Tanning in the Spermatophore of a Crustacean (*Penaeus trisulcatus*)

It now seems established that tanned structures are of widespread occurrence among the invertebrates[1,2]. A hitherto unsuspected structure, the spermatophore of a penaeid prawn, is here reported to owe its chemical stability to an enzymically-catalyzed tanning process, but one in which no free dihydroxyphenols are involved.

Sections through the proximal region of the vas deferens of *Penaeus trisulcatus* reveal a highly viscous fluid to mix, at first, with the sperms incoming from the testis. The sperm mass thus produced next acquires two covering layers (I and II) in the succeeding main region. They give rise to a cylindrical main body in but one duct, when in an accompanying wing duct of this region 3 other secretions (III, IV and V) simultaneously contribute to the formation of an accessory solid wing. No sooner do these ducts become confluent with one another than secretion V from the wing establishes a preliminary, but weak, connexion with the body after it has set down around its outer surface.

Important changes take place in the terminal ampoule. The tubular body is moulded into a globular form and the wing spread out into an extensive flattened sheet. A further yield of secretion III furnishes another, but more firm, connexion between these 2 parts. And lastly, protective layers, VI around the body and VII on both surfaces of the wing, are added.

Until this stage of formation the entire spermatophore is to be regarded as soft. Its conversion into a hard structure, initiated in the ampoule itself at the ontogenetically correct time, is believed to continue well after it has been transferred to the female. In agreement with observations on other tanned structures[3], only those layers (II, IV and V) that proved to consist of lipoprotein are involved in this change. It is interesting that the lipid, shown by the Liebermann-Burchardt test to be of steroid type, loses its ability to stain shortly before tanning is initiated, presumably as a result of polymerization. The protein moiety, on the other hand, is characteristically rich in phenolic groups owing to the presence of tyrosine. No free phenols could, however, be detected either histochemically or after prolonged extraction with suitable solvents, but a phenolase, readily demonstrable by the 'Nadi' reagent, does exist. If sections of soft spermatophores, before the onset of the tanning process, are incubated with catechol under suitable conditions pronounced darkening takes place, indicating that the existing phenolase is capable of promoting active oxidation of phenolic substrates. Since this effect is not produced in previously boiled sections or after treatment with cyanide, it is concluded that this enzyme complex, restricted as it is to the layers destined to be tanned, eventually oxidizes the tyrosyl residues of the naturally occurring substrate in situ, in the manner originally suggested by BROWN[4] and later shown to be chemically possible by HACKMAN[5].

Although the tanned layers fail to darken appreciably in colour, they strongly reduce ammoniacal silver hydroxide, undergo pronounced changes in affinity for stains and in isoelectric point, and develop a greater resistance to acids. All of these criteria, associated with tanning[1], clearly indicate that the crustacean spermatophore may owe its hardness, not to the mere exposure to sea water as has hitherto been thought[6-9], but to a definite enzymically-catalyzed chemical transformation. It is suggested, in conclusion, that this hardness, likewise existing in forms other than *Penaeus*, is the cause of the difficulty reported by earlier workers in sectioning spermatophores[9,10]. A full account of these and other aspects of the present work will be published elsewhere.

Résumé. Le spermatophore chez *Penaeus trisulcatus* (Crustacés Décapodes) a une structure très compliquée. Sa dureté est dûe à un tannage phénolique qui ressemble à ce qui a lieu en général dans les cuticules d'arthropodes, bien qu'il n'implique pas de polyphénols libres.

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1 A. G. RICHARDS, *The Integument of Arthropods* (University of Minnesota Press, Minneapolis 1951).
2 V. B. WIGGLESWORTH, A. Rev. Entomol. *2*, 37 (1957).
3 S. R. A. MALEK, Nature, Lond. *170*, 850 (1952).
4 C. H. BROWN, Nature, Lond. *165*, 275 (1950).
5 R. H. HACKMAN, Biochem. J. *54*, 371 (1953).
6 V. DAHLGREN and W. A. KEPNER, *Principles of Animal Histology* (Macmillan, New York 1908).
7 B. M. ALLEN, Calif. Univ. Publs., Zool. *16*, 139 (1916).
8 H. HELDT, C. r. hebd. Séanc. Acad. Sci., Paris *194*, 2162 (1932).
9 D. C. MATTHEWS, Pacific Sci. *5*, 359 (1951).
10 J. E. KING, Biol. Bull. *94*, 244 (1948).

Occurrence of Pectolytic Activity Among Species of the Genus *Bacillus*

The degradation of pectin by micro-organisms has engaged scientists largely because of its relation to phytopathogenesis[1-3], its role in softening stored fruits[4] or fermented olives[5] and because of its significance in retting plant fibres[6-8]. Biochemically, the retting of plant fibres from flax, hemp and jute stems is nothing more than the maceration of pectinous cell material to obtain clean fibres.

Pectolytic enzymes are found among fungi[1,9,10], yeasts[5], actinomycetes[11,12] and various types of bacteria[1,11,13]. Nevertheless, in the case of retting, a specific pectolytic flora, for the greater part consisting of aerobic and anaerobic spore-forming bacteria, has been claimed responsible[6]. Although pectolytic activity has been observed with some species of the genus *Bacillus*[14-16], neither the distribution of this property among the common soil-inhabiting *Bacillus* species, nor its use as a taxonomic aid in classifying unknown spore-forming bacilli has been studied so far.

Method. Using the gel liquefaction technique, a total of 99 different strains belonging to 18 different recognized *Bacillus* species, was examined for ability to degrade pectin. The following basal medium was found suitable. Peptone (Merck), 5.0 g meat extract (Merck), 3.0 g; yeast extract (Difco), 0.5 g; glucose, 0.5 g; NaCl, 0.1 g and $CaCl_2 \cdot 2H_2O$, 3.0 g; distilled water, 1000 ml; agar (Oxoid No. 1), 10.0 g/l. Before autoclaving, the pH of the basal medium was adjusted to 6.0, 7.0 and 8.0,