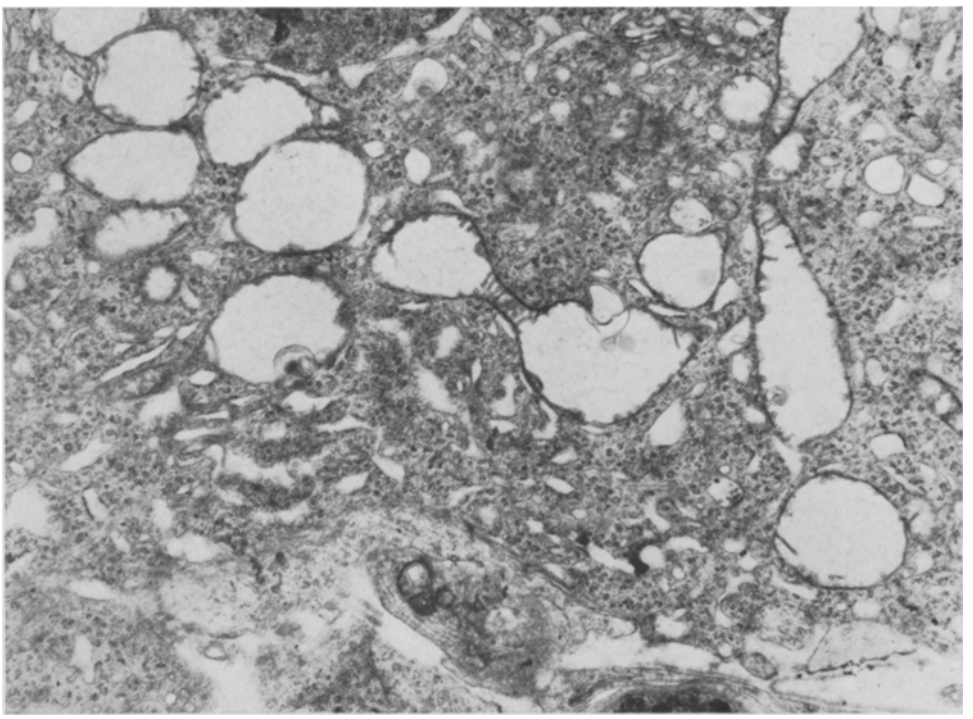

Part of a pinealocyte of the rat after 30 days of continuous illumination. Mitochondria are extremely swollen and deformed. × 18,000.

All animals kept under artificial lighting presented the typical appearance of swollen mitochondria. Swelling of mitochondria has been described by previous investigators both after in vitro and in vivo conditions in connection with cell injury. Animals kept in darkness or a light-dark cycle had intact mitochondria. Our electron micrographs of the rat hypothalamus provided findings similar to those found in the pineal body. These results will be reported in a later publication.

Since swelling of mitochondria could not be registered under conditions involving an increased metabolism of norepinephrine, we assume that the intensified metabolism of serotonin under continuous illumination is responsible for this effect. Mitochondrial swelling is a rather stereotypical reaction of the organelle under various conditions and in response to various agents. Absence of ADP (or ATP)[5], a decreased P:O ratio[6,7], or the uncoupling of oxydative phosphorylation[8,9] are some of the factors leading to this ultrastructural alteration. Since serotonin has been proved to suppress phosphorylation[10,11], it is possible that swelling of mitochondria can be accounted for by the increased serotonin metabolism induced by exposure to continuous illumination. A detailed discussion of this explanation, as well as of a possible physiological significance of the described effect, will be published elsewhere.

*Zusammenfassung.* Die Rattenepiphyse von Tieren, die 30 Tage lang einer ständigen Belichtung von 70 Lux Stärke ausgesetzt worden waren, wurde elektronenoptisch untersucht. Alle Tiere wiesen mitochondriale Veränderungen der Pinealozyten auf. Diese Veränderungen sind vermutlich auf den erhöhten Serotoninstoffwechsel zurückzuführen, der durch die ständige Belichtung induziert wird, da die Kontrolltiere keine derartigen Abartigkeiten zeigten.

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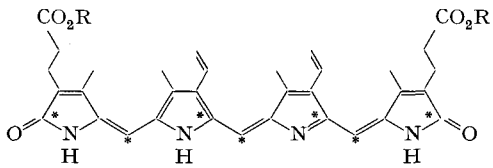
## On the Biosynthesis of Biliverdin-IX$\gamma$ in *Pieris brassicae*

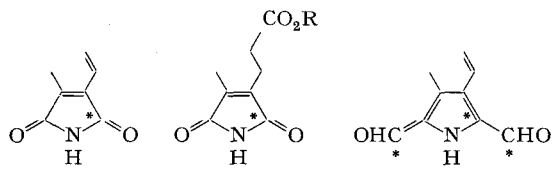
Recently[1] we identified pterobilin, the blue-green tegumental pigment of caterpillars of the cabbage butterfly *Pieris brassicae*, as biliverdin-IX$\gamma$ (Ia). Its biosynthesis is of particular interest because it is the first known natural bile pigment which has not the IX$\alpha$ structure. Bile pigments of vertebrates are formed from glycine and acetate via porphyrines and hemes[2]; glycine is also incorporated into biliverdin-IX$\alpha$ in Orthopteres[3]. The question arose whether pterobilin is formed from small precursors in the caterpillars or whether it is derived from a hitherto unknown pyrrole compound ingested with food. We therefore tested the possible incorporation of labelled glycine into biliverdin-IX$\gamma$.

220 caterpillars (5th stage)[4] are injected each with 1.36 $\mu$Ci of 1.2 $^{14}$C-glycine (specific activity 90 mCi/m$M$) dissolved in 5 $\mu$l of the Ringer solution for insects, and

kept alive for 52 h. The chromoproteid and pterobilin dimethylester (Ib) are then prepared as before[1]. After TLC[5] with benzene/petrol/methanol = 60:5:3 (solvent A) the pigment is rechromatographed with benzene/dioxane/acetic acid = 12:2:1 (solvent B) and again with solvent A. Methyl pheophorbides a and b are purified by rechromatography with petrol/ethyl acetate/diethylamine = 58:30:13. The degradation products of Ib, namely II, IIIb, and IV, are separated by repeated TLC with carbon tetrachloride/ethyl acetate/cyclohexane = 15:3:2 on silica gel $G_F$ (Merck); the spots are marked under UV-light and then eluted with methanol. For calculations the following molar absorption coefficients are used – imide IIIb: $\varepsilon_{222} = 19{,}300$ (determined for IIIa[6]); dialdehyde IV (maxima at 314 and 258 nm): $\varepsilon_{316} = 20{,}500$, $\varepsilon_{243} = 10{,}680$ (determined for the ethyl – instead of vinyl – derivative[6]).



Ia: R = H
Ib: R = $CH_3$



II

IIIa: R = H
IIIb: R = $CH_3$



IV

Table I. Incorporation of 1,2-$^{14}$C-glycine into biliverdin-IX$\gamma$

| Fraction | Biliverdin-IX$\gamma$ [$\mu M$] | Radioactivity [cpm] | Specific activity [cpm/$\mu M$] |
|---|---|---|---|
| 1 | 0.40 | 11,580 | 28,900 |
| 2 | 0.24 | 6,820 | 28,400 |
| 3 | 0.146 | 4,180 | 28,600 |

Fraction 1, purified by TLC in solvent A; fraction 2, rechromatography of fraction 1 in solvent B; fraction 3, rechromatography of fraction 2 in solvent A.

Table II. Distribution of radioactivity in products obtained by chromate oxidation of $13.4 \times 10^{-2}$ $\mu M$ biliverdin-IX$\gamma$

| Product | Amount recovered [$\mu M$] | Activity [cpm] | Specific activity found | calculated [cpm/$\mu M$] |
|---|---|---|---|---|
| Hematinic acid imide methylester (IIIb) | $8.86 \times 10^{-2}$ | 422 | 4,760 | 4,100 |
| Methylvinylpyrrole dialdehyde (IV) | $2.56 \times 10^{-2}$ | 370 | 14,500 | 12,300 |

The radioactivity is localized on chromatograms with the Dünnschicht-Scanner type II (Berthold, Wildbad/Germany) and determined in solutions in a liquid scintillation counter (Philips type PW).

Glycine is effectively metabolized by the animals: We find a total activity of $3.81 \times 10^6$ cpm in the chloroform layer after washing with water. Biliverdin-IX$\gamma$ (fraction 1, Table I) contains 0.3% of this activity. The specific activity remains constant during rechromatography (fractions 2 and 3, Table I) whereas pheophorbides a and b are inactive after rechromatography. We conclude that pterobilin is biosynthetized from small precursors by the caterpillars but pheophorbides are derived from chlorophylls ingested with food.

If the biosynthesis of pterobilin follows the 'normal' route of bile pigment formation, there should be 7 labelled carbon atoms in the molecule after incorporation of 1,2-$^{14}$C-glycine (cf. asterisks in formula I). After chromate oxidation, *one* of these carbons should be found in each molecule of imides II and IIIb while the dialdehyde IV should contain *three* of these per molecule. The chromatogram of the degradation products shows that the only detectable activity is – apart from that of unreacted pigment and polymerization products of vinyl compounds at the starting point – associated with the spots of hematinic acid imide methylester (IIIb) and the pyrrole dialdehyde IV. These compounds are eluted and their specific activities determined (Table II); their identity and purity are then verified by staining on a second chromatogram. The yield of the missing imide II was sufficient only for identification but not for determination of either amount or activity.

The specific activity of IV is three times that of IIIb as expected; furthermore the data agree with calculated figures obtained from the specific activity of biliverdin-IX$\gamma$. This means that glycine is incorporated into biliverdin-IX$\gamma$ in *P. brassicae* in a specific manner corresponding to the normal way of bile pigment biosynthesis.

*Zusammenfassung.* Radioaktiv markiertes Glyzin wird von Raupen des Kohlweisslings (*Pieris brassicae*) in Biliverdin-IX$\gamma$ (Pterobilin), nicht jedoch in die gleichfalls aus dem Tegument isolierten Phäophorbide eingebaut. Die Aktivitätsverteilung in den Abbauprodukten IIIb und IV zeigt, dass dieser Einbau entsprechend dem «normalen» Biosyntheseweg für Gallenfarbstoffe erfolgt.

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[4] Thanks are due to Prof. Hurpin and Mrs. I. N. R. A. Arnold-Chauffaux, La Minière-91, France, for the kind gift of the animals.
[5] TLC: Thin-layer chromatography on silica gel G (Merck).
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