Effect and fate of intravenously administered porphobilin in rats

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Summary. In rats after i.v. porphobilin, blood pressure and pulse rate remained stable, the animals were calm and moved freely with no symptoms or signs of nervous effect. Porphobilin was rapidly excreted in the urine.

The role of porphobilinogen (PBG) in the formation of porphyrin, in vivo, is well-known[1-8]. The metabolic pathway from uroporphyrinogen to protoporphyrin, and its incorporation into heme has also been established[2]. In both older and quite recent studies the mode of conversion of PBG to uroporphyrinogen III has been clarified[4-9]. This reaction includes formation of di-PBG, tri-PBG, and tetra-PBG intermediates[6]. These intermediates were prepared synthetically and their incorporation into uroporphyrinogen III and I has been confirmed experimentally, in vivo[8].

In the acute attack of acute intermittent porphyria (AIP), neurological symptoms are prominent[10], but in spite of great interest, the question of their cause remains unknown. In our own studies, increased levels of δ-aminolevulinic acid (ALA) and PBG (given i.v.) are nontoxic in rats, and those intermediates are rapidly excreted. An inhibitory effect of ALA, PBG and porphobilin (PB) on the neuromuscular junction has been reported[11], but is thus far unconfirmed in the intact rat. These studies[11], it should be noted, related to the rat diaphragm, and it is recognized that a comparison with experimental porphyria, even in the same species, is scarcely possible. The in vivo toxicity of PBG-following products has not been checked, therefore, we have prepared PB, a dark brown, almost black pigment derived from PBG, probably a dipyrrylmethene[4-8], and have studied its physiologic effect, toxicity and clearance in 7 intact rats after i.v. injection of relatively large amounts of PB.

Materials and methods. PB was prepared from crystalline PBG by an earlier method[11] of PBG polymerisation. It was purified on Sephadex G25 repeatedly, until only a single band was present on the column. This single fraction was collected and evaporated to dryness. The product was characterized by available analytical methods, e.g., UV, visible and IR spectrophotometry, mass spectra, molecular weight measurement (mol.wt 525), elementary analysis, electrophoresis, and thin layer chromatography. The probable dipyrrylmethene structure has been described previously[4-8].

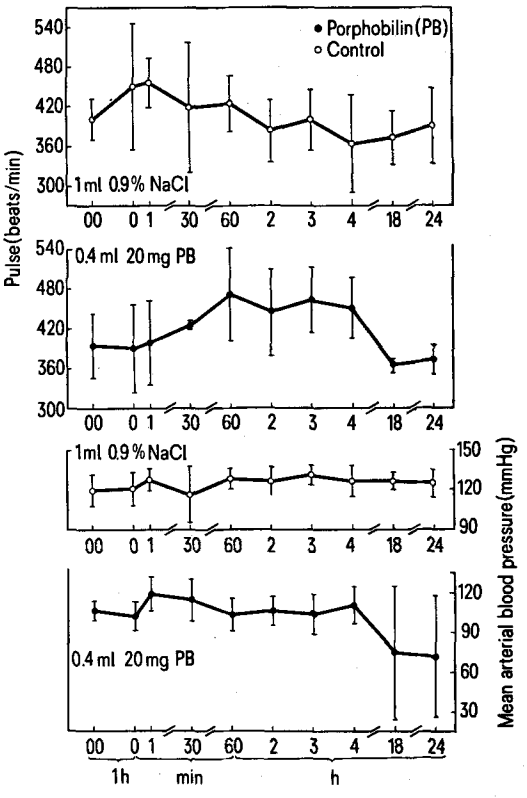

Fig. 1. Pulse rate and blood pressure after i.v. injection of 20 mg PB, and of 0.9% NaCl. The vertical bars indicate the standard error of the mean for 7 rats/group. 00, end of surgical catheter placement (carotid artery and jugular vein), 0, injection time.

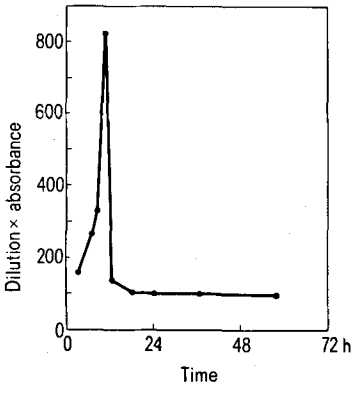

Fig. 2. Urinary excretion of PB administered i.v. as a 20 mg bolus at 0. Urinary absorbance and dilution factor (directly proportional to porphobilin concentration in urine) plotted against time after PB injection. The absorbance was measured at 400 nm.

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The final product, with extinction coefficient unaffected by further purification, was dissolved in water at a concentration of 50 mg/ml. PB, 20 mg were injected in rats as a bolus (see below), through a catheter previously inserted in the jugular vein. Male Wistar rats, 190–320 g b.wt, were used. Blood pressure and pulse rate were recorded via catheter in a carotid artery, using a P23 Db Statham pressure transducer. At the time of injection, the rats had recovered from the light ether anesthesia used for introduction of the catheter. The experiments were carried out on 7 animals, with an equal number as controls where 1 ml of 0.9% NaCl was injected. Blood, urine and feces were collected from all animals and examined for fluorescence in Wood's light and for the presence of PB. Most experiments were terminated 24 h after injection by i.v. air administration. Brain, heart, lung, liver, spleen and kidney were examined after sectioning and staining with hematoxylin and eosin.

Results. The i.v. administration of PB was well tolerated by all animals. During injection, and for a few minutes thereafter, a slight restlessness was observed, but no difference was noticeable between animals receiving PB or saline. Pulse rate and blood pressure, recorded for 24 h, showed no significant changes (figure 1). The urine samples passed within a few minutes and again several hours after injection of PB, were dark; the absorption spectra corresponded to PB. At 12 h after the injection of PB, urine had cleared (figure 2). Neither urine, feces, nor tissue showed any fluorescence under Wood's light. The microscopic examination showed no histologic change of brain, heart, lung, liver, spleen and kidney, as between animals given PB and controls.

Discussion. After i.v. administration of 20 mg PB to rats, blood pressure and heart rate remained stable, and the animals, after initial restlessness, were calm and could move freely. A transient darkening of the urine indicated PB excretion. The transit time of PB was less than 12 h. PB, with its rapid renal clearance, probably has little or no role in the symptomatology of those porphyrias of man in which nervous manifestations are prominent. The present results at least lend no support to this theory.

Pheromone XII. Männchenduftstoffe von Noctuiden (Lepidoptera)[1,2]

Male sex pheromones of noctuides

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Summary. By gaschromatographic-mass spectrometric methods 2-phenylethyl alcohol, benzylalcohol, phenylacetaldehyde and benzaldehyde were identified from scent brushes of male Noctuidae species (Lepidoptera).

Eine Vielzahl unterschiedlicher Arten männlicher Eulenfalter (Noctuidae, Lepidoptera) besitzt am zweiten, abdominalen Sternit einen paarigen Strahlhaarapparat mit Tasche, der einen starken Geruch verbreitet. Die damit ausgeschiedenen, flüchtigen Substanzen könnten bei der Kopulation der Insekten eine Bedeutung als männliches Sexualpheromon besitzen[3,4].

Wir haben die auf den Duftpinseln von Mamestra persicariae[5], Mamestra brassicae[5], Polia tincta und Agrocola helvola befindlichen flüchtigen Verbindungen untersucht. Dazu wurden jeweils 10 in Lichtfallen gefangenen Tieren die Duftpinsel herausgezogen, diese in 1 ml Hexan gegeben, die über den Haarpinsel stehende Lösung im Stickstoffstrom eingeengt und der Rückstand direkt mittels gekoppelter Kapillargaschromatographie-Massenspektroskopie analysiert. Die ungefähren Mengenverhältnisse erhält man durch Vergleiche der Peakflächen des Totalionenstrom-Chromatogramms. Bei den oben genannten Noctuidenarten fanden wir die im folgenden angeführten Verbindungen und Mengenverhälnisse:

Mamestra persicariae: 2-Phenyläthanol (**1**, 80%), Benzylalkohol (**2**, 3%), Phenylacetaldehyd (**3**, 15%), Benzaldehyd (**4**, 2%).

Mamestra brassicae: 2-Phenyläthanol (**1**, 96%), Benzylalkohol (**2**, 4%).

Polia tincta: 2-Phenyläthanol (**1**, 74%), Phenylacetaldehyd (**3**, 6%) und eine in ihrer Struktur noch nicht aufgeklärte Verbindung mit dem Molekulargewicht 164 (**5**, 20%).

Agrocola helvola: 2-Phenyläthanol (**1**, 100%).

Bedingungen für die Gaschromatographie: 20 m Glas-Dünnfilmkapillare OV 1, 0.25 mm i.D., 2 ml He/min. Bei Raumtemperatur splitlos injiziert, nach 2.5 min auf 80 °C, anschliessend 80–200 °C, 10 °C/min.

Retentionszeiten: 6.88 (**1**), 6.56 (**2**), 5.70 (**3**), 4.92 (**4**) und 9.90 (**5**) min.

Massenspektroskopie: Quadrupol-Massenspektrometer[6], 70 eV, Scanzeit 2.5 sec/Spektrum. m/e und relative Intensitäten (%):

2-Phenyläthanol (**1**)	39(55), 51(32), 65(54), 77(11), 91(100), 92(51), 122(15).
Benzylalkohol (**2**)	39(85), 51(100), 63(22), 65(19), 77(72), 79(90), 91(18), 107(25), 108(32).
Phenylacetaldehyd (**3**)	39(82), 51(29), 65(54), 91(100), 92(25), 120 (13).
Benzaldehyd (**4**)	39(40), 51(65), 77(100), 105(52), 106(60).
unbekannte Verbindung (**5**)	39(100), 51(70), 55(82), 65(50), 77(75), 91(60), 103(41), 122(18), 131(13), 133(11), 137(10), 147(2), 149(16), 164(28).

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2 Diese Arbeit wird vom Bundesministerium für Forschung und Technologie unterstützt.

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