THE INDOLE PIGMENTS OF MOLLUSCA

by

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SUMMARY

This study discusses occurrence and biosynthesis of melanins and indigoids, pigments with indole structure produced by mollusca. Indigotin and 6,6'-dibromoindigotin (DBI), constituents of « mollusc purple », are treated in some detail. Their colourless precursors in the organism are presumed to be of dietary origin. The hypothesis is formulated that formation of DBI is due to a detoxification mechanism ; it is suggested that purple-producing species have developed it into an enzymatically controlled defensive system against large predators. This first reaction step is then followed by spontaneously proceeding reactions involving oxygen and, for DBI, light, leading to the formation of the coloured pigment, which seems to be of no further use to the animal. Experiments with purple from three Mediterranean species are reported.

Keywords : melanin, mollusc purple, indigotin, biosynthesis.

RÉSUMÉ

Cette étude discute l'occurence et la biosynthèse des mélanines et indigoides, pigments à structure indolique produits par des mollusques. Les indigoides indigotine et 6,6'-dibro-meindigotine (DBI), présents dans la «pourpre des mollusques», sont traités en plus de détail. Les précurseurs incolores présents dans l'organisme sont supposés être d'origine diétique. L'hypothèse est formulée que la formation du DBI est due à un méchanisme de détoxification, et que les espèces produisant la pourpre ont développé ce méchanisme en un système enzymatiquement contrôlé de défense contre de grands prédateurs. Cette première réaction est suivie spontanément — en présence d'oxygène et, pour le DBI, de lumière — par des réactions produisants le pigment coloré. Ce pigment lui-même ne semble avoir aucune valeur pour l'animal. Des essais sur la pourpre de trois espèces méditerranéennes sont décrits.

INTRODUCTION

Two main physico-chemical phenomena may be responsible for colour phenomena in nature :

In the case of structural colours, also called schemochromy, the coloured object does not contain any pigment or dye responsible for the colour involved ; the visual impression of colour is only the result of purely physical effects such

as reflection, refraction and/or interference of light in thin layers of crystal-like structure. Examples are : the colours in the nacre of *Haliotis* shells, on the feathers of birds and wings of butterflies. Non-biological exemples are to be found on soap-bubbles and in the well-known colours of oil spread on water surfaces.

Pigmented objects, however, contain colourants, generally in a form insoluble in the matrix containing them. These pigments can, by means of appropriate chemical techniques, be extracted from the biological material, be purified and analysed so that their chemical structure can be inferred.
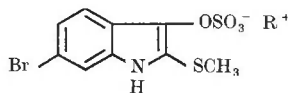
A large number of pigments have been isolated from animals and plants, and are classified according to their chemical structure. A survey of pigments present in mollusca can be found in e.g. Fox (1983). The present paper only deals with indole pigments.
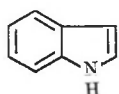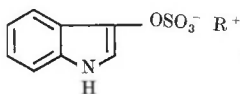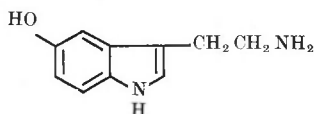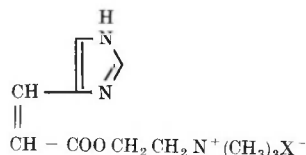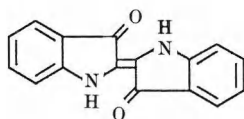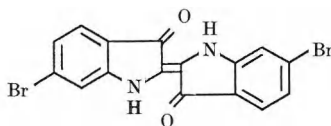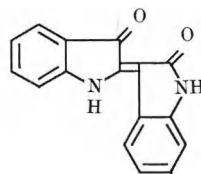

Fig. 1. — Structural formulae of compounds of relevance in this study.
I, indole base structure ; II, indoxylsulphate ($R = K^+$: metabolic indican) ; III, tyrindoxylsulphate ; IV, choline ; V, serotonine (= enteramine) ; VI, murexine (= urocanylcholine) ; VII, indigotin ; VIII, 6,6'-dibromoindigotin (= DBI) ; IX, indirubine.

Indole pigments are derived from the indole base structure (Fig. 1, I). In molluscs, two main groups can be distinguished : the *MELANINS* and the *INDIGOIDS*. The indole pigments are the only mollusc pigments that have historically been used by man because of their colouring properties : the melanin

from *Sepia* was used since Antiquity as writing-ink and as painter's pigment ; and the indigoid « mollusc purple » was the most expensive dye during some 30 centuries. Together with the anthraquinone pigments derived from coccid insects, the indole pigments therefore are unique among animal pigments.

FORMATION OF INDOLE PIGMENTS

The biosynthesis of melanins starts from tyrosine ; that of the indigoids from tryptophan. Both these aromatic amino acids have a partly common biosynthetic
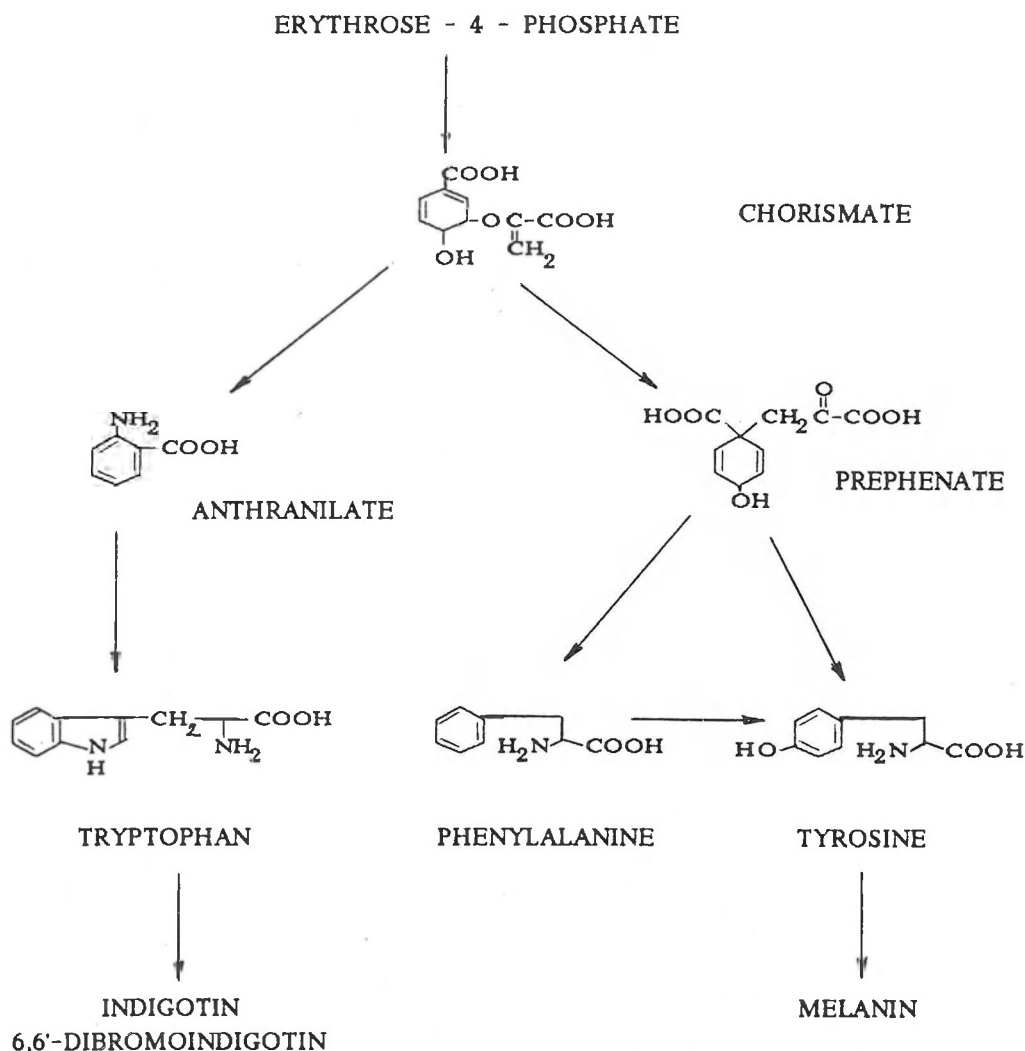

Fig. 2. — Biosynthesis of benzenoid aromatic amino acids.

pathway starting from the phosphorylated sugar erythrose-4-phosphate, as schematically shown in Fig. 2. The reactions shown are almost exclusively restricted to plants : apparently (higher) animals have lost the capacity to synthesize these compounds. Together with phenylalanine, another naturally occurring aromatic amino acid, tyrosine and tryptophan are necessary for the formation of animal proteins. Therefore, animals need plants as a dietary source of these compounds. This applies especially to tryptophan and to phenylalanine, that cannot be synthesized by animal tissue ; in contrast, tyrosine can be formed from phenylalanine by animals. For this reason, tryptophan and phenylalanine are termed « essential » amino acids, while tyrosine is a « non-essential » one.

This distinction may explain the large difference in occurrence between melanins derived from the non-essential tyrosine, and the indigoids formed from the essential amino acid tryptophan. Melanins are very widespread among animals, while indigoids occur very rarely in healthy animal tissues. Indigotin, the main representative of the indigoids, is in fact a pigment of typically vegetal origin ; but it is also produced from secretions of some molluscs (*vide infra*).

MELANINS

Melanin is a very common pigment in the animal kingdom : the darker colours of hair, skin, feathers, etc. are all melanins. Absence of melanin, caused by the lack of the enzyme tyrosinase, leads to albino forms.

The name melanin stands for a group of polymeric molecules formed from tyrosine, over dopa or dopamine, by reactions schematised in Fig. 3, resulting in two different products : dopa-melanin and dopamine-melanin. Recently, DUFF *et al.* (1988 : 7112) stated : « After years of study, no generally accepted molecular structures have been defined for these materials ». More details on its rather complicated formation are given by PETER and FOERSTER (1989). Eumelanin is the deep black product formed in the cited reaction ; Pheomelanin is the yellow to brown pigment mixture formed when also the sulphur-containing amino acid cysteine participates in the reaction (cfr. THOMSON, 1974), producing non-indole chromophores. Allomelanin is a nitrogen-free pigment : it does not contain the indole group. Consequently, pheomelanins and allomelanins fall outside the scope of this paper.

The most striking example of melanin in molluscs is evidently the so-called « ink » of the cephalopods, contained in the ink-gland of these animals. This ink-gland is well-developed in the Sepiacea, but absent in e.g. the Cirrotheutacea. To my knowledge, an ink-gland is also lacking in the Nautilidae and Argonautidae. Extinct cephalopods already had well-developed ink-glands, as is testified by some well-preserved fossils still featuring the dark colour of the melanin : e.g. *Belemnotheutis* from the Oxfordian (Malm, Jurassic) of Chippenham (WOODWARD, 1870 : 186) or *Geotheutis bollensis* from the Upper-Lias (FRAAS, 1910 : 184).

Ink of cephalopods may vary in colour from black over brown to reddish (Fox, 1983 : 294). It is stated that one *Sepia* is capable of obscuring 4,500 litres of water with its ink, but no definition of the term « obscuring » was given (GOLDSMITH, 1950 : 717). Moreover, some deep-sea cephalopods produce not a black, but a luminescent ink (Fox, 1983) of unknown composition.

The role of the ink could intuitively be assumed to be the formation of a visually protective cloud between a predator and the escaping animal ; however, this can hardly be the case at night or in dark environments. The ink is known to contain odorous compounds that may distract predators. A case has been reported of a moray « eel », searching frantically in and around an ink cloud, while the cephalopod has already long fled (Fox, 1983).

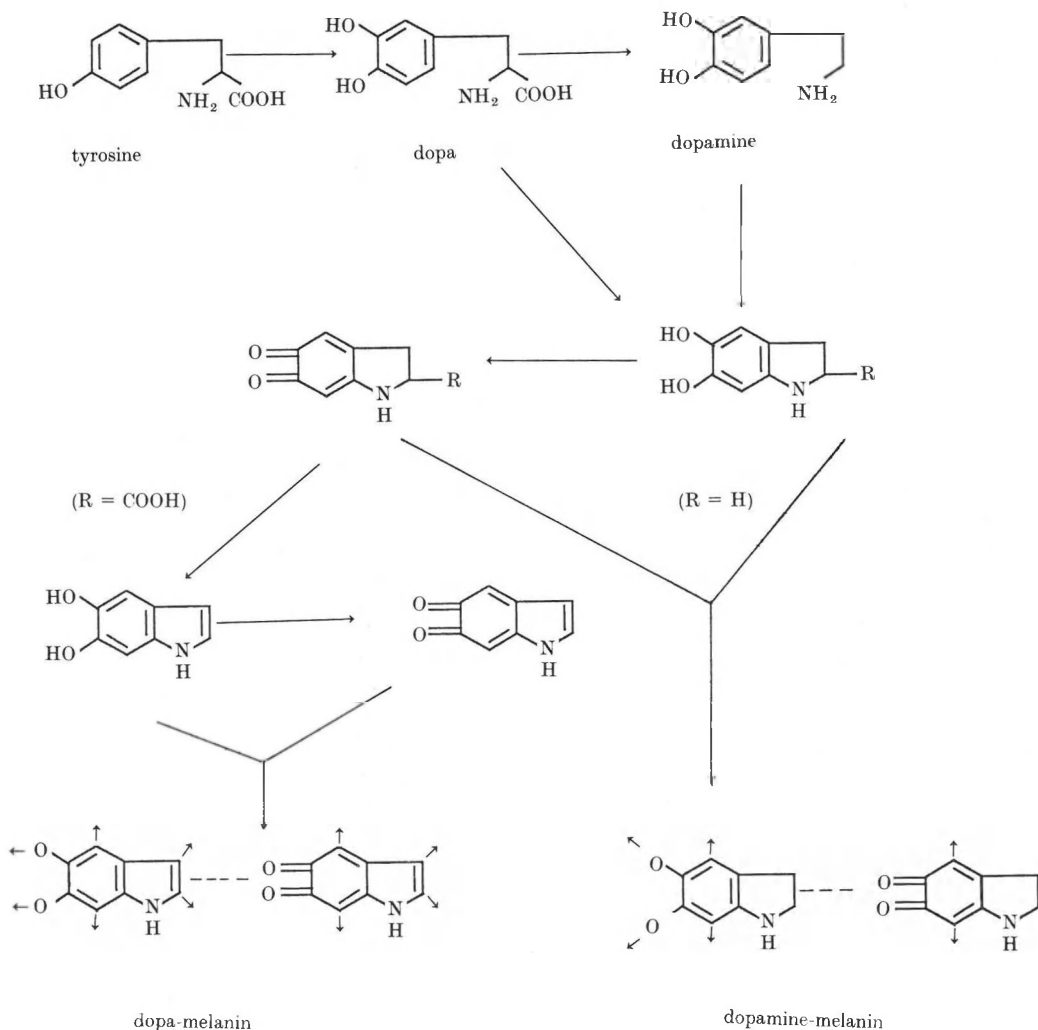

Fig. 3. — Schematics of melanin formation chemistry as understood at present.

As indicated by its name, the ink has been used by man for writing, but also for making a valuable brown-black painter's pigment known as « sepia ». Still today, this is the name of this brown-black colour shade ; it correlates the pigment and the animal (*Sepia officinalis*) used for its preparation in Italy until

recently. Only specimens from the Adriatic Sea contain the dark brown pigment ; animals from the Mediterranean Sea or the Atlantic Ocean produce a black ink unsuitable for the production of the painter's pigment (VERNICOLOR, 1930 : 106). Possibly, this difference may be attributed to changes in pheomelanin content.

<div align="center">INDIGOIDS</div>

The indigoids of importance in the present study are indigotin (blue), 6,6'-dibromoindigotin (purple) and, to a minor extent, indirubin (reddish).

Surveys of the chemistry of mollusc purple (= Tyrian purple), and of the history of its use by man, were given recently (ZIDERMAN, 1986. VERHECKEN, 1984. LAMMENS, 1984) ; they also contain references to the main chemical literature on this matter. Indigotin may be present in very low to very high concentration in the « purple » produced by the different species. The term « mollusc purple » used in this paper refers to the (mixture of) pigments (exact composition often still unknown) produced from the secretions of the molluscs ; « 6,6'-dibromoindigotin » (DBI) refers to the pure chemical compound.

Species producing mollusc purple.

First of all, it must be emphasized that the mollusc purple does not occur as such in the organism of living molluscs : it is only formed from the secretions produced by the animal. Muricids have a hypobranchial gland containing the colourless precursors, mainly indoxylsulphate (Fig. 1, II) and tyrindoxylsulphate (Fig. 1, III). In the reaction sequence of Fig. 4, the latter compounds can be transformed into the insoluble coloured indigoid pigment under the consecutive influence of (a) an enzyme, (b) oxygen and (c) light. The enzyme, purpurase, is a sulphatase produced in the hypobranchial gland but kept apart form the tyridoxylsulphate under normal circumstances.

The sulphated precursors II and III of Fig. 1 have as counter-ion some biologically active amines such as choline (Fig. 1, IV) and its derivatives (BAKER and DUKE, 1976), serotonine (Fig. 1, V) and murexine (Fig. 1, VI) (ERSPAMER, 1952 : 256). Some authors report potassium as counter-ion in the Mediterranean species ; but cited amines have indeed been detected in these species by other authors. This contradiction may be due to the ion-exchange steps in the purification procedure, or to similar effects in the paper-chromatography used in these investigations (e.g. BOUCHILLOUX and ROCHE, 1955).

However, *Murex trunculus* has in its hypobranchial gland also (or mainly, cfr. our experimental results) other, unbrominated precursors not containing sulphur compounds. In their reaction sequence (cfr. ZIDERMAN, 1986 : 49), light is not needed, and the pigment produced is the blue indigotin with possibly some reddish indirubin (BOUCHILLOUX and ROCHE, 1955 : 2).

Those are the general lines of the purple formation in Muricidae. This is not the place to enter in details of this rather complicated chemistry ; moreover, there are still some contradictions between the results of different workers. It must be pointed out that High Performance Liquid Chromatography (HPLC), one of the most successful analytical methods of the last decade, has not yet been applied to these problems. When a suitable HPLC method becomes available, our material (*vide infra*) will be analysed with this technique.

Fig. 4. — Formation of 6,6′-dibromoindigotin from tyrindoxylsulphate.

The great majority of species known to produce mollusc purple belong to the Muricacea, but it has not yet been established if all species of this superfamily, or even of the family Muricidae, possess the precursors. The following species have been reported to produce mollusc purple (the nomenclature has not been critically reviewed) :

Murex brandaris,	Mediterranean	(LACAZE-DUTHIERS, 1859)
M. trunculus	Mediterranean	(LACAZE-DUTHIERS, 1859)
M. cornutus	West Africa	(BAKER, 1974)
Thais haemastoma	West Africa	(LACAZE-DUTHIERS, 1859)
T. lapillus	Atlantic coast Europe	(LACAZE-DUTHIERS, 1859)
T. clavigera	Japan	(cited in ZIDERMAN, 1986)
T. bronni	Japan	(cited in ZIDERMAN, 1986)

T. savignyi	Indonesia	(WEE, *in litt.* to CHARTER, 10.06.1987)
Ocenebra erinacea	Atlantic coast Europe	(LACAZE-DUTHIERS, 1859)
Purpura madreporarum	Atlantic	(BAKER, 1974)
P. patula	Gulf of Mexico	(BAKER, 1974)
P. aperta	Gulf of Mexico	(BAKER, 1974)
Dicathais orbita	Australia	(BAKER, 1974)
Mancinella bufo	Australia	(BAKER and DUKE, 1973)
M. kieneri	Australia	(BAKER and DUKE, 1973)
M. distinguenda	Australia	(BAKER and DUKE, 1973)
Rapana bezoar	Japan	(BAKER, 1974)
Oliva bulbosa	Arabian Sea	(CHARTER, 1987)
O. paxillus	Hawaii	(THORSSON, 1988)
Mitra sp.		(SCHIMELMAN, 1982)
Epitonium sp.		(SCHIMELMAN, 1982)

Citations of *Janthina* sp. must probably be disregarded : my tests on *Janthina janthina* from Florida have shown that the pigment contained in the shell is not an indigoid, as it is destroyed when the shell is dissolved in cold dilute acetic acid ; the pigment secreted by the hypobranchial gland (FRETTER and GRAHAM, 1962 : 562) was not available for study.

Very recently, species of *Oliva* have been cited in the literature as producing a purple dye when held in the hand : *Oliva bulbosa* from Oman (*Charter*, 1987) and *O. paxillus* from Hawaii (THORSSON, 1988). The formation of purple by *Oliva* species was further confirmed by Dr. R. HOUBRICK, NMNH Washington (pers. comm.) Also species of Coralliophilidae, living completely embedded in coral, may produce purple stains on the hands when taken out of the coral alive, or leave spots in their dwelling cavity in the coral when left to dry in it (Dr. C. MASSIN, IRSNB, pers. comm.).

Practical experience with mollusc purple.

All cited data can be found in a fairly abundant literature. However, very few workers seem to have had practical experience with mollusc purple. Therefore, in june 1987 some experiments were performed in Spain with *Murex brandaris*, *M. trunculus* and *Thais haemastoma*. Both *Murex* species were purchased from fishermen in San Carlos de la Rapita, Tarragona province, where the first is commercially fished for consumption, and the second are specimens captured together with other material and used as bait for fishery. The *Thais* species was taken by skindiving, on rocks at a depth of two to three meters at the coast of the same locality, near the cement factory.

With some exercise it is possible to make a hole in the shell, near the hypobranchial gland, without smashing the whole animal (The shell is placed, aperture down, on a hard surface and the hammer is directed at a spot about 1/4 whorl higher than the aperture). The material of this gland was processed in two ways :

1. the mantle was cut open next to the hypobranchial gland, the gland tissue was taken with a rough brush and brushed open on a white cotton cloth. In contact with the air, and exposed to light, the dye is formed fairly rapidly from the

almost colourless precursors. For *Thais haemastoma*, in bright sunlight shortly after one o'clock p.m., this transformation was complete in 9 minutes (*).

Results : *M. brandaris* and *T. haemastoma* produce a similar redpurple colour ; *M. trunculus* gives shades varying from almost pure blue to bluish purple.

2. from some twenty animals the hypobranchial glands were dissected, collected together in about 50 ml of seawater to which 3.5 g of sodium chloride was added ; this was macerated for two weeks at ambient temperature (based on Antique texts ; cfr. VERHECKEN, 1984 : 169). A rather dark liquid was obtained. When exposing this liquid to sunlight, the pigment is formed ; it precipitates in the liquid and can be collected on a filter. Thus, a mixture of products is obtained, containing remains of animal tissue and the pigment(s) 6,6'-dibromoindigotin and/or indigotin.

These products are vat dyes : they can be made soluble by reduction in an alkaline bath, the soluble leuco-form thus obtained applied to textile fibers such as wool, and then reoxidised in the air, forming the insoluble pigment again. Also here, the experiment confirmed the difference between *M. brandaris* and *T. haemastoma* on the one hand (red-purple), and *M. trunculus* on the other (blue purple.).

These experiments led to the following conclusions :

(a) *M. brandaris,* the species generally cited as the main source of Antique purple, yields only a relatively small amount of pigment at this locality and time of the year.

(b) the results of ELSNER and SPANIER (as reported by ZIDERMAN, 1986 : 51) that in *M. trunculus* « individual males ... generally dye blue, while the femals mostly yield purple », could not be confirmed. The two animals that were anatomically checked as to sex yielded results exactly contrary to ELSNER and SPANIER'S, but evidently this may not be understood as a contradiction of their results, because of our extremely limited sample size. It must be remarked here that ERSPAMER (1952 : 256) found a similar distinction between *M. trunculus* specimens from the Adriatic sea (mainly purple) and the Tyrrhenian sea (mainly blue). Further research on this topic is needed.

Biological role of purple formation.

A major question resulting from all these observations involves the significance to the animal of these dye-producing reactions. The only influence the animal may have on these reactions is probably restricted to the first, enzymatic, reaction. As long as, in the hypobranchial gland, the Tyrindoxylsulphate and the purpurase enzyme are kept separate, no reaction occurs. Yet, it is not known if the animal has any direct control over bringing together precursor and enzyme, and thus starting the reactions. In fact, this seems improbable because of the anatomy of the hypobranchial gland as described by LACAZE-DUTHIERS (1859 : 40) a mere layer of oblong cells at the inner side of the mantle wall. Presumably, this layer of cells grows from the mantle on, and cells are liberated into the mantle cavity at the same rate. Only because of pressure (e.g. when the animal contracts vigorously) are the cells massively liberated. They

(*) This transformation sequence will be illustrated in colour photographs elsewhere (VERHECKEN, 1990).

contain a granulated matter ; when liberated, the cells burst open by mechanical or osmotic pressure, and their content is dispersed into the mucus (LACAZE-DUTHIERS, 1859 : 40).

These observations were later refined by the histological work of BERNARD (1890 : 297), who found a well-developed innervation in the gland, suggesting a role in perception.

It has been observed that in wounded or dying animals purple is formed, so that the separation precursor-enzyme must be lost. Dead *M. brandaris* and *M. trunculus,* as found in fishermen's nets drying in Mediterranean harbours, show a purple to bluish spot near the aperture. The fishermen from San Carlos de la Rapita, Spain, call this the « menstruation of the shell ». A case was reported of a fisherman from that locality who uses the animals of *M. trunculus* for bait, and to this end extracts the animals from their shell by means of his teeth : he has his lips coloured blue-purple (BRUNET NAVARRO, pers. comm.), caused by the mucus of the traumatised animals.

In order to obtain some insight into this problem, we consider in Fig. 5 the net result of the reaction sequence of Fig. 4. From its own resources the organism uses for these reactions only the precursor(s) (tyr)indoxylsulphate in ion-pair with choline-related amines ; thereby are produced free cholinesulphate, dimethyldisulfide, and the pigments DBI and/or indigotin.
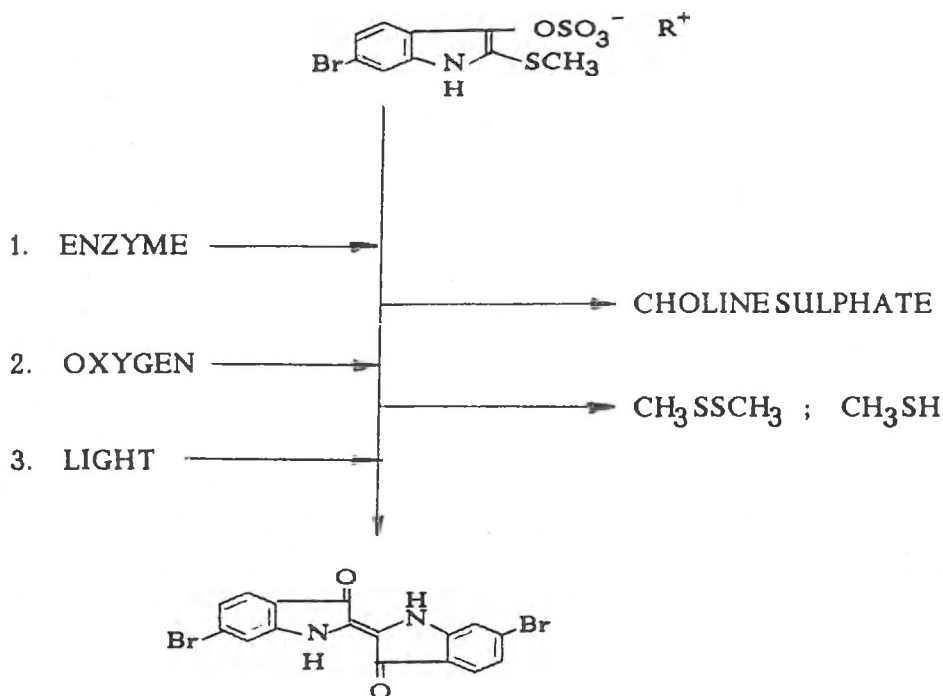

Fig. 5. — Net result of the purple-producing reactions of Fig. 4.

The biological effect of these pigments is unknown. The significance of dimethyldisulfide and methylmercaptane, which is easily formed from the disulfide by reduction, is not documented ; the last has no effect on experimental animals (JULLIEN *et al.*, 1941a : 1636). Interestingly, also in some plants (e.g. onion, garlic), related compounds are only formed enzymatically when the cells are crushed (*Kallio et al.*, 1989 : 174). The only products formed by this reaction sequence that might be of any biological use to the animal are the liberated sulphates of choline, serotonine (= enteramine), or related amines. Serotonine is a vasoconstrictor which also plays a role in neurotransmission ; its chemical structure is very close to that of hallucinogens like bufotenine (from the skin of toads) and psilocin from *Psilocybe mexicana* HEIM, the « sacred mushroom » of Mexico.

It has been shown (JULLIEN, 1948a : 10) that extracts from the hypobranchial gland of *M. trunculus* can be lethal when injected in e.g. fish. However, it was also shown (JULLIEN, 1948b : 102) that there is no effect when the molluscs, even with broken shell, are put in the water in which fish are swimming. So, there is no biological effect when these amines are diluted in seawater.

However, *Purpura patula pansa* from Western central America and *P. patula* from the Caribbean do produce, when captured, a mass of foamy mucus that soon colours purple. We experienced this at Barbados ; and the Indians of Oaxaca, Mexico, used this for dyeing cotton threads purple by passing them through the mucus (GERHARD, 1964). Similar observations were reported recently by CHARTER (1987) who held captured *Oliva bulbosa* from Dubai in his hand for some time : this resulted in a purple spot and an anaesthesized area on his hand. Also THORSSON (1988 : 5) reports the formation of purple by captured animals of *Oliva paxillus* in Hawaii and/or the Solomon Islands.

These arguments might indicate that the liberated amines have a defensive role (repulsion of predator) rather than an offensive one (paralyzing of prey).

Another explanation for the liberation of choline-like sulphates has been suggested : their use as a source of « acid sulphate », possibly useful in the boring of calcareous shells of prey. Muricids possess an accessory boring organ (FRETTER and GRAHAM, 1962 : 511) used alternatingly with the radula, and for which also some chemical action has been suspected, but not proven. As to the hypobranchial gland, it is not clear how the animal could direct its secretion to the spot to be bored. Moreover, since choline and the related amines are strong bases, their (bi)sulphates cannot have strong acid properties and, consequently, must be rather inactive in dissolving calcium carbonate. So, this possibility is fairly improbable.

On the other hand, DUBOIS (1909, cited by *Jullien*, 1948b) suggested that, after mechanically boring the shell of bivalves, the animal introduces the hypobranchial mucus into the bivalve, thereby paralysing the adductor muscles and thus opening the valves for consumption of its soft parts. This hypothesis could not be experimentally verified on *Mytilus* and *Tapes* (JULLIEN, 1948b : 103).

A last explanation for this rather complicated chemistry is that it only acts as a detoxification mechanism, by eliminating (brominated) indoles (as already suggested by DRIESSEN, 1943), choline or related compounds, and sulphur compounds. In this context it is interesting to remark that the molar concentration of chlorine in sea-water is about 660 times higher than that of bromine (calculated from data in Encyclopedia Brittanica, 15th edn. 1984, 16 : 193). Syn-

thetic 6,6'-dichloroindigotin has the same purple colour as has 6,6'-dibromoin-
digotin (SADLER, 1956 : 317), but the first product, or a precursor of it, has not
yet been demonstrated in molluscs. This could be interpreted as confirmation of
a detoxification mechanism for the hightly diluted, but probably biologically
unwanted, bromine derivatives. However, the fact that *M. trunculus* produces
mollusc purple containing mainly indigotion with only a minor part of the
brominated pigment complicates this picture.

For the sake of completeness, it must be mentioned that FISCHER (cfr.
FISCHER, 1950 : 180) thought the gland must have some role in the reproductive
biology of *Nucella,* by causing aggregations of animals attracted by the odour of
the hypobranchial gland secretions ; but this was not widely accepted (FRETTER
and GRAHAM, 1962 : 122).

Origin of precursors.

All this brings us to the question of the origin of the tyrindoxylsulphate. The
unbrominated indoxylsulphate (Fig. 1, II ; metabolic indican) is synthesized from
tryptophan (Fig. 6) by vegetal microorganisms, e.g. the intestinal flora of mam-
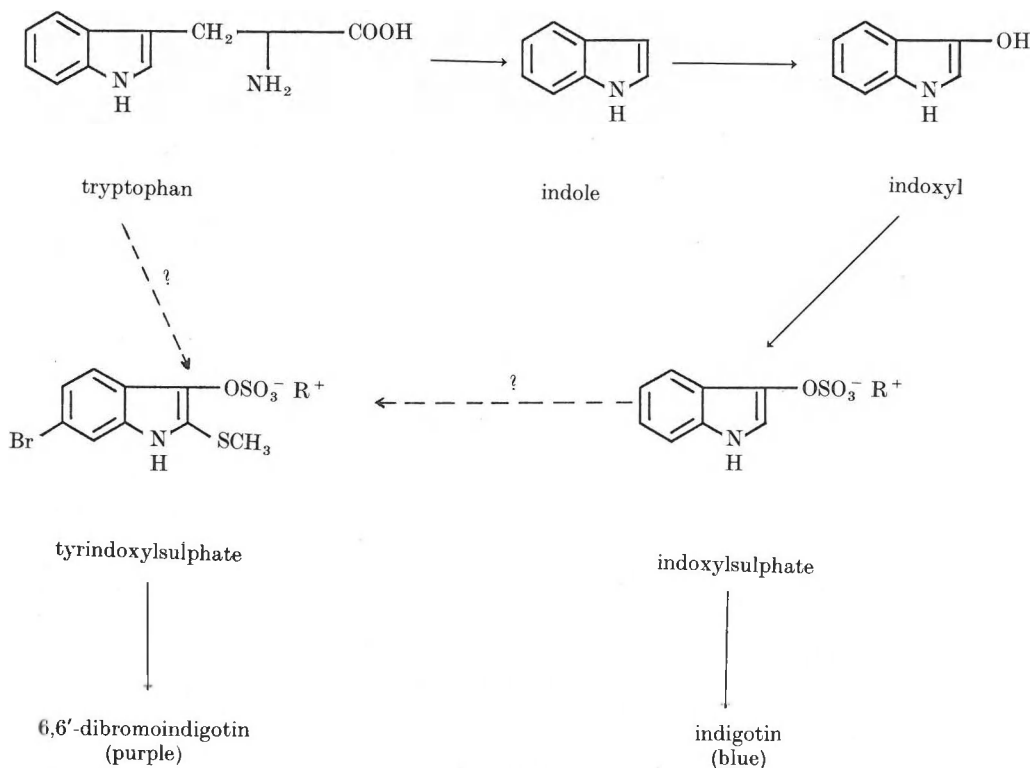mals. This may explain the presence of indigotin in the pigment from *M. trun-
culus.*

Fig. 6. — Schematic formation of indigoid pigments from tryptophan.

The brominated derivative, tyrindoxylsulphate (Fig. 1, III), with the Br in the 6-position of the indole compound, presents a problem. I am not aware of brominations of indole compounds in animal organisms. Brominated indoles have been found in sponges (TYMIAK and RINEHART, 1985) and in species of *Aplysia* (SCHMITZ *et al.*, 1982) : it is agreed that these products are secondary metabolites of the animals, who acquired them from their vegetal diet of e.g. algae.

For the species of Muricidae, Olividae, etc. known to produce purple, this is no explanation : to my knowledge, these animals are carnivorous, so that the presumption of secondary metabolites must be discarded. Are these cases examples of tertiary metabolites, or are vegetal symbionts present in these molluscs ? The real nature of the granules in the hypobranchial cells has not yet been determined : they may simply have some role in the production of the mucus, but they might be crystals or micelles of tyrindoxylsulphate, or even vegetal symbionts. The latter hypothesis, however, is unlikely since light cannot penetrate onto the hypobranchial gland and its possible symbionts so that, if they exist at all, these should be of another nature than e.g. the *Zooxanthella* in the mantle of *Tridacna* species. Photosynthetic vegetal symbionts producing bromoindoles have been suggested for sponges (DJURA *et al.*, 1980 : 1438) ; but the absence of light leaves practically only bacteria as possible (hypothetical) symbionts in the hypobranchial gland. Also this topic needs further research.

It must be pointed out that 6-bromoindoles are very rare in nature. Also, chemical (non-physiological) bromination of indoles produces 5-Br, 7-Br or 5,7-dibromo compounds, but not 6-Br. BOUCHILLOUX and ROCHE (1955 : 18) failed to produce tyrindoxylsulphate by bromination of indoxylsulphate.

Most bromoindoles isolated so far from marine organisms have the Br in 5- or 7- position ; very few have it also, or exclusively, in the 6-position. I have no data on biochemical bromination of indoles ; most probably the bromination occurs before the indole cyclisation. As stated by TYMIAK and RINEHART (1985 : 1044), « the bromoindoles ... appear to be biosynthetically related as alternative metabolites of tryptophan. However, the regiospecific bromination patterns appear to preclude a common bromoindole precursor and suggest divergent biosynthetic pathways ... ».

The fact that BOUCHILLOUX and ROCHE (1955 : 18-19) could not detect 6-Br-tryptophan in the hypobranchial gland of Mediterranean Muricidae is one more indication that tyrindoxylsulphate is not formed in the mollusc nor in hypothetical symbionts in its hypobranchial gland, but has a dietary origin.

Reports on purple produced by other molluscs.

Interesting data on *Cassidaria echinophora* from the Adriatic Sea were already given by OLIVI (1792 : 303). This species has, like many others, a hypobranchial gland similar to that of Muricidae (LACAZE-DUTHIERS, 1859 : 53). OLIVI states that the animal, when disturbed, produces a quantity of colourless viscous mucus that, however, does not turn into purple. Only when the animal in its shell is heated, the viscous mucus becomes fluid, and develops a purple colour. Although I could not experiment on this species, the following explanation is here proposed : the mucus of *C. echinophora* may also contain tyrindoxylsulphate, but not the enzyme purpurase, so that the first reaction of Fig. 4 cannot start. Tyrindoxylsulphate is unstable in heat (JULLIEN, *et al.*, 1941) and in acid medium (BOUCHILLOUX and ROCHE, 1955 : 10). On heating, the animal

probably exudes its salivary secretions known to contain 3 % sulphuric acid (FAENGE, 1984), which decomposes the precursor to tyrindoxyl, that further reacts with formation of mollusc purple. Acid hydrolysis of the mucus polymeric structure would also explain its loss of viscosity on heating.

Also other species of mollusca have been cited as producers of purple : species of *Epitonium* (confirmed by Dr. S. KOOL, MCZ Cambridge, USA, pers. comm.), *Arca* (OLIVI, 1792 : 156), *Mitra* (SCHIMELMAN, 1982), ..., but insufficient information is available on this subject, especially on the chemical nature of these pigments.

Hypothesis on the biological role of the formation of mollusc purple.

Based on the facts cited above, the following hypothesis is proposed :

The presence of the brominated precursors in the hypobranchial gland points to a detoxification mechanism for eliminating bromoindoles from — as yet unspecified — dietary origin. This mechanism may well be present also in several other groups of marine molluscs with a diet similar to that of e.g. muricids. Only some groups (Muricidae, Olividae, etc.) have developed a sulphatase enzyme (pur-purase) enabling them to use this detoxification mechanism also as a defensive system by liberating biologically active amines (choline, serotonine, ...) from the colourless precursors. The formation of the mollusc purple after this first, enzymatic, reaction is of no use to the mollusc ; it is but a mere artifact attributable to a rather coincidental combination of non-biochemical reactions evolving spontaneously in the circumstances available, according to the laws of organic chemistry.

The fact that *M. trunculus* has only very few brominated compounds practi-cally contradicts this hypothesis, unless we assume that the purpurogenic pathway of this species is an evolutionary branch derived from the one here presented.

Also, the production of purple by species of Caralliophilidae living completely embedded in coral and completely protected against predators by both the hard skeletons and nettle cells of the coral, is not directly in keeping with the proposed hypothesis, since there seems to be no need of a supplementary defensive system. Possibly, the embedded Coralliophilidae are an evolutionary branch derived form the free-walking species of this family, and they retained the defensive system they shared with the free-walking species.

Clearly, much more research is still needed in order to shed some light on the remaining problems.

ADDENDUM

Supplemental list of purple-producing species

The following list was compiled by Dr S. KOOL (MCZ), who kindly made it available for publication in the present paper. The list contains some species already mentioned earlier in the paper, but they are included here as confirmation of published data.

During his anatomical work on species of *Muricoidea* (especially *Thaidinae*) Dr. Kool found purplish colour production by living specimens of :

Drupa morum
Vexilla vexillum
Plicopurpura patula
Dicathais orbita
Nassa serta
Cronia amygdala
Neorapana muricata
Stramonita haemastoma
Mancinella alouina
Nucella lappilus
Morula uva
Morula nodulosa
Morula granulata
Drupella cornus

On specimens preserved in alcohol, Dr. Kool observed traces of purple on specimens of :

Concholepas concholepas
Purpura persica
Thais nodosa
Vasula melones
« *Thais* » *kiosquiformis*

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