THE PIGMENTATION OF MOLLUSCAN SHELLS

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I. INTRODUCTION

The coloured substances which occur in molluscs offer a remarkably wide and largely unworked field to the biochemist. Since pigments are among the most conspicuous and easily identified products of metabolism, pigment chemistry is almost always a remunerative study for the general biologist seeking information about metabolic patterns within a phylum. The colours of molluscs offer incentives and difficulties in almost equal numbers. The physiology of the group is of less practical importance than that of insects, and is proportionately little known. In a very large and diverse phylum, many of the most highly coloured groups, such as nudibranchs, are minute, perishable, and hard to obtain; few if any of the species described in such detail by field workers ever reach the laboratory in the right state or in sufficient quantity for chemical study. Molluscan shells, on the other hand, contain a wide range of pigments, and are virtually indestructible.

The phylogeny of molluscan species covers more living examples of development across the sea-land barrier than that of any other group: the shell itself, unlike most other secretions, is continuously deposited throughout life, and the intermittent deposition of pigment which produces its colour pattern is therefore a biochemical

diary of the individual mollusc from cradle to grave. This combination of circumstances should make it possible, given knowledge of the substances present, to follow two-dimensionally, in ontogeny and phylogeny, the whole process of chemical and metabolic evolution which the group has undergone, and in particular the changes in nitrogen metabolism associated with progress from free-swimming larva to cleidoic egg.

To do this we need a broad picture of the relationship of shell and body pigment to classification and growth. Nothing so coherent is at present obtainable, and if it is to be obtained a closer co-operation between zoology and chemistry will be necessary, both in obtaining material and in interpreting it. A large number of unidentified pigments have been described by chemists, some from unnamed or inadequately identified material: but in certain fields, particularly among the simpler shell pigments, broader outlines are already traceable. The purpose of this article is to summarize the information on the chemistry of molluscan shell pigments which is available to-day.

II. BIOLOGY OF SHELL PIGMENTS

The *patterning* of shells is a graphical representation, in time, of secretory activity along a line of cells, the mantle edge. The ground-colour is produced by the whole line; banding by the special activity of groups of cells, often sharply localized. Where the activity of these groups is cyclical, blotching results: where the active focus moves up and down the mantle edge, or where activity spreads from a focus, zigzag or hollow V-shaped patterns result. By 'unrolling' the pattern of spiral shells the whole process can be followed throughout life. The intensity of the pigmentation varies with the growth rate, periods of diapause giving rise very often to darker varices, and periods of rapid growth to paler zones. Groups of secreting cells also appear to possess rhythms of their own, independent of the growth rate, analogous to the inherent rhythms of feather melanoblasts. The simplest molluscan patterns are self-coloured or consist of axial continuous bands, corresponding to specialized pigment-secreting zones in the mantle. Irregular blotching with colour is already well-developed in the Archaeogastropoda.

The division between shell and body pigment is, of course, artificial. The site of formation of the coloured materials which occur in shells is not known. Melanins are probably formed in the secretory cells of the mantle edge, but other pigments such as pyrroles and porphyrins may well originate elsewhere. In most of the primitive molluscs, especially the Archaeogastropoda and the lower bivalves, shell pigments are almost certainly secreted in the shell as a means of disposal, being either derived from the diet or from unmanageable metabolic residues.

Significant colour is probably less common in these groups than was formerly believed. It is certainly present in many nudibranchs and cephalopods, in association with elaborate mechanisms of colour change and colour adaptation, and the melanin patterns at the siphonal end of bivalve shells are probably also significant. Since pigmentation which subserves a protective function usually involves specialization

in chemistry, the *ad hoc* pigments of many shells are highly complex, and for practical reasons most initial work has been directed to groups where the colour consists of metabolic products of small molecular size, which can be identified with reasonable certainty. There is a clear-cut break between the pigments of Aspidobranchia, tectibranch opisthobranchs, and pearl oysters, on one hand, and the shell colours of higher gastropods, bivalves, and pulmonates on the other. The first group are acid soluble, relatively simple substances, while the second group is intimately associated with the conchiolin of the shell and resists extraction. The first group comprises pyrrolic and perhaps indigoid substances, and a large number of pigments of relatively small molecular size but of unknown nature, while most of the second group appear to be chromoproteins, possibly with melaninoid prosthetic groups, for which no successful technique of extraction has yet been devised.

Pigmentary complexity and the formation of these insoluble materials seem to be characteristic of the shells of the more highly organized forms, both among marine and land molluscs. In pulmonates, all the shell-pigments are protein-bound, but no highly developed pigmentary patterns appear in phylogeny until the specialized helicids are reached. Land operculates show a closely similar linked progress of anatomical and biochemical specialization: here, however, even the simpler forms retain some of the patterning which is found in the marine groups from which they are believed to have risen. The pigments of the Littorinidae, for example, already belong to the non-primitive, protein-bound group.

In the development of the land pulmonates there is a reversion to primitive type among 'ancestral' forms preceding the crossing of the sea-fresh-water and sea-land barriers, with the loss of all the elaborate colour patterns found in the higher marine genera. In the tectibranch opisthobranchs, porphyrins and other acid-soluble pigments identical with those found in Archaeogastropoda account for most of the colour patterns which have been studied. Existing fresh-water forms, and land pulmonates of simple organization, except a few of the Ellobiidae, show little or no colour patterning. Most of these molluscs owe what shell-colour they have to non-pigmentary proteins, formed, perhaps, by quinone-tanning reactions, and giving yellow, brown or reddish shells. The primordial patterns in helicids, especially the single peripheral band of many *Helicella* and the five-band pattern of the larger *Helices*, are structural, that is, they are often distinct even in the absence of differential colouring and in albino shells. This relationship between pigmentation and shell structure, first noted by Wrigley (1948), appears to be a general characteristic of the phylum.

From a very extensive study of Recent and fossil shells, Wrigley (1948) has attempted to relate the loop-shaped and denticulate patterns which are so widespread in molluscs to the toothed or nodular sculpture of shells. In his view, some at least of these patterns in smooth shells may represent suppressed sculpturing. In the bivalve *Paphia ala-papilionis*, for example, Wrigley has observed interlocking of the pigmented and unpigmented parts of the growing valve edges, a pale area in one valve frequently falling opposite a coloured area in the other. He points to the

analogy between this type of pattern and the physical interlocking which occurs in bivalves whose toothed edges fit together (e.g. *Cardium*). In view of the relationship between depth of colour and differential growth rate, which is observable in many forms, this suggestion is of great interest. If, as in the feather-pigment cells, active melanophores in molluscs produced a diffusible inhibitor of adjacent pigment cells, many aspects of shell patterning might be so explained. The interlocking of dark and light patterns is not, however, constant in the shell edges of bivalves. In mottled examples of British *Paphia* (*pullastra, aurea*) interlocking of pattern is no commoner than coincidence of pigment in both shell edges.

III. MARINE SHELL PIGMENTS

(1) *Introduction*

Most of the information available upon marine shell pigments concerns the easily extracted acid-soluble group, although this accounts for a very small fraction only of the known species of sea-living molluscs. The non-extractable pigments can be studied by spectroscopy of the intact shell, but the spectra are not characteristic

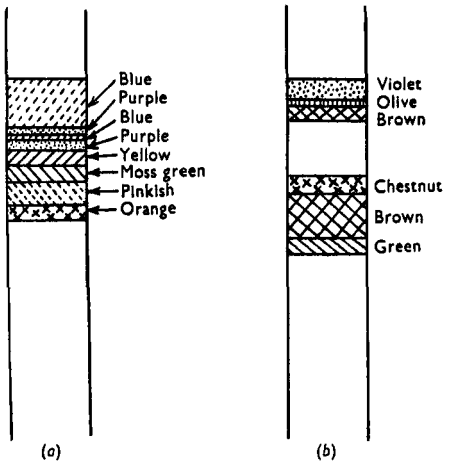

Fig. 1.

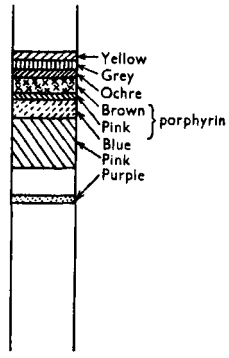

Fig. 2.

Fig. 1 (*a*) Undeveloped chromatogram on talc of acid extract of shell of *Haliotis cracherodii*. (*b*) Partly developed chromatogram on alumina of ether-soluble fraction of *H. cracherodii* extract.

Fig. 2. Chromatogram on talc of acid extract of *Pinctada vulgaris* shells.

of any well-defined group. Some of the most striking colours, such as those of the scallops, are therefore still unidentified. By contrast, the porphyrins are readily detected spectrographically and by fluorescence, and exist in crystallizable amounts.

Chromatograms of acid shell extracts on talc columns show a complex mixture of substances (Figs. 1, 2). The pigments so far identified with reasonable certainty include pyrroles, melanins and porphyrins. The carotenoids, which have a peculiar place in the economy of molluscan eggs and gonads, do not appear to occur

in shells either free or as adsorbed chromoproteins; quinones similar to those found in echinoderm tests have not been described in shell material, though they may well occur, while pterins and flavins are also absent.

(2) *Indigoids*

The hypobranchial gland of many Stenoglossa produces 6–6′:dibromoindigotin, from which this substance was formerly extracted for use as a dye. Purple markings are common in the shells of several species known to produce dibromoindigotin, but no confirmation of identity has been obtained. The free pigment is highly insoluble. The occurrence of purple or brown pigment in shells of *Nucella lapillus*, and of violet pyridine-soluble pigment in its eggs, have been shown to depend on a diet of mussels (Moore, 1936), individuals fed exclusively on *Balanus* being without brown or purple pigment. Ianthinine, the pigment of the shell and body of *Ianthina*, has been considered, probably incorrectly, to be a related substance (Moseley, 1877).

The shell of *Haliotis cracherodii* contains, among other substances, a blue, acid-soluble pigment, which has been the subject of more study than any other extractable shell pigment (Krukenberg, 1883; Schulz, 1904; Schulz & Becker, 1931; Kodzuka, 1921; Lemberg, 1931). Early work suggested that it might be pyrrolic, but its visible spectrum resembles that of the indigos, and its ultra-violet absorption shows fine structure almost identical with that of indigotin (Comfort, 1949*a*, *d*). Its solubilities differ from those of indigotin, and it has been regarded as a related but not identical pigment. Recent studies by Tixier & Lederer (1949), however, have shown that its empirical formula is incompatible with indigotin, and resembles that of a copro-mesobiliviolin. Several subsidiary pigments can be separated from extracts containing the main blue material. These are mainly yellow or greenish in colour: a yellow fraction derived by chromatography from this mixture forms a zinc complex similar to that of the linear tetrapyrroles, but possessing a striking four-banded spectrum suggesting a resonant structure (Comfort, 1950*b*) (fig. 3). It seems probable that the supposed indigoids of *Haliotis* will turn out to be unusual substances of the pyrrole family.

(3) *Pyrroles*

The naturally occurring pyrroles fall into two groups, the bile pigments, in which the pyrrole rings are arranged in chain, and the porphyrins in which they make up a four-membered ring. In higher animals the linear tetrapyrroles represent products of the degradation of haem, but they are absent from molluscan bile. In most molluscs haems are probably confined to the prosthetic groups of cytochrome, certain enzymes, the radular myohaemoglobins, haemoglobin, where this occurs, and helicorubin. It is therefore interesting to discover linear tetrapyrroles and porphyrins figuring widely in the pigmentation of shells of Archaeogastropoda, and intermediate bile pigments such as mesobiliviolin in the chromophore groups of the chromoprotein aplysiopurpurin, the pigment of *Aplysia* ink (Lederer, 1940; Lederer & Huttrer, 1942).

Linear pyrroles were found in the shells of a number of marine species by Krukenberg (1883, 1886) and by Schulz (1904), and several pigments of unknown structure have been referred to this group, among them 'rufescine' from the shells of *Haliotis rufescens* (Dhéré & Baumeler, 1928); similar pigments occur in *H. cracherodii*. Reliable evidence exists that one pigment of the shells of green species of *Turbo* is a glaucobilin; this material, from *T. regenfussi* and *T. marmoratus*, has been crystallized (Tixier, 1947).

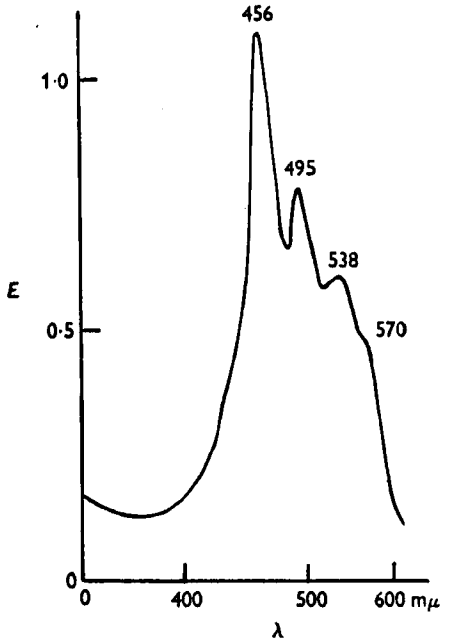

Fig. 3. Spectral absorption of the zinc complex of the yellow pigment of *Haliotis cracherodii* in chloroform.

More remarkable is the presence of extremely large amounts of free porphyrin. Fischer and subsequent workers isolated porphyrins from the shells of several species of *Pteria* and from *Trochus* (Fischer & Haarer, 1931; Fischer & Jordan, 1930, 1931; Fischer & Hoffmann, 1937; Waldenstrøm, 1937; Tixier, 1945). 8-carboxyl porphyrins of the uroporphyrin series predominated with traces of coproporphyrin. Fischer claims to have isolated from '*Pteria radiata*' a 5-carboxyl porphyrin, which, partly on account of the obscurity attending the identity of his material, has never been rediscovered. Nicholas & Comfort (1949) failed to detect it by partition chromatography in *P. radiata* Lamarck, and in a number of gastropod and pelecypod species which contained abundant uroporphyrin. Fischer's original material was found by them to contain a mixture of uroporphyrin and coproporphyrin.

The distribution of porphyrins closely follows the accepted anatomical classification of molluscs (Comfort, 1948, 1949*b*). They occur widely in the Archaeogastropoda, though not in *Pleurotomaria*, *Patella* or most species of *Haliotis*, being replaced in the Turbinidae by linear pyrroles. They also occur in several familes of Lamel-

lariacea, in several Cypraea (*Luria cinerea, isabella, pulchra*; *Erronea subviridis, Cypraea mappa*), in *Marginella ornata*, in several tectibranchs, and in *Umbraculum*; in several loricates, scaphopods, and among bivalves in the Anomiidae (*Placuna, Enigmonia*), *Pinctada, Malleus, Pinna* and a few isolated Veneridae. They are absent from all land and fresh-water shells which have been studied, except for a few species of the Neritinidae.

The main genera in which porphyrins have been shown to occur are listed below (Comfort, 1949*b*), the asterisk indicating that the property is widespread in the genus:

**Clypidina*	**Monodonta*	*Hydatina*
**Fissurella*	**Angaria*	**Bulla*
Lucapina	**Leptothyra*	*Acteon*
**Acamaea*	*Lithopoma*	*Haminea*
**Trochus*	**Tricolia*	*Aplustrum*
Clanculus	*Theodoxus*	**Umbraculum*
Ethalia	*Neritodryas*	**Enigmonia*
Isanda	*Neritina*	*Anomia*
**Monilia*	*Torinia*	**Pteria*
Thalotia	**Trivia*	**Pinctada*
Elenchus	*Erato*	**Malleus*
**Umbonium*	*Velutina*	*Isognomon*
**Gibbula*	*Cypraea*	**Pinna*
**Livona*	*Marginella*	*Venus*

The pattern of distribution in an individual species may or may not coincide with the visible pigment; in some forms it is generalized, in others confined to a single band or patch. In *Gibbula cineraria* it is usually only the uppermost whorl and protoconch that fluoresce.

The stability of porphyrins in shells is also remarkable. Marked porphyrin fluorescence was detected in *G. cineraria* from the Clyde Beds (post-Pleistocene), and also in *Pteria media* from the London Clay, and in several species from the Calcaire Grossier (Paris Basin, Upper Eocene), namely *Fissurella squamosa, Angaria calcar, A. lima, Tectus crenularis.*

Among British Recent Mollusca the following are known to deposit shell porphyrin (Comfort, 1948, 1949*b*):

Patelloida virginea	*Velutina velutina*
Gibbula magus	*Erato voluta*
Gibbula cineraria	*Trivia monacha*
Cantharidus striatus	*Acteon tornatilis*
Monodonta lineata	*Venus fasciata*

The origin of these pigments in molluscs is obscure, and uroporphyrin is present in unusually large amounts judged by the standards of other biological material. Uroporphyrins in higher forms have been regarded as by-products of the synthesis of the protoporphyrin of haem, but in molluscs some other explanation is required for their presence. The question of porphyrin biosynthesis has been much studied in mammals and bacteria, since the work of Shemin & Rittenburg (1945) established

their origin from ^{15}N-labelled glycine and it seems likely that the molluscs may provide a new line of attack. The absence of shell porphyrins from land forms, and from the higher molluscs generally, together with the discovery by McMunn (1886), and the confirmation by Dhéré & Baumeler (1928*a*, *b*) of a dermal porphyrin, probably protoporphyrin, in *Arion rufus*, where it co-exists with the redox pigment rufine, suggests the possibility of a direct dietary origin in marine forms rather than a relation to protoporphyrin synthesis, but there is no evidence that uroporphyrins can be produced from the porphyrin of chlorophyll or that they occur in sea water. Neither is there any relation between porphyrins in the shell and the feeding mode of the animal. W. Kuhnelt (personal communication, 1948) has detected porphyrin in the dart-sac of *Helix* during the secretion of the dart, and a relation between porphyrins and calcium deposition has been suggested in several higher animals. The physiology of the large amounts of uroporphyrin present in some thin-shelled forms such as *Enigmonia* is still, however, matter only for conjecture. If the porphyrin is dietary, deposition in the shell may depend upon inability to destroy it.

Acid solutions of the shells of most of the porphyrin-producing groups contain a number of blue, red and violet pigments separable by chromatography on talc. The identity of these substances is unknown, but they appear to make up a related series. The pink fractions derived by chromatography from *Pinctada vulgaris* and from several trochids appear to be spectroscopically identical. A blue fraction with red porphyrin-like fluorescence from the same species behaves very much like a mesobiliviolin (Comfort, 1949*c*, 1950*a*), in the formation of a zinc complex with a two-banded spectrum, and in the abolition of its fluorescence in acid solutions. The pigments of the porphyrin-accompanying series seem to be quantitatively interchangeable between individual specimens, the total amount of pigment being the same, but the proportions of the various individual pigments varying from shell to shell. They are probably tetrapyrrolic products of porphyrin catabolism, but they differ in solubilities and chemical behaviour from the bile pigments described in mammals. Their closest affinities are with the prosthetic groups of the bile pigment-chromoproteins of marine algae. Spectra of typical substances of this group, from acid extracts of pearl-oyster shell, are shown in Figs. 4 and 5.

(4) *Pterins*

These widespread insect pigments are most likely to be found in uricotelic land forms, if at all. The sensitive test devised by Ford (1947) for the examination of insect wings has, however, given negative results with many of the Helicidae. There is no experimental evidence that pteroylglutamic acid plays the part in molluscan metabolism which has been assigned to it in bacteria and higher vertebrates.

(5) *Quinones*

Pigments similar to those of sea-urchin tests have not been found in shells. Quinonoid pigments bound to protein may well be responsible for much of the colour of shells which resist acid extraction.

It is possible that the formation of pigment-protein complexes plays a part in the deposition of conchiolin (Trueman, 1950). C. H. Brown (personal communication, 1948) has demonstrated a close relationship between the presence of *o*-diphenols, acting as tanning agents, and the deposition of the byssus and periostracum of

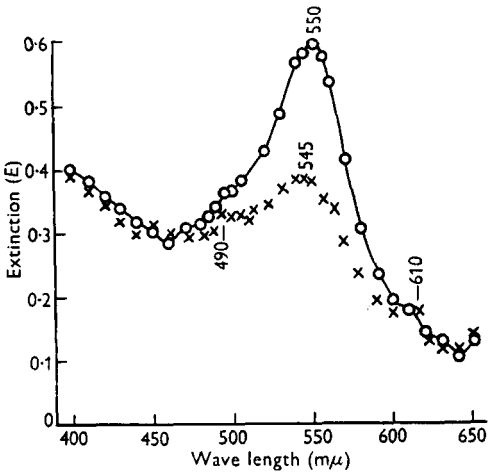

Fig. 4. Spectral absorption (in N-HCl acetone) of lower pigment fractions from chromatograms of shell extracts: o, *Pinctada vulgaris*; ×, *Malleus regula*.

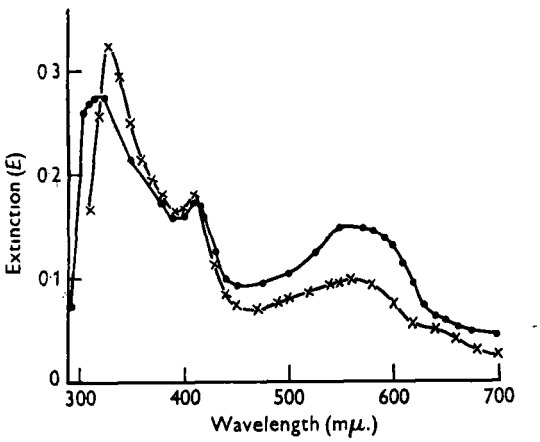

Fig. 5. Spectral absorption of violet pigment of *Pinctada vulgaris*. •, *Pinctada* '3' fraction (N-HCl); ×, *Pinctada* '3' fraction methyl ester.

Mytilus. Her work suggests that some at least of the brown shell-pigments, now loosely grouped as melanins, may be polyphenol-protein and quinone-protein complexes formed in the process of conchiolin secretion.

(6) *Melanins*

The term 'melanin' has been applied not only to the end-product of the tyrosine-DOPA cycle, but to a number of black biological pigments whose exact structure

is unknown. Of these some appear to exist as protein complexes. The name should be reserved for the group of black, brown, and reddish materials derived from tyrosine, but containing an indolic chromophore (Arnow, 1938).

Melanins are widely distributed in molluscs, as in other phyla, existing both as part of the general colour mosaics and in association with photo-receptor mechanisms. As visceral pigments they occur in the mantle and hepatopancreas, along the course of nerves, in cephalopod inks and in relation to eye spots. Melanins tend to be distributed in areas exposed to light, as in the siphonal tips of *Pholas*, and the protective patterns at the siphonal end of many bivalves, e.g. *Paphia pullastra*, are almost certainly melanotic. Squid retina contains a unique melanoprotein which superficially resembles the lipochrome-protein rhodopsin of higher animals, but which is not photosensitive (Escher-Desrivières & Verrier, 1938). Apart from their relation to light-receptors, melanins are almost certainly common in shells, but they have been demonstrated by alkali elution and acid precipitation only in *Lymnaea* (André, 1895) (a melaninoid giving a purple reaction with nitric acid) and *Mytilus* (Wetzel, 1900).

The range of non-extractable colours existing in variants of *Littorina littorea* suggests a series of related melanoproteins which may well be analogous to those found in feathers (Nickerson, 1946). Experiments are in progress to identify the pigments spectroscopically in films of decalcified shells.

The causes of melanin deposition and of the occurrence of melanotic molluscan populations are not known, though molluscs appear to exhibit both climatic and insular melanism (Germain, 1928; Rensch, 1928). The study of shell pigmentation in relation to habitat has been most fully pursued in *Cochlicella* (Aubertin, Ellis & Robson, 1931): marine forms, which are less amenable to field study, have been little investigated.

IV. SHELL COLOUR IN PULMONATES

(1) *Distribution*

Well-developed shell pigments of unknown nature, mostly brown, purplish or orange, occur in the Ellobiidae (*Pythia*, *Cassidula*). Apart from these, almost all genera phylogenetically lower than *Helix* tend to be self-coloured. Brownish and reddish tints predominate, though violet appears occasionally (*Urocoptis cylindrus*) and a few forms are banded. Reddish radial markings which do not coincide with the angle of the growing lip, and therefore represent a fairly complicated secretory mechanism, occur in several Polyplacognatha (*Goniodiscus*, *Patula*). More elaborate structural banding is uncommon (*Macroceramus*, *Anoma*).

In the lower helicids, the unifasciate or pentataeniate pattern appears in a developed form. The band or bands differ in consistency from the ground pattern of the shell, and genera can be grouped into those producing white bands on a reddish ground (*Hygromia*, *Trichia* spp., *Theba*), and those where the bands are typically melanotic (*Helicella*). Almost all the highly organized helices fall in the second of these groups.

Really elaborate patterns of pulmonate shell colour are virtually confined to these higher helicids, especially tropical forms (*Helicostyla*, *Papuina*, *Polymita*, *Camaena*) and a few temperate ones (*Monadenia*, *Cepaea*, *Helicigona*), to the bulimoids (*Liguus*, *Orthalicus*, *Porphyrobaphe*, *Placostylus*) and related forms, and to *Partula* and *Achatinella*. Highly pigmented forms are strikingly commoner among arboreal genera.

(2) *General characters of helicid pigmentation*

The typical characters of helicid shell pigments are that they are deposited continuously or intermittently in bands, the site of which is constant for the species, that the concentration of pigment tends to increase with age, and to vary with the growth rate, and that they are chemically associated with the outer or middle shell protein. The ground pattern which appears most frequently in all groups, is of melanotic banding on a self-coloured ground. In forms exhibiting this type of colouring there are two grades of albinism; of the shell, with translucency of the structural bands, dissociated from albinism of the animal, and of animal and shell together (Oldham, 1928). The yellow non-melanotic form of *Helix aspersa* has an unbanded shell showing only structural markings, but the animal is pigmented. Total albinism is also on record. In *Hygromia odeca* (Huggins, 1922) and in most of the species of *Oxychilus* or *Retinella*, by contrast, albinism of the shell is only recorded in company with albinism of the animal.

Another typical feature of pulmonate shell colouring is the tendency to deposit a *coloured lip*. In most forms where this takes place, the lip pigment appears to be derived from a different source from the main shell pattern. It is usually continuous with the inner nacreous layer, and the whole interior of the shell may be lined with the same pigment, which is deposited thickly in the lip only after growth is completed, or during an arrest of growth. Lip colouring of this kind may take the form seen in *Cepaea nemoralis* or in *Placostylus miltocheilus* where, as a rule, one pigmented lip only is formed, or it may less commonly appear at each arrest of growth, forming definite varices (*Polymita*, *Liguus*, *Amphidromus*). Varices due to changes in growth rate are also common in the melanotic bands. In this case the colour of the pigment seems to depend upon the growth or quiescence of the animal.

Banding

Pigment is deposited in specific bands, presumably by specialized secretory cell groups. A 'band' in any form is, in fact, a zone of potential deposition which may function continuously, intermittently, or not at all. The time of onset of pigment production in the bands of *Cepaea* is known to be genetically determined. Regular cyclical deposition occurs in many forms, giving a series of blotches, the length of each of which along the axis of growth is a function of growth rate. This periodicity bears a close similarity to that occurring in barred feathers (Rawles, 1948), the cellular mechanism of which is at least partly known. A gene-combination producing this type of pattern occurs from time to time in many continuously banded species.

(3) *Colour associations and interchangeability of pigments*

Where the chemistry of shell pigments cannot be studied directly, either because of complexity or of difficulty in extraction, the association of colours, and the range found in a given band of a known species, provide evidence of structural similarity. The large family of melanotic pigments in higher animals differ in colour with particle size, degree of polymerization, side-chain structure, and the internal pH of the depositing cells. The variations in ground colour which have been studied genetically are probably of the same type.

In terms of spectral absorption, most helicid shells are highly efficient filters against the shorter wave-lengths, with relatively little absorption in the red. This may possibly explain the predominance of yellows, browns, reds and oranges in land-shell colouring. The albinism of xerophiles is probably also protective. The least prevalent helicid colour is green, although this is common in aquatic genera. Clear greens or blue-greens are virtually confined to one or two arboreal forms (*Helicostyla* spp., *Achatinella* spp.), the finest examples occurring in the genus *Corasia* (*reginae*, *elizabethae*, *psittacina*). Olive pigmentation is considerably commoner. As in the marine *Ianthina*, whole genera occasionally exhibit a predominant pigment, though with less uniformity (cf. the orange lip pigment of many *Placostylus* and *Diplomorphe*).

A certain amount of information can be obtained by observing the interchangeability of colour patterns in variable and highly coloured forms such as *Polymita picta* or *Cepaea nemoralis*. Attempts to attack the chemistry of the pigments with melanin inhibitors such as phenyl thiourea have not so far succeeded, and the determination of colour appears to be largely genetic, resting upon the action of a limited number of genes, as in the case of flower pigments.

(4) *Pigmentation of* Polymita picta

The pigmentary system of the helicid *P. picta* (Comfort, 1951) is extremely complicated, and the genetics of the species, which is confined to Cuba and fails to thrive in captivity, are unknown. The variation of the pigment seems to occur in two systems; ground colour; and the sutural and columellar bands, the lip, interior, and growth varices. The second system normally produces melanin, but in the var. *roseolimbata* the whole of this system is pink. The pink pigment appears to be genetically interchangeable with the black, but this interchange does not usually extend to the whorl bands of the pentataeniate pattern. In most pink examples, these bands remain black if they appear at all. In most but not all pink and banded shells, a fine black band follows the suture. In some examples injury to the pink sutural band leads to the deposition, from that point on, of black instead of pink pigment, and the corresponding portion of the lip is black. This may be due to a change in pigment, or, more probably, to the replacement of pink-secreting cells by melanin-secreting cells from the sutural band. In others, the area laid down after injury is white, but some restoration of pattern appears close to the lip. Unfor-

tunately the experiments of Andrews (1936) on repair in this species were based on excision of pieces of shell, not upon damage to the mantle.

The degree of interchangeability shown by the pigments in this species is very similar to that of hair and feather pigments. No conclusive evidence can, however, be got from inspection to show that the pink system and the black system are derived from one another, since there is more than a suggestion that in the type form both exist, the pink being obscured by the melanin. This becomes clearer during acid digestion, when pink zones may be seen to overlie the structural melanin bar ds.

When shells are digested in a water bath with 2% aqueous acetic acid, the ground colour pigment can be peeled from the decalcified shell as thin protein lamina. The melanotic bands are deeper seated, and the pink material is decolorized. The yellow ground pigment is partly extractable by ethylene chlorhydrin, forming a yellow solution. This behaviour resembles that of the hair and melanoma melanins (Lea, 1945). The murexide test is negative for whole shells, and none of the bands exhibit specific ultra-violet fluorescence

The mantle groove of *P. picta* is apparently pigmented (Andrews, 1932), but live material has so far been unobtainable for extraction. The snails are extremely hard to keep under experimental conditions (Andrews, 1932, 1936).

(5) *Pigmentation of* Cepaea nemoralis

The variation of this species is already familar to geneticists. The pigment system shows three main genetic variables: ground colour, banding absent or present, and the position and character of the bands (Aubertin & Diver, 1927; Boettger, 1921, 1931; Diver, 1931, 1939; Rotarides, 1929, 1932, etc.). To these may be added colour of lip, which has been incorrectly regarded as a specific character. Very little can be said of these colour patterns in terms of chemistry. The black pigment ('melanocochlin') is alkali-soluble (Helmcke, 1935). Melanin banding of the shell frequently, but not always, goes with corresponding pigmentation in the mantle surface (Distaso, 1908; Aubertin & Diver, 1927). This pigmentation may be black or reddish.

We have attempted to relate yellowness of the shell to the concentration in the foot and mantle of a pigment of unknown nature, stated (Kubišta, 1950) to be a flavone, which *C. nemoralis* shares with *Helix aspersa* and *H. pomatia*. This alcohol-soluble pigment resembles the flavones in forming an insoluble lead salt, and in showing intensification of colour with alkalis. It was first noticed because of its intense yellow fluorescence in ultra-violet light (Turchini, 1926). Like many flavones the similar pigment in *H. aspersa* lakes with alumina and is a permanent dye. Flavones are widespread plant pigments but are not otherwise described in molluscs. No reduction in concentration of mantle pigment was detected in starved helices, or in helices fed on cellulose. Apart from the flavones, the substance has some resemblance to the hydroxy-indole derivative (8-methyl-5:6-dihydroxy-indoxyl) formed by alkalis acting upon adrenaline or adrenochrome (Lund, 1949), but is far more stable. We have failed to oxidize it to melanin, while the yellow fluorescent

adrenaline pigment deposits melanin readily on standing. If specimens of *Cepaea nemoralis* are immersed in weak alkali, the foot and mantle of yellow-shelled forms appear to give a deeper tint than those of red-shelled. Neither the yellow shells, however, nor the decalcified framework of protein with pigment, nor extracts of this in ethylene chlorhydrin, show any sign of the typical fluorescence of the body pigment.

In vars. *rubella* (red) and *libellula* (yellow) the colour of the decalcified pellicles is indistinguishable. The red form appears to contain an additional pigment which is pink or purplish, and is present in the calcareous part of the shell. In many shells this process of secondary deposition can be followed as growth proceeds. Examination of wind-blown shells from the extensive deposits at Bundoran and Strandhill in Ireland also suggests that the difference between yellow and red forms lies in the possession by the red form of an additional pigment. The red pigment is not recoverable after etching with acid.

(6) *Conclusion*

Diver (1939) has pointed out the wide validity of genetic colour patterns, once these can be properly analysed. The study of shell pigments makes it clear that the variety of patterns is really based on a comparatively limited number of factors within the species. It would be reasonable to guess that splitting of bands and production of an identical pattern in unrelated forms, e.g. *Polymita versicolor* and *Euparypha pisana*, may be the work of analogous genes. The chemistry of the genetic control of melanoblast periodicity is a fruitful but untouched field of study. The spectroscopy of whole shells may ultimately prove to be of value in detecting the pigments present. Serra (1943) has shown that the absorption spectra of human hair pigments derived from blond, brown, red and black hair differ little in form, the common feature being an inflexion at 5000A. Stary & Richter (1938) could detect no quantitative differences in analysing the same pigments, though the iron-containing material extracted from red hair by Rothman & Flesch (1943) appears chemically distinct. The differences between the visible colours of these melanins would appear to depend upon physical state and concentration. Protein complexes (melanokeratin and leucokeratin) have been postulated. Melanoproteins based on conchiolin probably exist in the structural bands, since in such forms as *Cernuella virgata* and *Planatella itala* var. *hyalozona* the melanotic bands are replaced by clear protein, and all intermediate shades between white and black may be found in the band region. In *Cernuella virgata* pinkish bands are recorded. The feather pigments studied by Goernitz (1923) present an almost identical series.

In an attempt to detect porphyrin bands in marine shells, spectroscopic studies of a large number of species were made; in almost all the forms whose pigments were insoluble in acid, absorption tends to be maximal at 5000A., with little regard to the predominant colour. An exception occurs in the green species of *Corasia* (*elizabethae*, *reginae*, *psittacina*), all of which show an additional band at about 6290A., at a rather shorter wave-length than that of the green bilitrienes (Glaucobilin IX*a* methyl ester 6460A., biliverdin 6650, turboglaucobilin 6550). No material has yet been obtained to see whether this pigment is extractable.

The general picture which emerges from recent work on shell pigments is one in which the porphyrins and atypical pyrroles of the primitive marine forms give place first to bile pigments, and later in phylogeny to chromoproteins of increasing complexity bound to the conchiolin of the shell by a process of tanning. In no case does the primitive pattern of pigment metabolism succeed in crossing the sea-land barrier. The possession of a cleidoic egg, or viviparity on land, may preclude the type of metabolism on which these simpler pigments depend. Unlike the insects, land molluscs do not seem to have developed nitrogenous disposal pigments of the pterin type. The most elaborate patterns in land forms, while following the basic common pattern of molluscan shell marking, are commonest in diurnal and arboreal groups and are probably examples of significant colours.

V. SUMMARY

1. The methods of studying shell pigments, the literature and some of the experimental data are reviewed.

2. The pigments of shells are sharply divisible into acid-soluble and acid-insoluble groups.

3. Acid-soluble pigments occur in archaeogastropods, opisthobranchs, and the lower bivalves, as well as in isolated groups and species scattered throughout the marine genera.

4. These pigments include porphyrins, pyrroles, and a number of unidentified pigments of small molecular size.

5. The biological origin of porphyrins and pyrroles in marine molluscs is unknown. The porphyrin is mainly uroporphyrin. The existence of pentacarboxylic porphyrins ('conchoporphyrin') has not been confirmed.

6. In most higher molluscs and all pulmonates the pigment is firmly attached to the conchyolin of the shell. It appears to be incorporated during shell secretion, possibly by a process of quinone tanning.

7. The variation and behaviour of typical pigmented land species suggest that some at least of these pigments are similar chemically to the melanins of higher animals.

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