calculée pour la superficie centrale circulaire Mg–N_4, de la chlorophylle, mais elle est, très nettement plus petite que la superficie du noyau carré de la porphyrine, qui est de 105 (Å)[2]. Ce résultat est interprété comme preuve supplémentaire pour la théorie photoélectrique de photosynthèse, selon laquelle l'auteur a postulé que c'est l'atome Mg de chlorophylle qui est excité électroniquement par absorption de lumière.

Cytochrome *h* from *Aplysia depilans* L.

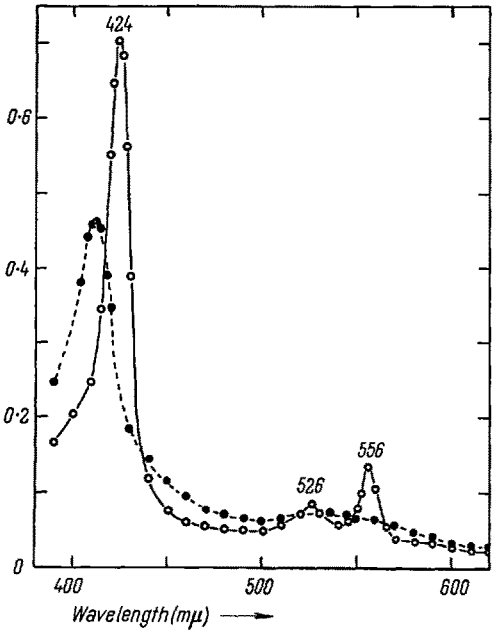
An hemochromogen very similar to Helicorubin has been recently extracted and purified from the digestive gland of *Helix pomatia*[1]. The pigment, which was found to have several properties in common with the cytochromes of the *b* and *c* groups, has been named Cytochrome *h*. According to KEILIN, Cyt. *h* is genetically related to Helicorubin, this latter probably representing its extracellular from.

In the course of an investigation on the cytochrome system of marine Gastropods[2] we have found that, by a method similar to that described for Helix, a pigment can be extracted and purified from the Hepatopancreas of Aplysia giving a spectrum very similar to Cyt. *h* both in the reduced and in the oxidized forms. An account of the isolation and purification of the pigment, which is here indicated as Aplysia Cytochrome *h*, followed by some of its chemical and biological properties, is given in this note.

Aplysia is an herbivorous marine Gastropod which has no oxygen carrier pigment in the blood and no Helicorubin in the intestine nor in any other part of the body[3]. Two species, which are very common in the Bay of Naples, were used: *A. depilans* and *A. limacina*. The best source of material was found to be *A. depilans* and this was therefore preferred for most of the experiments.

The animal was dissected under sea water and the hepatopancreas liberated from its ligaments and from the intestine. For each preparation 500–600 g of tissue from 20–30 animals was used. Fresh organs or acetone powders were used. Acetone powders were found more practical on account of the great amounts of green and yellow pigments which were removed during the preparation. They were obtained by homogenization in a Waring blendor for 1–2 min in cold (– 20°C) acetone, followed by rapid filtration and washing until no color was extracted. The powdered material was extracted for 2 h with alkaline (pH 8) water and centrifuged; the sediment was discarded and the supernatant was treated with 1–2% basic lead acetate. The exact concentration depends upon the dilution of the extract and is determined for each individual experiment. The precipitate was removed by centrifugation and the excess of lead in the supernatant was precipitated at pH 6·0 with sodium sulfate. Treatment with $(NH_4)_2SO_4$ at 65% saturation gives a precipitate which contains the hemochromogen. The precipitate was filtered on a Hyflo Cell bed, washed with 65% $(NH_4)_2SO_4$ and dissolved in water. The purification was repeated several times with ammonium sulfate from pH 5 to 8, followed by negative adsorption on calcium phosphate gel or Cy alumina, and finally the pigment was dialysed against distilled water and concentrated under *vacuo*. Heating at 68°C, as required for the preparation of Helix Cyt. *h*, was not necessary, since Aplysia hepatopancreas does not contain cellulase.

In its oxidized form, the pigment extracted from Aplysia's hepatopancreas shows a peak at 410–412 mμ in the Soret region of the spectrum and a broad band with a maximum around 536 mμ in the visible region. After reduction with dithionite, three bands appear with peaks at 423–424, 526–528 and 556 mμ (Fig.). The *γ* band, both



Absorption spectra of Aplysia Cytochrome *h* in the oxidized (dodded line) and in the reduced (full line) forms.

in the oxidized and in the reduced forms of the pigment, is slightly shifted towards higher wavelengths compared with the values obtained by KEILIN for Helix Cyt. *h* (Table).

Table

Position of absorption bands (mμ) of Cytochrome *h* from Aplysia and Helix Hepatopancreas

		Band	Band	Band
Aplysia	Oxidized	—	536–540	410–412
	Reduced	556	526	424
Helix*	Oxidized	562	536	408
	Reduced	556	526·5	422

* From J. KEILIN[1].

Aplysia Cyt. *h* is reduced by dithionite, lithium hydride, ferrous oxalate, cysteine, ascorbic acid, and glutathione. It is oxidized by ferricyanide, ferric oxalate, and hydrogen peroxide. After treatment of Cyt. *h* with NaOH 0·2 *N* and pyridine, followed by reduction with dithionite, a spectrum is obtained with peaks at 415, 520, and 550 mμ. Attempts to extract iron porphyrin with acidic acetone were not successful, even when treatment with HCl 0·5 *N* had been made previously. The spectrum of the Cyt. *h*-cyanide compound, in its reduced form, has peaks at 424–426, 530–532, and 560–562 mμ.

[1] J. KEILIN, Biochem. J. *64*, 663 (1956); Nature *180*, 428 (1957).
[2] L. TOSI, A. GHIRETTI-MAGALDI, and F. GHIRETTI, R. C. Accad. Lincei *23*, 447 (1957).
[3] E. A. PHEAR, Proc. zool. Soc., London *125*, 383 (1955).

After reduction with ascorbic acid at pH 7·4, Aplysia Cyt. *h* is oxidized by a beef heart cytochrome oxidase preparation in absence of any Cyt. *c*[4]. Added Cyt. *c* increases the rate of oxidation, but is not necessary for the reaction as was found for the Helix pigment[1]. Beef heart preparation catalyses the reduction of Aplysia Cyt. *h* in the presence of cyanide and of DPNH. When succinate is used, cyanide is not necessary.

From the above data it seems that the pigment extracted from Aplysia Hepatopancreas can be identified with Helix Cyt. *h*. Although they differ from each other in certain respects, both pigments have many properties in common.

More work is required to establish if the term of Cytochrome *h* can be maintained, or if the pigment must be listed in one of the already known groups of cytochromes. Whereas its spectrum is similar to that of mammalian Cyt. b_5, Aplysia Cyt. *h*, like the Helix pigment, has several properties in common with Cyt. *c*. A cytochrome having the same spectrum as mammalian Cyt. *c* can, however, be extracted from Aplysia's muscles by the method of KEILIN and HARTREE[5], while all attempts to extract Cyt. *h* from organs of the animal other than the Hepatopancreas, were unsuccessful.

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Riassunto

Nell'epatopancreas di due Gasteropodi marini: *Aplysia depilans* e *A. limacina* è presente un emocromogeno assai simile al citocromo *h* di Helix. Il pigmento è stato estratto e purificato e le sue proprietà fisiche, chimiche e biologiche sono state confrontate con quelle dell'emoproteina di Helix.

[4] D. KEILIN and E. F. HARTREE, Proc. Roy. Soc., London [B] *125*, 171 (1938).

[5] D. KEILIN and E. F. HARTREE, Proc. Roy. Soc., London [B] *122*, 298 (1937).

Toxicity of Benzenethiol and its Derivatives on *Musca domestica* (DDT-Resistant Strain) and on *Periplaneta americana*

In the course of our research on the insecticidal properties of a series of halogenoacetates[1] on the resistant flies, it has been observed that benzenethiol chloroacetate and benzenethiol bromoacetate are markedly more toxic than all other esters tested.

The greater toxicity of such products might be due to to benzenethiol itself; in fact, benzenethiol and some of its derivatives have been employed in the past as insecticides[2] and are also known as bactericidal agents[3].

[1] M. BOCCACCI and S. BETTINI, R. C. Ist. sup. Sanità *19*, 1237 (1956); in press (1958).

[2] D. E. H. FREAR, *A catalogue of insecticides and fungicides*, vol. 1 (Waltham, Mass., U.S.A. 1947). – W. V. KING, *Chemicals evaluated as insecticides and repellents at Orlando, Fla.*, U.S. Dept. Agric., Agric. Handbook No. 69 (1954).

[3] A. BALLIO and E. CINGOLANI, Boll. Soc. ital. Biol. sper. *29*, 622 (1953).

We have studied the toxicity of these substances on insects under the same conditions as employed for halogenoacetates.

A highly DDT-resistant strain (K_1) of *M. domestica* and a strain of *P. americana*, both bred in laboratory, have been used. Acetonic solutions of the substances have been injected into roaches. A contact method to be described[4] has been used for the housefly: slightly volatile substances only have been tested.

Toxicity of benzenethiol and some of its derivatives on *P. americana* and on *M. domestica*

Compound	*P. americana* LD_{50} µg/g, (Injection)	*M. domestica* LD_{50} g/m² (Contact)
Benzenethiol chloroacetate . . .	100	—
Benzenethiol bromoacetate . . .	100	—
Benzenethiol	45	—
Phenyl disulfide	50	> 1
Benzenethiol benzoate	400	> 1
Benzenethiol caproate	150	0·9
Benzenethiol caprylate	>680	0·5
Benzenethiol caprate	300	0·5
Benzenethiol laurate	>680	>1·0
Benzenethiol myristate	>680	>1·0
p-Toluenthiol	130	—
p-Tolyl disulfide	600	>1·0
p-Toluenthiol caprate	>680	0·9
p-Acetyl benzenethiol	500	—
p-Acetyl benzenethiol chloroacetate	470	>1·0
p-Nitrobenzenethiol chloroacetate	>680	>1·0

As can been seen from the Table, benzenethiol shows a toxicity superior to that of its chloro- and bromoacetates. Phenyl disulfide only, among the derivatives, maintains a toxicity equal to that of benzenethiol; the thioesters are much less toxic; the addition of a group in the para position causes a marked loss of activity.

Very little is known yet on the mode of action of these substances. Recently VAN EYS and KAPLAN, and VAN EYS *et al.*[5] have shown that thiol compounds may react with pyridine nucleotides (DPN) and TPN) interfering with the activity of the dehydrogenases which depend from them.

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Riassunto

Gli autori hanno studiato la tossicità del tiofenolo a di alcuni suoi derivati per gli insetti. Il tiofenolo si è rivelato notevolmente tossico sia per *Periplaneta americana* che per *Musca domestica* (ceppo resistente al DDT), mentre la maggior parte dei suoi derivati è fornita di scarsa tossicità.

[4] S. BETTINI, M. BOCCACCI, and GIUSEPPINA NATALIZI, J. Econ. Ent. in press (1958).

[5] J. VAN EYS and N. O. KAPLAN, J. biol. Chem. *228*, 305 (1957). – J. VAN EYS, F. E. STOLZENBACH, L. SHERWOOD, and N. O. KAPLAN, Biochem. Biophys. Acta *27*, 63 (1958).