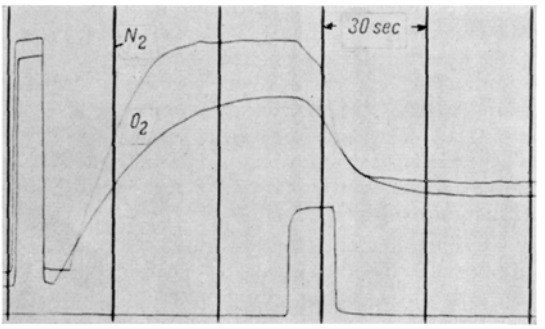
BRINKMAN and LAMBERTS[1] and LAMBERTS[2] have described a phenomenon abolished by the radioprotector cystamine discovered by BACQ and HERVE[3]. We have observed that it is not sensitive to the presence of O_2.



Pressure records. One injection needle is supplied with normally oxygenated saline, the other with oxygen-depleted solution. In each case, calibration of the apparatus to 100 mm Hg; then the pressure is slowly built up until a plateau is reached. The lower curve indicates the time of X-irradiation (175 r/sec). Both pressures fall in the same manner.

Thanks to the help of Dr. LAMBERTS, we have built in Liège the apparatus used by the Dutch scientists. It consists of two very fine injection needles (no. 20) inserted in the skin of a young adult mammal (rat, man) in the deep layer of the dermis where the mucopolysaccharide fibres are numerous. One builds a pressure of about 100 mm Hg by slow constant injection of 0·9% NaCl solution; the pressure is limited by a system of valves and registered on photographic paper by means of electronic tubes which transform pressure in potential changes. The skin is irradiated locally just above the tip of the needle with a soft X-ray beam (30 kV, 15 mA, 175 r/sec); after a latency of only 1 sec, the pressure falls, presumably because hyaluronic acid depolimerizes. This effect is observed in a living or a recently dead rat. We have confirmed BRINKMAN and LAMBERT's observations. In order to be sure that oxygen is not involved, we have worked with rats anaesthetized by Nembutal, killed in a plastic box where a slight pressure of pure nitrogen was maintained. The oxygen dissolved in the injected solution was carefully removed by a vacuum pump and 3 or 4 washings with pure nitrogen. The effect of X-irradiation in this condition of strict lack of oxygen is the same as that seen when no precautions are taken to exclude oxygen (Fig.). We have seen that the radioprotector tryptamine (HCl, 0·01 M) inhibits the fall of pressure just as cystamine does.

Thus the oxygen effect in this case cannot explain the action of cystamine or tryptamine which is better interpreted by the theory of trapping of free radicals as proposed by ALEXANDER, BACQ *et al.*[4].

Z. M. BACQ, P. CICCARONE*, and J. RENSON

Laboratories of General Pathology and of Civil Security, University of Liège (Belgium), November 28, 1958.

[1] R. BRINKMAN and H. B. LAMBERTS, Nature *181*, 774 (1958).
[2] H. B. LAMBERTS, *Chemische bescherming tegen beschadigende bestraling* (Thesis, Groningen 1958), p. 124.
[3] Z. M. BACQ and A. HERVE, Bull. Acad. Roy. Méd. Belg. [VI^e série] *17*, 13 (1952).
[4] P. ALEXANDER, Z. M. BACQ *et al.*, Radiation Res. *2*, 392 (1955).
* Fellow of the Belgo-Italian Commission for Cultural Relations.

Résumé

La chute de pression sous l'influence des rayons X dans une poche de solution saline injectée dans la peau du rat (phénomène décrit par BRINKMAN et LAMBERTS) se produit aussi en l'absence d'oxygène. L'action de certains radioprotecteurs, mise en évidence par cette technique, est donc également indépendante de l'oxygène.

Occurrence of a Pigment Layer in *Gastrothylax crumenifer* (Creplin, 1847)

Pigments in helminths have been known for a long time but during the last decade or two some of these have been shown to resemble haemoglobin (WHARTON[1], STEPHENSON[2], VAN GREMBERGEN[3], DAVENPORT[4], and ROGERS[5]). The present paper gives an account of a pigmented layer in an amphistome, *Gastrothylax crumenifer* (CREPLIN, 1847) commonly parasitizing the rumen of cattle.

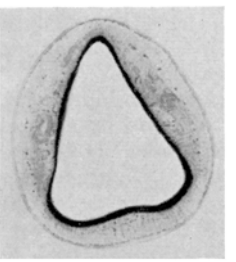


Fig. 1. – Photograph of a thick transverse section of *Gastrothylax crumenifer* to show the pigment layer lining the cavity of the ventral pouch.

The body of this amphistome appears red or pink in fresh condition when removed from the rumen, but in a transverse section of this amphistome (Fig. 1) the colour can be traced to its ventral pouch which is a large cavity showing a red coloured layer. This pigmented layer lies just over the layer of the oblique muscles next to the general parenchyma and appears to be slightly vacuolated. As the pigmented layer extends along the length of the ventral pouch, the entire body of the amphistome appears red. The pigmented layer can be peeled off from the cut surface in an amphistome longitudinally bisected through its ventral pouch (Fig. 2) and parts of the body of the amphistome without this layer appear almost greyish-white. The pigment seems to be concentrated in this layer, there being little dispersal of the pigment elsewhere.

Extraction of this pigment from amphistomes was done by a simple method. Live worms along with some contents of the rumen maintained at about 37°C in a thermos flask were brought to the laboratory from the slaughter house. The worms were thereafter thoroughly rinsed in distilled water and then cut longitudinally into two halves across the cavity of the ventral pouch. The cut worms were

[1] G. W. WHARTON, J. Parasit. *27*, 81 (1941).
[2] W. STEPHENSON, Parasitology *38*, 128 (1947).
[3] G. VAN GREMBERGEN, Enzymologia *13*, 241 (1949).
[4] H. E. DAVENPORT, Proc. R. Soc. London *B 136*, 255 (1949). – R. F. H. FREEMAN and J. LLEWELLYN, J. Mar. Biol. Ass. *37*, 435 (1958).
[5] W. P. ROGERS, Australian J. sci. Res. *B 2*, 287 (1950). – D. KEILIN, Proc. R. Soc. London *B 98*, 312 (1925).

washed quickly with two to three changes of distilled water and then left in distilled water in a frigidaire. Within 48–72 h most of the pigment was extracted in water and the amphistomes became colourless. Peelings of the pigmented layer were obtained from the ventral pouch of the amphistome and treated in a similar way. This gave the pigment solution with little contamination with the animal tissue fluid. In fact this latter method was more

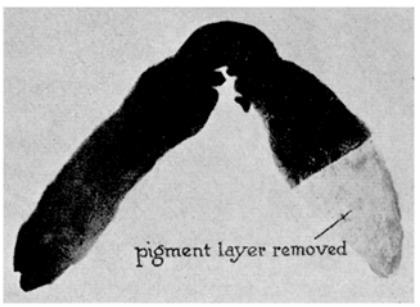


Fig. 2. – Photograph of a specimen of *Gastrothylax crumenifer* split longitudinally into two halves to show the pigment layer lining the ventral pouch. Part of the layer removed in one half to show the grey body.

satisfactory as most of the pigment is confined to the lining of the ventral pouch. The pigment solution was centrifuged and the supernatant clear red fluid was separated and stored in a frigidaire. When 4% trichloracetic acid or 90% alcohol was added to this fluid, a brownish red precipitate, mainly of protein, was formed and a colourless solution was left. This suggested the association of pigment with protein. In another experiment, glacial acetic acid was added to the aqueous solution of the pigment and the mixture shaken in a test tube with a little ether and allowed to stand for some time. Three distinct layers or

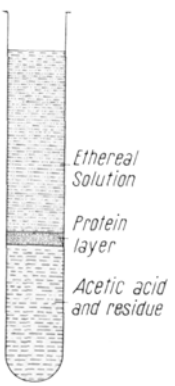


Fig. 3. – Diagram to show the three layers after treatment of the pigment solution with acetic acid and ether.

columns of liquid (Fig. 3) appeared in the test tube (HAWK, OSER, and SUMMERSON[6]). The upper column contained a reddish etherial solution of the pigment, below this was a narrow zone of precipitated protein which was devoid of colour (the colour having been isolated from the protein by the action of glacial acetic acid and ether), and the bottom column was a mixture of acetic acid and residual water. Both the aqueous and etherial solutions were tested for the presence of iron. A few drops of these solutions were evaporated to dryness and the residue ignited and extracted with dilute hydrochloric acid. A distinct blue coloration was developed in the solution on addition of a drop of dilute potassium ferrocyanide solution, thereby indicating the presence of iron. No such coloration was obtained in the controls.

When a dried film of the aqueous solution of the pigment on a glass slide was flooded with a drop of glacial acetic acid containing a few crystals of potassium iodide and examined under a polarizing microscope, minute boat-shaped birefringent crystals of protoiodoheme were observed which resembled protoiodoheme crystals prepared from human blood (GLICK[7], p. 62). In another experiment, a small drop of the aqueous solution of the pigment greatly diluted with distilled water was put on a glass slide. Then a fraction of a drop of 0·75% NaCl was added to it and the fluid carefully evaporated to dryness over a microburner. A cover glass was put over the dried spot and a drop of glacial acetic acid was run underneath. The slide was warmed gently until the formation of gas bubbles. Another small drop of glacial acetic acid was run beneath the coverslip and the preparation on cooling was examined under the microscope. Parallelogram-shaped hemin crystals were observed which also resembled the hemin crystals prepared from human blood (HAWK, OSER, and SUMMERSON[6]).

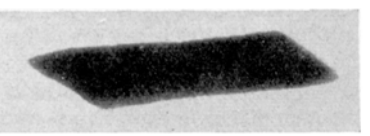


Fig. 4. – Photomicrograph of a greatly enlarged parallelogram-shaped hemin crystal obtained from the pigment of *Gastrothylax crumenifer*.

Pigment extracted from 20 amphistomes in about 2 ml of water and diluted 1:4 was subjected to a spectroscopic examination by Hilger's spectrograph and a microspectroscope. This revealed two absorption bands, a-band at 5735–5850° Å and b-band at 5410–5535° Å (diffuse). In order to determine the peaks within these ranges, a Bausch and Lomb spectrophotometer was employed which indicated the absorption peaks at 5750 and 5450° Å respectively, corresponding to the two bands. Addition of Takayama's fluid (HAWK, OSER, and SUMMERSON[6]) to the aqueous solution of the pigment did not materially alter the position of the original two bands; but, when the mixture was heated and examined by a microspectroscope, two characteristic bands different from the normal, one at 5550–5650° Å (sharp) and the other 5200–5300° Å (faint) were seen. These apparently conform to the characteristic bands of pyridine haemochromogen produced when the haem pigments are treated with Takayama's fluid.

The pigment obtained from the lining of the ventral pouch of *Gastrothylax crumenifer* is associated with protein. This fact is proved firstly by the coloured protein precipitate obtained after treatment with trichloracetic acid, secondly by the fact that the pigment appears to dissolve more readily in ether after acetic acid treatment, and thirdly by the liberation of the coloured product from the protein during this treatment. That the pigment might probably be of a haemin type was also made clear when an aqueous solution of the pigment was subjected to a spec-

[6] P. B. HAWK, B. L. OSER, and W. H. SUMMERSON, *Practical Physiological Chemistry* (Toronto 1954).

[7] D. GLICK, *Techniques of Histo- and Cytochemistry* (New York 1949).

troscopic examination. This revealed the usual two absorption maxima at 5750 and 5450. Å. With the addition

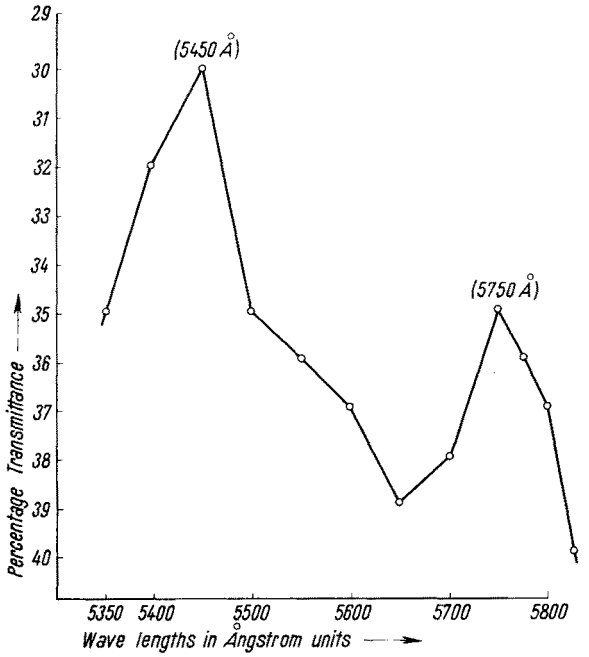


Fig. 5. – A graph showing the absorption peaks of the pigment solution of *Gastrothylax crumenifer*.

of Takayama's fluid and application of heat, the characteristic pyridine haemochromogen bands at 5200–5300° Å (faint) and 5550–5650° Å (sharp) were also detected on spectroscopic examination. The chemical detection of traces of iron and the positive deposit of boatshaped protoiodoheme and parallelogram-shaped hemin crystals comparable to those obtained from human blood, suggest a haem base in this pigment. The solubility tests also point to a non-carotenoid nature of the pigment. It is, however, not possible for the writer at this stage to explain the peculiar disposition of the pigmented layer in the ventral pouch and its possible role in the physiology of this amphistome.

M. B. LAL

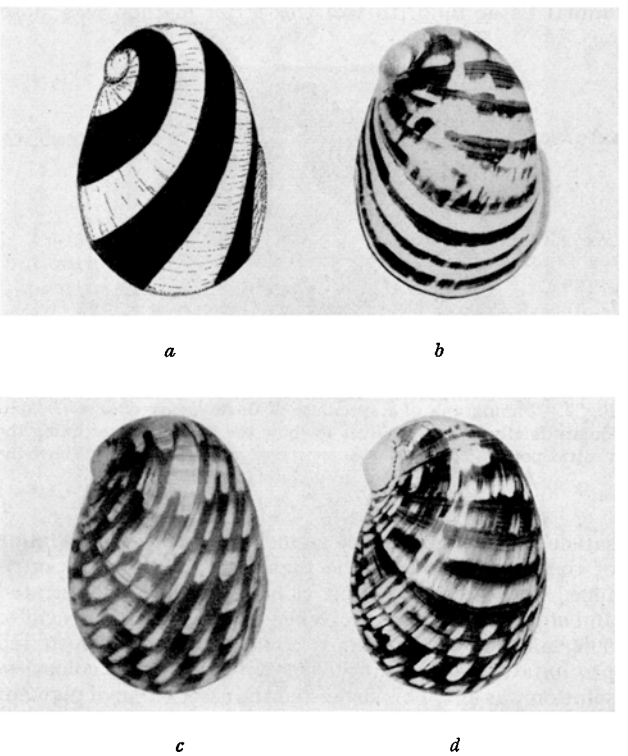
Department of Zoology, The University, Lucknow (India), December 1, 1958.

Résumé

Il s'agit de l'étude d'une couche rouge pigmentée formant la surface intérieure de la poche ventrale de l'amphistome *Gastrothylax crumenifer* (CREPLIN, 1847). Les expériences de solubilité démontrent la nature non-caroténoide de ce pigment. L'examen spectroscopique et les expériences chimiques prouvent la présence d'une base (haem) dans le pigment.

Farbmusterumschlag auf der Molluskenschale

Zahlreiche Untersuchungen an Farbmustern von Schmetterlingen, Vögeln und Säugern haben gezeigt, dass die tierischen Farbmuster geeignete Modelle für eine entwicklungsphysiologische Analyse einfacher Gliederungsvorgänge sind (HENKE[1]). Die Farbmuster der Molluskenschale sind dagegen aus Mangel an einem geeigneten Laboratoriumstier bisher wenig berücksichtigt worden. Ihre morphologische und experimentelle Untersuchung konnte neuerdings aufgenommen werden, nachdem die Züchtung der einheimischen Flussdeckelschnecke *Theodoxus fluviatilis* L. (abgekürzt *Th. fl.*) gelungen war.



Farbmuster von *Theodoxus fluviatilis* aus der Werra. *a–c:* die drei Farbmustertypen (Erläuterungen siehe Text); *d:* sukzessiver Musterumschlag zwischen dem synchronen Querstreifenmuster und dem phasenlängenkonstanten Längsmuster. Vergrösserung 1:5.

Das Farbmuster von *Theodoxus* gehört der zwischen Periostracum und Hauptkalkschicht gelegenen Musterkalkschicht an (BECKER[2]). In ihr finden sich im Bereich dunkler Schalenteile braune Einlagerungen, das Schalenpigment[2], und im Bereich heller Schalenteile farblose Einlagerungen, die Schalengranula. Die Musterkalkschicht erfährt einen Zuwachs nur längs des Schalenrandes und ist einer gesonderten Bildungszone des Mantelrandepithels zuzuordnen[2]. Aus der gleichbleibenden oder wechselnden Verteilung von pigment- und granulabildenden Zellbereichen dieses Musterkalkschicht-Epithels fügt sich im Wachstumsverlauf das Schalenmuster von *Theodoxus* zusammen. Bei *Th. fl.* können bisher drei Farbmustertypen gegeneinander abgegrenzt werden, denen qualitativ voneinander verschiedene Gliederungsvorgänge zugrunde liegen. 1. Das Sektorenmuster (Abb. 1 *a*, halbschematisch) ist mit dem Muster der einheimischen Bänderschnecken vergleichbar. Es entsteht, wenn die Verteilung der bevorzugt pigment- und granulabildenden Bereiche bereits frühzeitig in der Entwicklung determiniert und anschliessend während des weiteren Schalenwachstums unverändert beibehalten wird. 2. Das synchrone Querstreifenmuster (Abb. 1 *b*) erscheint auf der Schalenfläche, wenn Pigment- und Granulabildung zeitlich nacheinander längs des gesamten Schalenrandes mit-

[1] K. HENKE, Naturwissenschaften *35*, 176, 203, 239 (1948).

[2] K. BECKER, Biol. Zbl. *68*, 263 (1949).