3.7 ± 0.2
<i>Diemictylus viridescens</i>	Dorsal	11.4	11.3	10.3
	Ventral	15.8	15.8	15.8
<i>Rana pipiens</i>	Dorsal	37.3 ± 1.0	38.4 ± 0.6	37.4 ± 1.9
	Ventral	38.2 ± 1.1	39.0 ± 0.5	36.9 ± 0.8
<i>Rana catesbiana</i>	Dorsal	21.9 ± 1.0	18.5 ± 0.6	21.3 ± 0.9
	Ventral	23.5 ± 0.6	24.2 ± 0.4	21.8 ± 0.5
<i>Bufo marinus</i>	Dorsal	38.9 ± 1.7	31.6 ± 1.1	41.0 ± 0.9
	Ventral	34.0 ± 1.1	31.8 ± 1.1	35.3 ± 1.0

<sup>a</sup> Expressed in % of total L-tyrosine converted. <sup>b</sup> See footnote <sup>b</sup>, Table II.

**Results and discussion.** Six amphibian species showed very high integumental tyrosinase activities when compared with previously studied vertebrates<sup>1</sup>. As sex differences in tyrosinase activity were not discernible, all data for a species were consolidated. The enzymatic activity in both the dorsal and ventral skin of *Amphiuma* and *Diemictylus* and that in the ventral skin of *Ambystoma* (Table II) was greater than that occurring in any other normal vertebrate integument studied to date and approached or exceeded the enzymatic activity levels in the vertebrate melanomas previously studied<sup>1</sup>. The very high specific activities in these amphibians indicate the relatively high concentration of tyrosinase in the protein pool of the skin. In addition, it is also possible that a highly effective tyrosinase activator(s) or that a less effective tyrosinase inhibitor(s) is present.

The integumental tyrosinase activity levels among the amphibians were, in descending order, *Amphiuma*, *Diemictylus*, *Ambystoma*, *Bufo*, *R. pipiens*, *R. catesbiana* and *Necturus*. In the dorsal skin, the highest activity present in *Amphiuma* was 76-fold higher than that present in the lowest, *Necturus*. In the ventral skin, the highest activity present, in *Ambystoma*, was 132-fold higher than that present in the lowest, *Necturus*. While the tyrosinase activity in the dorsal skin was 1.90-, 1.79- and 1.28-fold greater than that in the corresponding ventral skin of *Necturus*, *Diemictylus* and *Amphiuma*, respectively. The tyrosinase activity in the ventral skin was 7.87-, 1.36-, 1.32- and 1.17-fold greater than that in the corresponding dorsal skin in *Ambystoma*, *R. pipiens*, *R. catesbiana* and *Bufo*, respectively. In *R. pipiens* and *R. catesbiana* the unusual anatomic distribution of tyrosinase activity and the possible mechanism involved have been discussed previously<sup>3</sup>. The findings in regard to *Bufo* and *Ambystoma* may be explained similarly.

The subcellular distribution study of tyrosinase revealed that the enzyme was present in both the soluble and particulate fractions in all species (Table II). However, the enzymatic activity was greater in the particulate fraction of *Diemictylus*, *Ambystoma*, *R. pipiens* and *Necturus* skin. The highest ratio of particulate activity to soluble activity occurred in the dorsal skin of *Diemictylus* and the lowest in the ventral skin of *Amphiuma*.

The incorporation of tyrosine without decarboxylation in % of total L-tyrosine converted, ranged from 3.6–41.0%

(Table III). The highest occurred in *Bufo* and *R. pipiens* and the lowest in *Ambystoma*. However, *Diemictylus* and *Necturus* also showed a comparatively low carboxyl incorporation. The incorporation of tyrosine carboxyl groups into melanin supports the attraction hypothesis in melanogenesis as proposed previously<sup>2,4</sup>.

**Summary.** The anatomic and subcellular distribution of integumental tyrosinase activity and the tyrosine carboxyl group incorporation into melanin in 7 species of amphibia were investigated. *Necturus maculosus*, *Diemictylus viridescens* and *Amphiuma means* showed higher tyrosinase activity in the dorsal skin compared to the ventral skin. However, *Ambystoma tigrinum*, *R. pipiens*, *R. catesbiana* and *Bufo marinus* showed the unusual feature of a higher enzymatic activity in the light ventral skin when compared to the dark dorsal. The integumental tyrosinase activity was present in both subcellular fractions, soluble and particulate, in all species. The particulate fraction of *Diemictylus*, *Ambystoma*, *R. pipiens* and *Necturus* was enzymatically more active. In the other species studied, the soluble fraction contained the greater enzymatic activity. The highest normal vertebrate integumental tyrosinase activity and specific activity reported to date, occur in the Amphibia. Tyrosine carboxyl incorporation into melanin ranged from 3.6–41.0%. Amphibia appear to be unusually interesting experimental animals in pigmentation studies<sup>5</sup>.

**Résumé.** L'analyse radiométrique de tyrosinase intégrumentale a démontré que les amphibiens sont les vertébrés les plus intéressants dans les études de mélanogénèse.

Y. M. CHEN and W. CHAVIN

Department of Biology<sup>6</sup>, Wayne State University, Detroit (Michigan 48202, USA), 7 August 1967.

<sup>3</sup> Y. M. CHEN and W. CHAVIN, in manuscript (1967).

<sup>4</sup> Y. M. CHEN and W. CHAVIN, Nature 210, 35 (1966).

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<sup>6</sup> Contribution 179, Department of Biology.