which is suggestive of a N,N-dimethylamino moiety, led to the postulation of skeleton I as a working hypothesis. This structure combines the observed data with the attractiveness of a structure closely related to the common analgesic, propoxphene hydrochloride (Darvon, II). A sample of propoxphene was then obtained and its mass-spectrum and retention time (coinjection) proved indeed to be identical to those of component F.

The suggested molecular composition of $C_{19}H_{23}N$ as well as the mass-spectrum of component E suggest structure III, the product of either thermal, electron impact or metabolic dehydration of the carbinol resulting from hydrolysis of the ester function in propoxyphene. The by far most abundant component of the extract is fraction G. Its mass-spectrum exhibited an ion at *m/e* 307 ($C_{21}H_{25}NO$) which apparently loses $C_4H_9NO$ to form an ion at *m/e* 220. This indicates loss of both heteroatoms (N and O) along with 4 carbon atoms and implies structural proximity of the nitrogen and oxygen atoms in G compared to II. Furthermore, the most intense peak appears in this spectrum at *m/e* 44 rather than *m/e* 58 which requires the absence of one of the N-methyl groups. The most likely structure would be IV, the result of an intramolecular acyl migration in N-desmethyl propoxyphene (VI) followed by dehydration of the intermediate amide (V). Compound VI has indeed been found to be the major metabolite of propoxyphene hydrochloride in humans[10], and its facile conversion to (V) is also known[11,12].

The amount of time required for the identification of the drug causing the patients condition would have been considerably reduced if a more extensive library of mass-spectra of commonly encountered drugs and their metabolites had been available. The compilation of such a library is currently in preparation[13,14].

*Zusammenfassung.* Mit Hilfe von hoch- und niederauflösender Massenspektroskopie sowie Daten eines Gaschromatograph-Massenspektrometer-Computer-Systems wurden die Droge Darvon[3] sowie eine Reihe ihrer Metaboliten im Harn eines Überdosispatienten nachgewiesen.

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[14] This investigation was supported by National Institute of Health Research Grants Nos. RR00317 (from the Division of Research Resources) and GM09352. Some of the authors are supported by N.I.H. Training Grant No. GM01523. Part of the equipment used was purchased with funds from a N.A.S.A. Research Grant (No. NAS-22-009-102).
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## Distribution of Integumental Tyrosinase Activity in Red-Eared Turtle, *Pseudemys scripta elegans*

The integumental tyrosinase activity in 4 species of reptiles, *Trionyx ferox* (Florida softshell turtle), *Caiman sclerops* (spectacled caiman), *Opheodrys aestivus* (vine snake) and *Anolis carolinensis* (American chameleon), has been described[1,2]. In these species the tyrosinase activity of the dorsal skin is higher than that of the ventral skin. In 3 species[3] the subcellular distribution of tyrosinase activity follows the amniote pattern, i.e., tyrosinase activity is confined to the particulate fraction. However, in *Opheodrys aestivus* the tyrosinase activity occurs in both particulate and soluble fractions as in most anamniotes[3]. As previous studies have dealt with general integumental areas, a detailed investigation of the distribution of tyrosinase activity in a number of skin areas from another reptile, *Pseudemys scripta elegans* (red-eared turtle), was undertaken in order to more precisely evaluate the enzymic activity in anatomically diverse regions.

*Materials and methods.* 5 non-melanistic adult red-eared turtles, 3 males weighing 720, 851 and 964 g and 2 females weighing 785 and 907 g, were utilized. The animals were sexually quiescent. The animals were decapitated and the different skin areas (Table) were removed and frozen (−27 °C). The enzyme preparation, radiometric assay procedures and substrates utilized have been reported previously[4,5]. The activities of the enzyme preparations were DOPA dependent, completely inhibited by sodium diethyldithiocarbamate (6 m*M*) and stable for at least 2 weeks at 0–4 °C. The methods of protein analysis and statistical evaluation also have been presented previously[4]. Data are expressed in the form $\bar{X} \pm \sigma_{\bar{x}}$ except those of the red patches (head) as the patches of all animals were combined prior to assay. Approximately 2000 assays were performed.

*Results and discussion.* As sex differences in tyrosinase activity were not discernible, the data for each skin area were consolidated. The tyrosinase activity varied considerably in the different integumental areas studied (Table). High enzymic activities occurred in the head, tail,

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Integumental tyrosinase activity ($\bar{X} \pm \sigma_{\bar{X}}$) in red-eared turtle, *Pseudemys scripta elegans*

| Integumental area | TU[a]/mg skin | | | Specific activity[b]/mg skin | | | Tyrosine carboxyl incorporation[c]/mg skin | | |
|---|---|---|---|---|---|---|---|---|---|
| | H[d] | P | S | H | P | S | H | P | S |
| Head, dorsal | 454 ± 7 | 347 ± 18 | 154 ± 9 | 51.1 ± 1.5 | 56.7 ± 2.2 | 61.1 ± 1.3 | 37.1 ± 2.5 | 36.1 ± 1.6 | 24.6 ± 1.0 |
| Head, ventral | 385 ± 5 | 243 ± 8 | 144 ± 4 | 64.1 ± 1.1 | 48.9 ± 1.0 | 87.3 ± 1.9 | 29.5 ± 1.7 | 30.1 ± 1.1 | 29.1 ± 2.0 |
| Head, red patch | 436 | 327 | 110 | 47.5 | 51.7 | 29.1 | 24.0 | 25.6 | 21.4 |
| Neck, entire skin | 246 ± 4 | 136 ± 7 | 114 ± 4 | 55.2 ± 1.3 | 55.7 ± 0.7 | 65.9 ± 1.0 | 32.2 ± 1.4 | 31.7 ± 2.1 | 31.0 ± 1.4 |
| Carapace | 239 ± 7 | 210 ± 3 | 22 ± 4 | 25.2 ± 4.8 | 32.1 ± 1.3 | 7.2 ± 0.8 | 38.8 ± 3.0 | 43.3 ± 2.7 | 23.5 ± 1.2 |
| Plastron | 196 ± 4 | 191 ± 7 | 3 ± 1 | 18.5 ± 1.6 | 29.8 ± 1.7 | 1.0 ± 0 | 50.0 ± 1.8 | 49.3 ± 1.3 | 50.0 ± 2.1 |
| Forelegs: | | | | | | | | | |
| anterior (dorsal) | 434 ± 9 | 343 ± 8 | 89 ± 10 | 92.3 ± 1.6 | 123.8 ± 3.3 | 39.9 ± 3.8 | 28.9 ± 1.1 | 30.8 ± 1.9 | 25.0 ± 1.1 |
| posterior (ventral) | 415 ± 7 | 313 ± 5 | 98 ± 4 | 82.0 ± 2.0 | 104.0 ± 3.3 | 44.0 ± 2.2 | 30.2 ± 1.4 | 32.5 ± 0.8 | 18.7 ± 0.7 |
| Hind legs: | | | | | | | | | |
| anterior (dorsal) | 283 ± 4 | 175 ± 7 | 107 ± 5 | 54.7 ± 1.0 | 42.3 ± 1.6 | 123.0 ± 1.9 | 23.0 ± 1.1 | 20.9 ± 1.7 | 23.2 ± 0.8 |
| posterior (ventral) | 298 ± 7 | 159 ± 5 | 136 ± 4 | 47.5 ± 2.1 | 30.5 ± 1.6 | 523.1 ± 6.5 | 21.9 ± 1.3 | 23.0 ± 0.4 | 16.4 ± 1.1 |
| Tail, entire skin | 421 ± 5 | 168 ± 4 | 253 ± 5 | 103.4 ± 6.0 | 66.7 ± 2.2 | 186.0 ± 2.9 | 36.3 ± 1.2 | 36.4 ± 1.9 | 36.1 ± 1.0 |

[a] TU (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 TU/μg protein nitrogen. [c] Expressed in % of total L-tyrosine converted. [d] H, skin homogenate; P, particulate fraction; S, soluble fraction (fractionation of skin homogenate at 0–4 °C, 144,000 × *g*, 40 min).

forelegs and red patches (head). It is interesting that the laterally located red patch skin showed a tyrosinase activity level similar to that of the dorsal head skin. The enzymic activity of the carapace or plastron integument was low, approximately half of that in the head. The tyrosinase activities of the dorsal skin areas were higher than those of the ventral areas except for the hind legs. However, the subcellular distribution of tyrosinase activity differed among the anatomical areas of the skin. Although both the carapace and plastron integuments contained very small amounts of tyrosinase activity in the soluble fraction (9.2% and 1.5%, respectively), the other skin areas ranged from approximately 20% to 60% of the total enzymic activity in the soluble fraction: forelegs, 20.5% (d), 23.6% (v); hind legs, 37.8% (d), 45.6% (v); head, 33.9% (d), 37.4% (v); red patch, 25.2%; neck, 46.3%; tail, 60.1%.

The specific activities of tyrosinase observed in the various integumental areas of the red-eared turtle were high when compared to those of other reptiles or amniotes[3], indicating a higher tyrosinase content in these enzyme preparations. In the ventral skin of the hind legs and in the tail, the specific activities of the soluble fractions were 523.1 and 186.0, respectively. Thus, among the reptiles or the amniotes studied to date, these integumental areas may provide a comparatively rich source of the enzyme for isolation and purification studies. In plastron integument, tyrosine carboxyl group incorporation approached 50%. This is the highest incorporation yet found in normal vertebrate skin or in melanomas of any vertebrate studied[2,3]. A comparatively low carboxyl group incorporation occurred in the hind legs (d, v) and red patch integuments.

Comparisons of the tyrosinase activities of the red-eared turtle carapace and plastron skin to the corresponding parts of the softshell turtle (*Trionyx ferox*) reveal higher enzymic activities in the former species despite the high degree of keratinization. Further, small amounts of tyrosinase were present in the soluble fraction of these integumental areas in the red-eared turtle but no such activity was observed in the corresponding areas of the softshell turtle. The enzymic activities in the carapace and plastron integuments of the red-eared turtle were similar to those occurring in the homologous anatomical areas of the skin in the American chameleon (*Anolis carolinensis*).

*Résumé.* L'activité enzymatique de la peau d'un Chélonien a montré que la distribution de la tyrosinase n'es pas uniforme. Ce sont des facteurs anatomiques infracellulaires et biochimiques qui controlent l'activité de la tyrosinase.

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