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ORIGIN OF URINARY PORPHYRINS IN EXPERIMENTAL HEXACHLOROBENZENE-INDUCED PORPHYRIA

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The basic pathobiochemical mechanism of porphyria, caused by the fungicide hexachlorobenzene (gamma-BHC) is inhibition of uroporphyrinogen decarboxylase (EC 4.1.1.37; UPD) in the liver [3, 7]. Very large amounts of uroporphyrin and smaller amounts of heptacarboxyporphyrin accumulate in the liver. Their excretion in the urine is greatly increased. Inhibition of UPD also has been found in the kidneys of rats with gamma-BHC porphyria [2, 7]. The hypothesis thus arose that the kidneys are the main source of porphyrins in the urine in this condition [2].

It has not yet been established whether inhibition of renal UPD develops in animals other than rats, treated with gamma-BHC. Nor has the quantity of porphyrins deposited in the kidneys been compared with the quantity of porphyrins in the liver. The discovery of large accumulations of uroporphyrin in the kidneys would support the hypothesis that porphyrins in the urine in gamma-BHC poisoning are synthesized primarily in the kidneys. The final elucidation of this problem would allow suggestions to be put forward regarding the origin of the porphyrins in the urine in the disease porphyria cutanea tarda in man, for gamma-BHC porphyria is a model of that disease.

The aim of this investigation was to discover whether chronic gamma-BHC poisoning leads to inhibition of renal UPD activity in mice, and to examine the relationship between the degree of uropoyphyrin accumulation in the liver and kidneys.

EXPERIMENTAL METHOD

Twelve male C57BL/6 mice weighing about 15 g and eight female Wistar rats weighing about 150 g were used. Six mice received a single dose of 0.25 ml inferon (12.5 mg of iron) intraperitoneally and were maintained on a standard diet ad libitum, containing 0.02% of gamma-BHC. The animals were killed 8 weeks later. Four rats were killed after being kept for 7 weeks on a standard diet containing 0.3% of gamma-BHC. The remaining six mice and four rats (control) received the standard diet without gamma-BHC. Immediately after decapitation of the animals the liver and kidneys were removed and homogenized in a glass homogenizer of the Potter–Elvehjem type with Teflon pestle. The homogenizing solution contained 0.1 M K_2HPO_4/KH_2PO_4 and 0.1 mM EDTA-Na_2, pH 6.8. The dilution of the homogenates was 1:5 (w/v).

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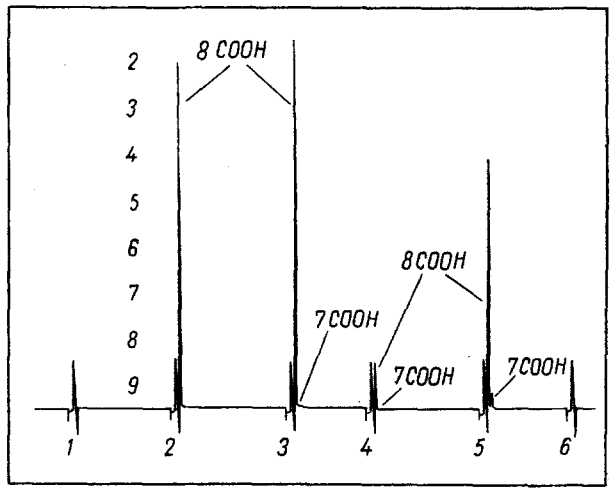

Fig. 1. Chromatogram of determination of UPD activity and uroporphyrin concentration in liver of a mouse treated with gamma-BHC, and control. 1) Zero sample, 2) initial quantity of substrate, 3) gamma-BHC (amount of substrate at end of reaction + endogenous uroporphyrin), 4) zero sample of treated mouse (endogenous uroporphyrin), 5) sample from control mouse (amount of substrate at end of reaction); 6) zero sample from control mouse (endogenous uroporphyrin absent), 8COOH) uroporphyrin and 7COOH) heptacarboxyporphyrin, formed during enzymic decarboxylation. Remaining products of decarboxylation (hexa- and pentacarboxyporphyrin) not recorded on chromatogram under the conditions chosen.

UPD activity in the homogenates was determined with the use of uroporphyrinogen III as the substrate. Uroporphyrinogen not used up in the enzymic reaction was oxidized to uroporphyrin, and measured as the free acid by means of reversed-phase HPLC. The value of enzyme activity was obtained from the difference between the initial amount of substrate and its amount at the end of the reaction. Endogenous uroporphyrin was measured through a zero sample not containing the substrate. Its value was subtracted from the total quantity of uroporphyrin in the sample (Fig. 1). The conditions of this method were described previously [1]. Total protein in the homogenates was determined as in [6]. The results were subjected to statistical analysis with a level of significance of $p < 0.05$.

EXPERIMENTAL RESULTS

Renal UPD activity was found to be inhibited not only in rats, but also in mice of a line susceptible to gamma-BHC poisoning (Table 1). Normally UPD activity in the liver is several times higher than in the kidneys. The degree of inhibition of enzyme activity was almost the same (by about 2.5 times) in the liver and kidneys of animals of both species. Deposition of porphyrins in the kidneys was less marked than in the liver (on average by about 9 times in mice and 5 times in rats).

Uroporphyrin is a highly hydrophilic substance, containing eight carboxyl groups, and for that reason it is excreted mainly through the kidneys and only to a very small extent through the bile. Irrespective of the fact that in gamma-BHC porphyria there is a certain increase in the uroporphyrin content of the feces [4], the presence of such large deposits of it in the liver suggests that they must be excreted chiefly, through secretion from hepatocytes, into the blood plasma, and thereafter through renal excretion in the urine. Very high correlation has been found between porphyrins in the blood plasma and urine in porphyria cutanea tarda [5]. This fact indicates that some of the porphyrins in the urine in gamma-BHC poisoning are synthesized in the liver.

The possibility cannot be ruled out that porphyrins deposited in the kidneys are the result of reduced UPD activity in these organs. However, it can be submitted that some of these deposits arise from the blood plasma (i.e., ultimately from the liver) and are formed by reabsorption of the primary urine. Renal porphyrins accumulate in the proximal tubules and the loop

TABLE 1. UPD Activity (in pmoles/min/kg protein) and Uroporphyrin Concentration (in nmoles/g tissue) in Liver and Kidneys ($M \pm m$)

Parameter	Mice		Rats	
	control (6)	gamma-BHC (6)	control (6)	gamma-BHC (6)
UPD activity in liver	60,9± ±4,35	23,9± ±7,35	42,5± ±1,11	15,1± ±8,3**
UPD activity in kidneys	22,3±4,0	8,7±5,5*	8,6±2,8	3,2± ±1,3**
Uroporphyrin in liver	0	383,5± ±96,8*	0	200,5± ±56,6*
Uroporphyrin in kidneys	0	43,6± ±14,4*	0	49,2± ±9,6*

Legend. Number of animals given in parentheses. *$p < 0.001$, **$p < 0.01$ compared with control.

of Henle [4]. It is perfectly possible that they are secreted there into the urine, thus determining the renal origin of some of the urinary uroporphyrin.

Consequently, it can be postulated that porphyrins in the urine in gamma-BHC-induced porphyria are both hepatin and renal in origin. However, bearing in mind the far higher level of accumulation of uroporphyrin in the liver and the much greater intensity of heme synthesis in that organ, which we established, as well as its larger size, are taken into account it can be regarded as most likely that most of the uroporphyrin in the urine in gamma-BHC poisoning is synthesized in the liver, and not in the kidneys.

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