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THE OCCURRENCE OF PORPHYRINS IN CERTAIN MARINE INVERTEBRATES

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(Text-fig. 1)

Kennedy & Vevers (1953*a*, *b*) confirmed the presence of a porphyrin in the integument of the starfish *Asterias rubens*, reported by MacMunn (1887), and showed that it was protoporphyrin. A yield of 33·6 mg of protoporphyrin dimethyl ester was obtained from 548·5 g of *Asterias* integument. Following this work, and the investigation of porphyrins in mollusc shells by Nicholas & Comfort (1949), it was naturally of interest to examine the distribution of porphyrin pigments in the soft and skeletal parts of other marine animals. A survey has therefore been made of the occurrence of porphyrins in certain invertebrates common in the waters off Plymouth.

Methods

The animals were examined first in the fresh state under ultra-violet light (Osira 125 W black glass lamp) and then homogenised (in an Atomix blender) and extracted by the methods used previously (Kennedy & Vevers, 1953*b*). The extracts in turn were examined by ultra-violet light and the occurrence of red fluorescence noted. Where extracts showed red fluorescence the source of this was investigated chemically and, where possible, the pigments responsible for this fluorescence were isolated and characterized by the following methods:

(1) *Solubility* in ether, chloroform, ethyl acetate, 0·1N hydrochloric acid, and 0·1N sodium hydroxide.
(2) *Partition* between ether and increasing concentrations of aqueous hydrochloric acid from 0·1% (w/v), this is the HCl number (Salzsäurezahl) of Willstätter & Stoll (1913).
(3) *Column chromatography*, Nicholas (1951) and Kennedy (1953*a*).
(4) *Paper partition chromatography* Nicholas & Rimington (1949), Kennedy (1953*b*) and Chu, Green & Chu (1951).
(5) *Absorption spectra*, using a Beck-Hartridge Reversion spectroscope, and a Unicam S.P. 500 spectrophotometer.
(6) *Formation of derivatives* and conversion to other porphyrins, when sufficient material was available.

TABLE I. OCCURRENCE OF RED FLUORESCENCE IN EXTRACTS FROM CERTAIN MARINE INVERTEBRATES

(Extracts of whole animal except where otherwise stated.)

	Red fluorescence due to		
	Porphyrins	Chlorophyll and derivatives	Remarks
PORIFERA			
Sycon coronatum	−	−	
Grantia compressa	−	−	
Tethya aurantium	?+	−	Not yet identified
Halichondria panicea	−	+	Algae
COELENTERATA			
Velella spirans (skeleton)	−	−	
V. spirans (soft parts)	−	−	
Alcyonium digitatum	−	−	
Actinia equina	−	−	
Anemonia sulcata	−	+	Algal symbionts
Tealia felina	−	−	
Metridium senile	−	−	
Calliactis parasitica	−	−	
ANNELIDA			
Aphrodite aculeata	−	−	
Nereis diversicolor	+	+(*a*)	
Perinereis cultrifera	−	−	
Chaetopterus variopedatus	+	+(*b*)	
Arenicola marina	−	−	
Myxicola infundibulum	−	−	
M. infundibulum (purple tentacles)	−	−	
M. infundibulum (alimentary system)	+	−	
ARTHROPODA			
Portunus depurator	−	−	
Carcinides moenas (soft parts)	−	+	From diet in alimentary tract
C. moenas (carapace)	−	−	
Cancer pagurus (eggs)	−	−	
C. pagurus (viscera)	−	−	
C. pagurus (claw shell)	−	−	
C. pagurus (carapace)	−	−	
Eupagurus bernhardus (soft parts)	−	−	
MOLLUSCA			
Mytilus edulis	−	−	
M. edulis (shell)	−	−	
Chlamys opercularis	−	−	
C. opercularis (mantle)	−	−	
Cardium edule (soft parts)	−	−	
Buccinum undatum (soft parts)	−	−	
Scaphander lignarius (bursae)	−	−	
Philine aperta (bursae)	−	−	
Aplysia punctata (integument)	+	−	
Jorunna tomentosa	−	−	
Archidoris britannica	−	−	
A. britannica (eggs)	−	+	Chlorophyll from Algae
Duvaucelia plebeia (upper integument)	+	−	
Loligo forbesi	−	−	
Parasepia elegans	−	−	

TABLE I (*continued*)

	Red fluorescence due to		
	Porphyrins	Chlorophyll and derivatives	Remarks
ECHINODERMATA			
Antedon bifida	–	–	.
Astropecten irregularis (integument)	+	–	.
Luidia ciliaris (integument)	+	–	.
Porania pulvillus (integument)	–	–	.
Palmipes membranaceus (integument)	–	–	.
Solaster papposus (integument)	–	–	.
Henricia sanguinolenta (integument)	–	–	.
Asterias rubens (integument)	+	–	.
Marthasterias glacialis (integument)	–	–	.
M. glacialis (spicules)	–	–	.
Ophiothrix fragilis	–	–	.
Ophiocomina nigra	–	–	.
Psammechinus miliaris (test)	–	+	Chlorophyll from Algae on test
Holothuria forskali (integument)	–	–	.
TUNICATA			
Ascidiella aspersa (viscera)	–	–	.
Ciona intestinalis (viscera)	–	–	.

(*a*) Phaeophorbide *a*; (*b*) Phaeophorbides *a* and *b*.

The results of this survey are given in Table I, and further details of those animals in which porphyrins were found are given in Table II. Where positive identification of pigments was possible the evidence upon which this was based is described under the name of the pigment.

TABLE II. OCCURRENCE OF PORPHYRINS IN CERTAIN MARINE INVERTEBRATES

Phylum	Species	Porphyrin	Distribution
Annelida	*Nereis diversicolor*	Coproporphyrin III	Viscera
	Chaetopterus variopedatus	Coproporphyrin III and a pentacarboxylic porphyrin	Viscera
	Myxicola infundibulum	Coproporphyrin III	Viscera
Mollusca	*Aplysia punctata*	Uroporphyrin I	Integument
	Duvaucelia plebeia	Uroporphyrin I	Integument
Echinodermata	*Astropecten irregularis*	Chlorocruoroporphyrin and protoporphyrin	Integument
	Luidia ciliaris	Chlorocruoroporphyrin and protoporphyrin	Integument
	Asterias rubens	Protoporphyrin	Integument

IDENTIFICATION OF PIGMENTS

Coproporphyrin III

This pigment was noticed during the course of paper partition chromatography (Nicholas & Rimington, 1949) of chloroform extracts of the three polychaetes—*Nereis*, *Chaetopterus* and *Myxicola*—in an investigation of the

green pigment 'chaetopterin' (with J. A. C. Nicol), to be reported in the near future. The long-paper method of Kennedy (1953*b*) was employed, with 2:6-lutidine and water as solvent phases. A well defined spot appeared, with strong red fluorescence at R_F 0·65, which suggested a 4-COOH porphyrin, and coproporphyrin in particular. Further chromatograms were run under the same conditions, but including on each paper a spot of coproporphyrin I as a marker, in both adjacent and mixed spots. Only one spot appeared at R_F 0·65 on the mixed spot papers, and on the papers with adjacent spots there were two spots side by side at R_F 0·65. This confirmed the presence of a 4-COOH porphyrin, and strongly suggested coproporphyrin.

There was very little material for experiment so the spots of all the long-paper chromatography experiments were cut out carefully, the pieces soaked in a little dry pyridine and examined spectrophotometrically. The spectrum showed the maxima:

I	II	III	IV
623·6	569·0	533·0	499·0 mμ

further indicating that this porphyrin is indeed a coproporphyrin.

Concentrated solutions of the extracts from the three worms were then evaporated to dryness *in vacuo*, the residues esterified with methanol/hydrochloric acid and examined by the double-development paper-chromatography method of Chu *et al.* (1951), using chloroform: kerosene/*n*-propanol: kerosene at 19° C. Well-marked spots were observed with centre-dense red fluorescence at R_F 0·81, corresponding to coproporphyrin III. The test was repeated with markers of coproporphyrin I tetramethyl ester and coproporphyrin III tetramethyl ester (the latter very kindly supplied by Prof. C. Rimington, F.R.S.) in both adjacent and mixed spots. On those papers bearing the extracts and coproporphyrin I in mixed spots, two clear sets of spots were seen, indicating that the two porphyrins were not identical. On those papers bearing adjacent spots of coproporphyrin I and extract, there was a wide difference between the R_F values.

Papers carrying mixed spots of the worm extracts with authentic coproporphyrin III showed only one set of spots at R_F 0·80 confirming that the worm porphyrin was, in fact, identical with coproporphyrin III. Papers bearing adjacent spots of the coproporphyrin III ester and the worm extract showed that both pigments travelled together in parallel, and took up the same R_F position, 0·80. This established that the worm porphyrin was present as coproporphyrin III.

A Penta-carboxylic Porphyrin

In the course of these long-paper chromatography experiments just described, extracts of *Chaetopterus variopedatus* produced a spot above that of coproporphyrin with an R_F value of 0·5. This is indicative of a porphyrin with five carboxyl groups. This was repeated several times and confirmed, but there

was insufficient material for exact characterization. Further material is being collected for a detailed examination of this porphyrin.

Uroporphyrin

The upper integument of the nudibranch gastropod *Duvaucelia* (*Tritonia*) *plebeia* yielded no red fluorescent material to ether/acetic acid, but on placing the tissue in a mixture of absolute methanol (19 parts) and concentrated sulphuric acid (1 part) overnight the extract was found to be slightly red fluorescent to ultra-violet light. This fluorescence increased very much in intensity on diluting the extract with water, shaking up with chloroform and then examining the chloroform hypophase under ultra-violet light.

The chloroform extract was roughly dried by filtration and evaporated to dryness, and the residue was then re-dissolved in chloroform. This chloroform extract was passed through a column of alumina grade IV (Nicholas, 1951), packed by sprinkling into chloroform, and a broad red fluorescent band formed at the top of the column, with a diffuse pinkish non-fluorescent band below. The top band was immovable with ethanol/chloroform mixtures, ether, ether/pyridine mixtures, acetone or ethyl acetate, but was eventually eluted with the methanol/sulphuric acid (19:1) esterification mixture. The pigment was collected as a purple-red solution, intensely red fluorescent. A greyish non-fluorescent band was left on the column.

The acid/methanol solution was diluted with two-thirds of its volume of water, and the pigment was extracted with chloroform to form a purple hypophase with intense red fluorescence. The hypophase was then separated, washed several times with 2% sodium chloride, and finally with water. The solution was roughly dried by filtration through chloroform-soaked paper and evaporated to dryness on the water-bath. The pigment was redissolved in dry chloroform and the absorption spectrum determined spectrophotometrically (Unicam S.P. 500), and plotted as $E_{1\,cm}$ against wave-length. The maxima were:

I	II	III	IV	Sorêt band	
624	572	535·5	501	406	mμ

This suggested uroporphyrin and an authentic specimen of uroporphyrin I, when examined in chloroform in the same spectrophotometer, gave maxima at:

I	II	III	IV	Sorêt band	
624	570·5	535	501	406	mμ

These peaks show very good agreement, as may be seen in Fig. 1. A mixture of uroporphyrin I octamethyl ester and the *Duvaucelia* porphyrin ester were made in equal proportions as far as possible and examined in the spectrophotometer. (If two dissimilar substances are examined spectrophotometrically the peaks of the absorption curve are usually very rounded and the

curve presents an undulating instead of a sharp appearance.) The maxima in this case were:

I	II	III	IV	Sorêt band	
624	570·5	535	501	405	mμ

The peaks of the curve (Fig. 1) are very sharp and the curve suggests the presence of one pure substance only.

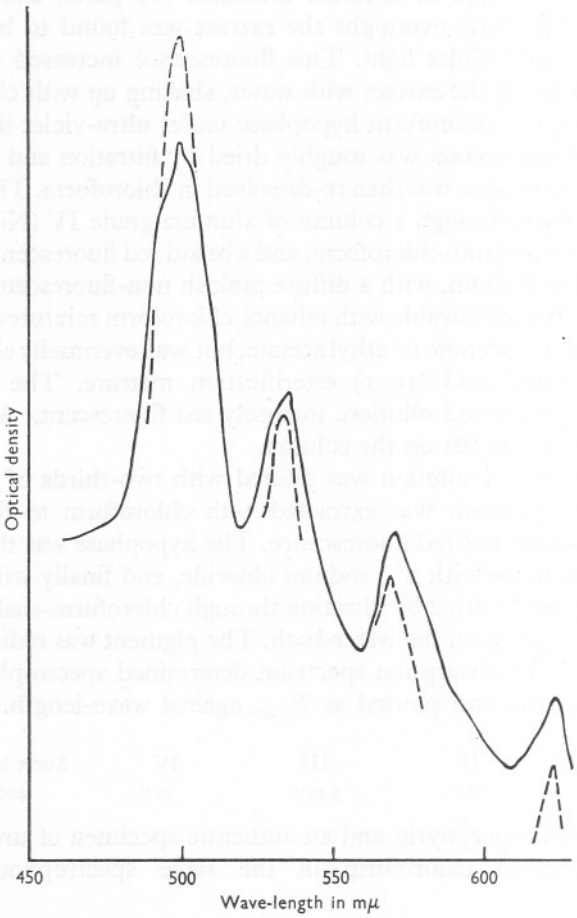

Fig. 1. Absorption spectra of *Duvaucelia* porphyrin in chloroform. ——, absorption spectrum of *Duvaucelia* porphyrin alone; - - - - -, absorption spectrum of a mixture of *Duvaucelia* porphyrin and authentic uroporphyrin I.

A little of the porphyrin ester solution from *Duvaucelia* was evaporated to dryness and hydrolysed by dissolving it in 7N-HCl and standing at room temperature for 36 h. At the end of this time the excess acid was removed by placing the dish in a vacuum desiccator over solid potassium hydroxide until

dry. The residue was dissolved in a little chloroform, spotted on a long paper (Kennedy, 1953*b*) and developed at 23° C with 2:6-lutidine/water (5:3) in an atmosphere of ammonia. The resulting chromatogram showed a single spot at R_F 0·15, indicating a porphyrin with eight carboxyl groups. This test was repeated using pure uroporphyrin I as a marker. With mixed spots only one large spot appeared at R_F 0·15 with a small spot above it at R_F 0·09 (the 'second spot' of uroporphyrin). In chromatograms with authentic uroporphyrin I as marker, parallel spots formed at R_F 0·15 and one small spot on the marker side at R_F 0·09.

These results together with the absorption spectrum confirm that the *Duvaucelia* porphyrin is uroporphyrin. The uroporphyrin was characterized as isomer I by the two-dimensional paper chromatogram technique of Falk & Benson (1953), and (at Sheffield) by its decarboxylation to coproporphyrin I identified by the Chu, Green & Chu (1951) technique.

The Porphyrin of Aplysia punctata

The upper integument was carefully removed from fifteen *Aplysia punctata*, all collected from Looe by Dr B. C. Abbott, to whom we are indebted for his kindness. The material was washed in several changes of fresh water to remove as much as possible of the 'aplysiopurpurin' pigment. The integuments were then dried roughly on filter-paper and extracts made in exactly the same way as for *Duvaucelia*. The final chloroform extract was evaporated to dryness and redissolved in dry chloroform and chromatographed on magnesia grade III (Nicholas, 1951). The material quickly resolved itself into a series of bands as follows, from the top downwards:

(1) Greyish.
(2) Brown-purple.
(3) Green.
(4) Blue.
(5) Purple (red fluorescent).
(6) Yellow (blue fluorescent).

It proved impossible to separate bands 5 and 6 from one another, and they were collected together. The other bands, although interesting, were discarded for the present, to be investigated in the future. The red fluorescent chloroform extract containing bands 5 and 6 was evaporated to dryness, to give a fatty residue. This was redissolved in dry chloroform and the absorption curve determined in the spectrophotometer. The following maxima were obtained:

I	II	III	IV	Sorêt band
624	570	534	501	406 mμ

This strongly suggested uroporphyrin, an authentic specimen of which gave the maxima described under the *Duvaucelia* pigment.

Some of the *Aplysia* porphyrin ester was hydrolysed by standing in 7N-HCl for 48 h and the acid removed by standing over solid KOH *in vacuo* over-

night. The residue was dissolved in 2:6-lutidine and examined in the long-paper chromatograph in a 2:6-lutidine/water system in an atmosphere of ammonia at 23° C. Three papers were run, including the *Aplysia* porphyrin alone, and with authentic uroporphyrin I as marker in mixed and adjacent spots. On all papers two spots were obtained at R_F 0·09 and 0·15, indicating that the porphyrin of *Aplysia* was a uroporphyrin.

The Chu *et al.* (1951) technique, followed by that of Falk & Benson (1953) established the uroporphyrin as isomer I, confirmed (at Sheffield) by its conversion to coproporphyrin I.

The Porphyrins of Luidia ciliaris

The upper integument of the starfish *L. ciliaris* was extracted in the usual way with a methanol-sulphuric acid (19:1) mixture and the porphyrin brought into chloroform. After evaporation to dryness on the water-bath this extract was redissolved in dry chloroform and chromatographed on magnesia grade III (Nicholas, 1951). The column was monitored by ultra-violet light. A blue fluorescent band of fatty material passed rapidly down the column and was discarded. Two purplish bands, both red fluorescent, then appeared and passed very slowly down the column about 2 cm apart. These were eventually eluted with chloroform-methanol (100:3) (band 1) and chloroform-methanol (100:5) (band 2) respectively.

Band 1. This was identified as protoporphyrin by spectrophotometry in pyridine (including comparison with authentic protoporphyrin) and by conversion to mesoporphyrin.

Band 2. This band in chloroform solution was evaporated to dryness in portions. One of these was dissolved in pyridine and examined in the spectrophotometer. The following maxima were obtained:

I	II	III	IV	Sorêt	
648	590	562	519	425	mμ

The curve obtained was clearly of the rhodo-type (that is, the optical density of the peaks of absorption decreased in the order III, IV, II and I). This fact, together with the positions of the maxima of absorption, strongly suggested chlorocruoroporphyrin. The oxime was prepared (at Sheffield) and a spectrophotometric examination in pyridine showed a characteristic shift of the absorption maxima towards the blue:

I	II	III	IV	
638	580	548	507	mμ

This is good evidence for the presence of a formyl (—CHO) group. Authentic chlorocruoroporphyrin, prepared from *Myxicola–Sabella* blood, gave an oxime with maxima at:

I	II	III	IV	
639	579	543	507	mμ

To determine the presence of vinyl groups, the porphyrin was treated with diazo-acetic ester followed by hydroxylamine. This produced a shift to the blue, but the rhodo-type spectrum was retained. The maxima in pyridine were:

I	II	III	IV	
645	586	557	516	mμ

This is consistent with the presence of one vinyl group, and is confirmatory evidence for the porphyrin being chlorocruoroporphyrin.

As an additional check on the identity of the main porphyrin of *Luidia*, the following experiments were carried out.

Fresh *Luidia* tissue was extracted by the ether : acetic acid procedure, and after the usual purification, the porphyrins were esterified by standing dissolved in methanol : HCl for 48 h at 0° C. The esters, after purification, were dissolved in dry chloroform and separated by chromatography on a column of alumina packed in a mixture of equal parts of chloroform and ether (Lemberg & Parker, 1952). The band of protoporphyrin dimethyl ester passed rapidly down the column well in advance of the main band of chlorocruoroporphyrin ester, and was discarded.

The chlorocruoroporphyrin band was collected, filtered, and the solvent removed *in vacuo* without heat. The residue was dissolved in glacial acetic acid saturated with sodium chloride and the chlorocruorohaematin prepared, the iron being introduced by the method of Paul (1950).

The chlorocruorohaematin was converted into the haemochromogen by the method of Warburg, Negelein & Haas (1930), viz.: the haematin was dissolved in aqueous pyridine (pyridine 1 ml. : water 3 ml.) and one-tenth of the volume of hydrazine hydrochloride solution added (hydrazine hydrochloride 0·7 g in 5 ml. water + 5 ml. of 2N-NaOH). The haemochromogen gave the following bands in the Hartridge Reversion Spectroscope:

I	II	Intensity
584·4	545·9 mμ	I > II

This result agrees well with that obtained for chlorocruorohaemochromogen by Lemberg & Falk (1951):

I	II	Intensity
583·1	545·1 mμ	not given

The haemochromogen from the 'haem *a*' obtained from ox-hearts by Rawlinson & Hale (1949) gave only *one* band in the visible spectrum at 587 mμ (Lemberg & Falk, 1951; Falk & Rimington, 1952; Rimington, Hale, Rawlinson, Lemberg & Falk, 1949).

These experiments distinguish clearly between the main porphyrin obtained from *Luidia* and the porphyrin *a* from haem *a* of heart muscle. In addition, the spectrum of the *Luidia* porphyrin—chlorocruoroporphyrin—is of the rhodo-type—intensities III, IV, II, I—whereas that of the porphyrin *a*

is of the oxorhodo-type—intensities III, II, IV, I. The porphyrin in *Luidia* occurs free, and not as a haem.

The Porphyrins of Astropecten irregularis

The integuments of fifty specimens of *Astropecten irregularis* were carefully stripped off and extracted with methanol/sulphuric acid overnight in the usual way. The pigments were taken into chloroform and the extract washed and evaporated by the conventional technique. The evaporated chloroform extract was redissolved in dry chloroform and chromatographed on a column of magnesia grade III (Nicholas, 1951), developing with chloroform with increasing concentrations of methanol, as for *Luidia*. The same type of chromatogram was obtained, and the two red fluorescent bands were shown to be protoporphyrin III type 9 and chlorocruoroporphyrin in the same way and by the same tests as were applied to the porphyrins of *L. ciliaris*. *Astropecten*, however, appeared to have much less porphyrin (and particularly protoporphyrin) in the integument than *Luidia*, although no quantitative investigation was carried out at this stage.

Discussion

This survey of a number of marine invertebrates from eight phyla shows that porphyrins are present in eight species for certain (with one doubtful species *Tethya aurantium*), and that these eight species belong to only three phyla—Annelida, Mollusca and Echinodermata. Porphyrins were not found in any of the coelenterates, crustaceans or tunicates examined, although MacMunn (1887) found haematoporphyrin in the madreporarian corals *Flabellum variabile* and *Fungia symmetrica*.

The finding of uroporphyrin in the upper soft integument of *Duvaucelia plebeia* (better known as *Tritonia*) and *Aplysia punctata* is of considerable interest in view of the presence of this pigment in some mollusc shells (Nicholas & Comfort, 1949). MacMunn (1887) found 'haematoporphyrin' in the black slug *Arion empiricorum* (a synonym introduced by Férussac, Férussac & Deshayes, 1819–51, to cover the numerous colour and pattern varieties of the slug more generally known as *A. ater*). Thus the only molluscs which have been shown definitely to contain a porphyrin in their soft parts are either species without shells (*Duvaucelia* and *Arion*) or species with uncalcified shells (*Aplysia*), whereas uroporphyrin, with traces of coproporphyrin, occurs in several of the shells of shell-bearing species. This suggests that in molluscs the porphyrin is normally laid down in the shell (an integumentary product), or failing that in the uncalcified integument. The former would be in keeping with the deposition of uroporphyrin I in the bones and teeth in congenital porphyria, and in the bones of the Pennsylvanian fox-squirrel *Sciurus niger* (Turner, 1937). Turner connected the formation of the

uroporphyrin I with the megaloblasts of the bone marrow, and postulated that in *Sciurus* there exists a unique persistence into the adult span of the normal method of foetal haemoglobin synthesis.

Protoporphyrin III type 9 occurs in the shells of the eggs of the hen and of a plover (Fischer & Kögl, 1924). Borst & Königsdorfer (1929), Fikentscher (1932), Fraenkel (1924) and Hammer (1930) found that small amounts of porphyrin occur in the bones of the human foetus, as well as in those of newborn babies and other newborn mammals, mainly in the centres of ossification. It is also interesting to recall the experiments of these workers with growing animals, in which injected uroporphyrin I and haematoporphyrin III type 9 were quickly deposited, the latter being required in large amounts before deposition takes place. Bingel (1937) showed that uroporphyrin III was also deposited in bones and teeth. Coproporphyrin I, mesoporphyrin III type 9 and protoporphyrin III type 9 appear to have a slight tendency to deposit in this way. The bones of the foetus do not become impregnated with porphyrins injected into the mother, according to Borst & Königsdorfer (1929) and others, but Kench, Langley & Wilkinson (1953) have shown that porphyrins are transmitted through the placenta but are rapidly excreted. This is surprising in view of the known affinity of one porphyrin at least—haematoporphyrin—for actively growing tissues (Figge, Weiland & Manganiello, 1948; Kennedy, 1952). The deposition of porphyrins in the asteroids is perhaps somewhat similar, although it is difficult to demonstrate whether the porphyrins are present in the spicules or not, owing to the difficulty of separating the spicules from the integument without destroying any porphyrin they might contain. The presence of free chlorocruoroporphyrin in *Luidia ciliaris* and also in *Astropecten irregularis* is of great interest, since this porphyrin has hitherto only been found to occur naturally in the form of its haemoglobin, chlorocruorin, in the blood of sabellid worms (Fox, 1926, 1949). Lemberg & Legge (1949) have suggested that 'since this pigment is found only in a small group of worms which live in the same type of environment as do others containing erythrocruorin with protohaem IX as prosthetic group, the peculiarity does not appear to be of adaptive importance, and may be an evolutionary relic'.

In the three species of echinoderms shown to contain porphyrins, *Asterias rubens* has only protoporphyrin (Kennedy & Vevers, 1953*b*) which has two vinyl groups, while *Luidia ciliaris* and *Astropecten irregularis* have chlorocruoroporphyrin, which has one vinyl and one formyl group. *Luidia ciliaris* and *Astropecten irregularis* also have protoporphyrin in the integument. Warburg (1932) considered that the presence of a carbonyl (=CO) group in a side chain (which later becomes a vinyl group) is a primitive characteristic. The occurrence of chlorocruoroporphyrin in *Luidia* and *Astropecten*, both phanerozonian asteroids, may therefore be regarded as an additional argument for classifying the Phanerozonia as less specialized than the Forcipulata such

as *Asterias rubens* (Grassé, 1948, p. 237). Apart from this, however, it would appear that the distribution of integumentary porphyrins in starfishes is not only random in relation to their taxonomic position but also in relation to their ecology and mode of life. Thus the presence of protoporphyrin in *A. rubens* and its absence in *Marthasterias glacialis* is surprising, for not only are these two starfishes classified in the same family (Asteriidae), but their larvae are almost identical in form and scarcely distinguishable (Mortensen, 1927), and the adults feed in the same way on the same type of food. In areas where their geographical ranges overlap, as they do off Plymouth, these two species may, in fact, be said to occupy the same ecological niche.

In the polychaetes the porphyrin is probably present in the viscera, but this is less certain as it is often difficult to separate integument from viscera in sufficient quantity for analysis. In *Chaetopterus*, in which the digestive tract was separated from the rest of the animal for extraction, the porphyrins certainly came from the viscera.

Summary

A survey of forty-eight species of British marine invertebrates has shown that porphyrins which have not previously been described in these animals are present in three representatives of the Annelida, two of the Mollusca and two of the Echinodermata. The occurrence of protoporphyrin in *Asterias rubens* was confirmed in a previous paper (Kennedy & Vevers, 1953*b*). A red-fluorescent extract was obtained from the sponge *Tethya aurantium*, but it contained too little material for further investigation. No trace of a porphyrin was found in the coelenterates, crustaceans and tunicates examined, although Moseley found 'polyperythrin' (which MacMunn later identified with the 'haematoporphyrin' of *Asterias* and *Arion*) in *Flabellum* and *Fungia*.

Chlorocruoroporphyrin was found free in the starfishes *Luidia* and *Astropecten*. Uroporphyrin was extracted from the soft dorsal integument of *Duvaucelia* (= *Tritonia*) *plebeia*, and from the integument of *Aplysia punctata*, observations of interest in view of the widespread occurrence of uroporphyrin in mollusc shells.

Coproporphyrin III was characterized from extracts of the viscera of *Myxicola infundibulum*, *Nereis diversicolor* and *Chaetopterus variopedatus*. Evidence of the occurrence of a penta-carboxylic porphyrin was obtained from paper chromatography of the extract from the gut of *Chaetopterus*.

These findings are discussed in comparison with the distribution of porphyrins in birds and mammals.

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