

only minor abnormalities in spindles from pathological materials. Apparently, they are 'disease-resistant', become involved late in disease so that they were not yet present in our material, or in fact never develop. Similar observations have been made in man and other animals¹². For example, observations on experimental division of the sciatic nerve in dogs cats, monkeys lead to the conclusion that (1) intrafusal muscle fibers may survive independent of a nerve supply from both afferent and efferent fibers for at least 6 months and that (2) atrophic changes in extrafusal fibers are greater than in muscle spindles, although in a number of human muscular and neuromuscular diseases including denervation atrophies, progressive muscular dystrophy, polymyositis, etc., the degree and extent of spindle lesions is related to the stage of the disease¹². Since the spindle is unaffected by nerve section, the muscle spindle is able to guide the regenerating nerve back to it¹³⁻¹⁵.

Zusammenfassung. Der Aussendiameter der Muskelspindeln variiert in Mäusen von Muskel zu Muskel. Der Grössenunterschied ist altersunabhängig. In Mäusen mit

vererblichen neuromuskulären Erkrankungen wurden nur geringe Abnormitäten der Spindeln gefunden. Anscheinend sind sie erkrankungsresistent oder entwickeln sich erst im späteren Erkrankungsprozess.

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¹² G. CAZZATO and J. N. WALTON, *J. Neurol. Sci.* 7, 15 (1968).

¹³ T. R. SHANTA, M. N. GOLAZ and G. H. BORUNE, *Acta Anat.* 69, 632 (1968).

¹⁴ This work was supported in part by Public Health Service research grant No. NB 06448 from the National Institute of Neurological Diseases and Blindness, a grant from the Muscular Dystrophy Associations of America, Inc., and a grant from the United Medical Research Foundation of North Carolina.

¹⁵ The principles of laboratory animal care as promulgated by the National Society for Medical Research are observed in this Laboratory.

Quinone-Tanning in the Reptilia and Aves

The presence of protein tanned by an orthoquinone has been established in the cuticle of arthropods¹, cysts of nematodes², shells of helminths³, chetae of earthworm⁴, byssus of the bivalve mollusc, *Mytilus edulis*⁵ and central capsule of radiolarian, *Thalassicola* (Protozoa)⁶. Among the Chordata, the hardening of egg cases of the selachian, *Chiloscyllium griseum*⁷ seems to involve a process very like sclerotization. During the course of histochemical studies on the eyes of the leaf-toed gecko, *Hemidactylus turicus turicus* (Linnaeus) and the white-bellied petrel, *Fregetta grallaria*, it was found that the conical papilla of the reptile and pecten of the bird are phenolically tanned.

Material and methods. Required amount of conical papilla and pecten were dissected and fixed in 5% neutral formalin. Routine paraffin sections were made at 7 μ thick and stained with Mallory's triple stain. Histochemical tests employed include Millon's, potassium iodide, potassium bichromate, argentaffin, Nadi, Sudan Black-B and Liebermann-Burchardt tests. Unidimensional ascending chromatograph on Whatman No. 1 paper of acid hydrolysis of the materials was also used.

Results. The conical papilla and pecten are deep amber in colour. The papilla appears as a small cone measuring about 1-2 mm in length, while the pecten is larger than the cone formed of a series of parallel ridges. Transverse sections through the cone stained with Mallory's triple stain show that the cone is a solid structure with a circle contour. The outer portion of the core is amber-coloured and refractile to stains, while the inner central portion shows affinity to the red of acid fuchsin. The same picture is afforded by the ridges of the pecten.

Maceration of the frozen-sections of the cone and ridge of pecten with mineral acids show that the outer amber region is more resistant than the inner fuchsinophil zone. The Millon's, xanthoproteic, potassium iodide and potassium bichromate tests give positive results in the outer and inner regions indicating the existence of tyrosine, tryptophane and other phenolic compounds. Evidence

that the amber-coloured region is tanned is given by the fact that, even after boiling, it induces a rapid oxidation of the Nadi reagent which has been used to indicate orthoquinones. The argentaffin reaction for polyphenols and polyamines is most marked in the outermost region of the amber zone. However, Sudan Black-B and Liebermann-Burchardt tests give negative reactions indicating the absence of simple as well as steroid lipids. Besides, upon detanning the cone and pecten by treating in diaphanol the amber regions become soft and white in colour. Histological inspection of such diaphanol-treated materials show that the region corresponding to amber and fuchsinophil regions are coloured red and blue respectively when stained with Mallory's stain. In view of these observations it may be assumed that the cone and ridge of the pecten are quinone tanned. This assumption is further supported by the chromatographic analysis for amino acids of them indicating the presence of aromatic amino acids like tyrosine and phenylalanine.

Discussion. The foregoing observations denote that the papillary cone and pecten are hardened by phenolic tanning, a process comparable to that found in the cuticle of insects and other arthropods. The outer mechanically resistant amber region is homologous to the amber-exocuticle, while the inner fuchsinophil portion to the mesocuticle of other insects. However, certain points of difference are significant: (a) unlike in the insect cuticle the substrate involved in tanning seems to be a protein rich in phenolic groups without a lipid component;

¹ A. G. RICHARDS, *The Integument of Arthropods* (University of Minnesota Press, Minneapolis (1951)).

² C. ELLENBY, *Nature* 157, 302 (1946).

³ W. STEPHENSON, *Parasitology* 38, 128 (1947).

⁴ R. DENNELL, *Nature* 164, 370 (1949).

⁵ C. H. BROWN, *Q. Jl. microsc. Sci.* 93, 487 (1952).

⁶ C. H. BROWN, *Nature* 165, 275 (1950).

⁷ G. KRISHNAN, *Biol. Bull. mar. biol. Lab., Woods Hole* 177, 298 (1959).

(b) chitin is absent; and (c) there is no differentiation into epi- and procuticle. In these respects, they recall ootheca of the cockroach, *Blatella orientalis*⁸ and spore walls of the fungus, *Aspergillus* sp.⁹.

Reference to previous literature shows that the papillary cone of reptiles and pecten of birds are homologous organs. Though the role of the former is not known, the latter is reported to increase the efficiency of visual powers of birds. It has been suggested that the shadows of the ridges of pecten, falling on the retina, act as a grille, and that small or distant moving objects are more readily discerned as their images pass from one component to another on this grille¹⁰.

The presence of sclerotin in the vertebrates is very unusual. Among the vertebrates, as reported earlier, the egg case of selachians (Pisces) is sclerotized⁷. The inner lining of the gizzard of birds (Aves) is said to be partially tanned¹¹. Among the Prochordata, walls of the stolon of the Pterobranch, *Rhabdopleura* sp. has been found to be sclerotized¹². Since the primary mode of hardening of the integument and its derivatives of the vertebrates is by keratinization and calcification¹³, the significance of the occurrence of sclerotin in the papillary cone and pecten seems to be an enigma. A point of interest to be mentioned in this connection is that the rhabdomeres of

the compound eyes of the housefly, *Musca vicina* Macquart also are derivatives of polyphenols^{14,15}.

Résumé. Pour la première fois on rapporte la présence de sclérotine dans la papille conicale du gecko, *Hemidactylus turicus turicus* (Reptilia) et dans la pectine du pétreil, *Fregetta grallaria* (Aves).

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⁸ M. G. M. PRYOR, Proc. R. Soc. London B 128, 378 (1940).

⁹ A. SANNASI, Mycopath. Mycol. Appl., in press (1969).

¹⁰ A. S. ROMER, *The Vertebrate Body*, 2nd Edn (Saunders Co., London 1955), p. 504.

¹¹ G. SUNDARA RAJULU, personal communication.

¹² A. SANNASI, unpublished observations.

¹³ L. PICKEN, *The Organisation of Cells and Other Organisms* (Oxford University Press, 1960).

¹⁴ K. SUZUKI, Nature 195, 994 (1962).

¹⁵ Acknowledgments. I am thankful to Dr. M. S. BLUM for his continued interest in my work.

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Dopa-Decarboxylase and Monoamine Oxidase Activities in a Transplantable Islet Cell Tumour of the Golden Hamster

A storage of biogenic monoamines in the endocrine pancreas has been demonstrated in many mammalian species by means of the histotechnical fluorescence technique of FALCK and HILLARP in combination with chemical analyses. The adult golden hamster is remarkable, however, because its islets contain a well-developed plexus of adrenergic nerve terminals, whereas the endocrine cells do not store any histochemically demonstrable monoamines. On the other hand, a transplantable, insulin-producing islet cell tumour of the golden hamster, originally described by KIRKMAN¹, has recently been found to contain dopa, dopamine, and 5-hydroxytryptamine, and also an unidentified, possibly monoamine-like, substance² which condensed with formaldehyde in tissues and on chromatograms to an intensely fluorescent derivative. The presence of dopamine and 5-hydroxytryptamine in the tumour is of particular interest since these are the 2 amines commonly found in mammalian

islets³. These findings have prompted an analysis of the tumour cell enzymes involved in the synthesis and breakdown of monoamines. The present study reports the dopa-decarboxylase and monoamine oxidase (MAO) activities in the tumour.

The tumour was transplanted subcutaneously⁴ to 16 golden hamsters and was allowed to grow for 4–10 weeks. 5 tumours were used for determination of dopa-decarboxylase and monoamine-oxidase activities, and the contents of dopa and dopamine were estimated fluorimetrically⁵ in 7 tumours. 4 animals were pretreated with the dopa-decarboxylase inhibitor NSD 1015 (m-hydroxybenzyl hydrazine; Smith and Nephew through Ferrosan AB, Sweden) 100 mg/kg, 1½ h before killing, and the dopa-decarboxylase activity and the contents of dopa and dopamine in the tumours were then determined⁶. The identity of dopa was further established with the use of paper chromatography (phenol: 0.1 NHCl; 85:15)⁶. The dopa-decarboxylase activity was estimated by means of the formation of dopamine from L-dopa⁶, and the monoamine oxidase activity by means of the formation of indoleacetic acid (IAA)⁷. Small tissue specimens from

	Tumours of non-treated animals M ± S.E.M. (n)			Tumours of NSD 1015 treated animals M ± S.E.M. (n)			
Dopa-decarboxylase activity µmol formed per g tissue/h	70	± 3.7	(5)	16	± 0.9	(4)	p < 0.001
MAO activity µmol IAA formed per g tissue/h	0.74 ± 0.05 (5)						
Dopa µg/g	0.20 ± 0.06 (7)			3.8 ± 0.21 (4)			p < 0.001
Dopamine µg/g	3.0 ± 0.27 (7)			2.2 ± 0.14 (4)			p > 0.05

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² L. CEGRELL, B. FALCK and A. M. ROSENGREN, Acta physiol. scand., in press (1969).

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⁴ T. A. I. GRILLO, A. J. WHITTY, H. KIRKMAN, P. P. FOA and S. D. KOBERNICK, Diabetes 16, 409 (1967).

⁵ A. ANTON and D. SAYRE, J. Pharm. exp. Ther. 145, 326 (1964).

⁶ Å. BERTLER and E. ROSENGREN, Acta physiol. scand. 47, 350 (1959).

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