# Conversion of Porphobilinogen to Porphyrin by Liver Homogenates of Rats with Experimental Hepatic Porphyria.* (23172)

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The companion paper(1) discussed the metabolic role of porphobilinogen (PBG) and its ready conversion to uro-, copro-, proto-, and other porphyrins by normal rat liver homogenate. PBG is excreted in large amount by patients with acute intermittent porphyria and by rats and rabbits with experimental porphyria induced by allyl-isopropyl-acetyl-carbamide (Sedormid)(2). It is believed that this abnormality in PBG and porphyrin metabolism is related primarily to a disturbance in liver metabolism(2,3), and there are two possibilities which might account for the excessive PBG excretion: (1) the liver in these conditions is unable to metabolize PBG at the normal rate, or (2) the production of PBG is increased beyond the capacity of liver to metabolize it.

To investigate these possibilities, studies similar to those previously described for normal rat liver(1) were carried out with livers of rats treated for various time intervals with allyl-isopropyl-acetyl-carbamide.§ A quantitative procedure was developed for the analysis of hepatic PBG and its rate of disappearance from liver homogenates was studied. The incubation data thus obtained were correlated with data on the accumulation of PBG and porphyrin in the livers of treated animals as described below.

*Material and methods.* Adult male rats were fed allyl-isopropyl-acetyl-carbamide at 100 mg/day as described previously(2). Actual measured intake in individual rats averaged from 70 to 100 mg/day. Preparation of homogenates and methods employed for quantitative analysis of porphyrins have been described(1). The homogenates were incubated without shaking unless noted otherwise. Because the volumes of liver homogenate employed in the different studies varied from 7 to 25 ml, the data are reported in terms of $\mu$g PBG or porphyrin/g of liver in the homogenate.

(1) *Quantitative determination of PBG in rat liver homogenate.* Various preliminary procedures employing dilute acetic, citric, or hydrochloric acids, ammonia and sodium hydroxide were discarded because of turbidity obtained on subsequent addition of Ehrlich's reagent with or without sodium acetate, or because of poor recovery. The following procedure, however, yielded clear solutions with reproducible recovery of added PBG.‖ Five ml of 7.5% trichloracetic acid were added to 2.5 ml of liver homogenate to yield a final concentration of 5% trichloracetic acid. The well mixed suspension was shaken for several minutes and centrifuged. To 2.5 ml of supernatant solution were then added 7.5 ml of Ehrlich's reagent prepared as recommended by Valquist(4).¶ The absorption of this solution was determined with an Evelyn colorimeter employing a 565 m$\mu$ filter. Because the color is somewhat unstable, the maximum absorption reading, obtained within 1 minute, was used in all cases for quantitative analysis.

In two series of recovery experiments solutions of different batches of PBG crystals were added to different liver homogenates, extracted with trichloracetic acid, and analyzed as described above. Results are shown in A Fig. 1. Curve B was obtained by diluting solutions of PBG directly with 5% trichlor-

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‖ The possible use of such a method was discussed by one of us (S.S.) with Dr. Robert Aldrich, University of Oregon, in the summer of 1953. His results with a similar procedure are in general agreement with those reported here (personal communication).

¶ 20 g *p*-dimethylaminobenzaldehyde dissolved in 500 ml conc. HCl plus 500 ml $H_2O$.

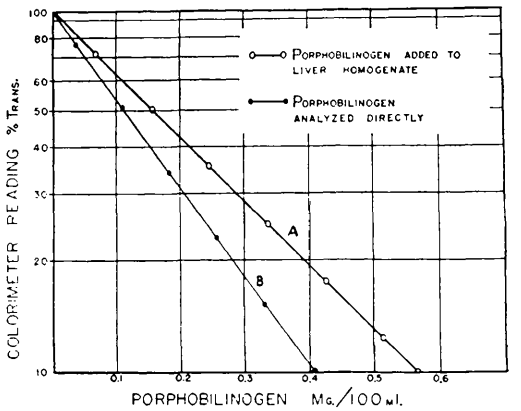



FIG. 1. Colorimetric estimation of porphobilinogen. A: Porphobilinogen added to homogenate of normal liver. B: Porphobilinogen added to 5% trichloracetic acid and analyzed directly. Readings taken with Evelyn Colorimeter, employing a 565 mμ filter.

acetic acid (without liver homogenate) prior to addition to Ehrlich's reagent. A consistent difference was found between the 2 curves at concentration levels tested. The reason for this has not been established. Curve A was employed for the analyses reported herein.

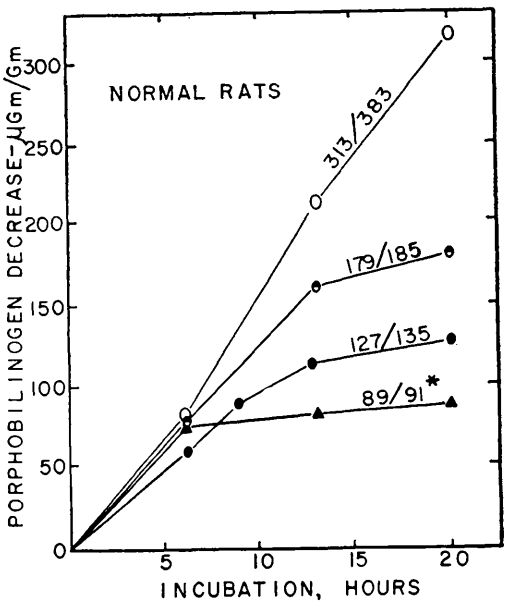
(2) *Estimation of hepatic PBG in rats treated with allyl-isopropyl-acetyl-carbamide.* Groups of 3-5 rats were fed allyl-isopropyl-acetyl-carbamide for periods up to 13 days. As shown in Table I no detectable PBG was present during the first 3 days. In general, increased amounts were found from the fifth to thirteenth days though values varied considerably in individual rats.

(3) *Fate of PBG incubated with homogenates of livers from normal and from "porphyric" rats.* a. Disappearance of PBG. Groups of 5 rats each were fed allyl-isopro-

TABLE I. Concentration of Liver Porphobilinogen in Rats Fed Allyl-Isopropyl-Acetyl-Carbamide.

| Feeding period, days | Porphobilinogen, μg/g | |
|---|---|---|
| | Range | Mean |
| 0 | 0– 0 | 0 |
| 2 | * | 0 |
| 3 | 0– 0 | 0 |
| 5 | * | 115 |
| 6 | * | 98 |
| 7 | 0–168 | 89 |
| 10 | 12–234 | 122 |
| 13 | * | 227 |

* 5 rat livers pooled for assay. 3 to 5 individual livers analyzed at other time intervals.



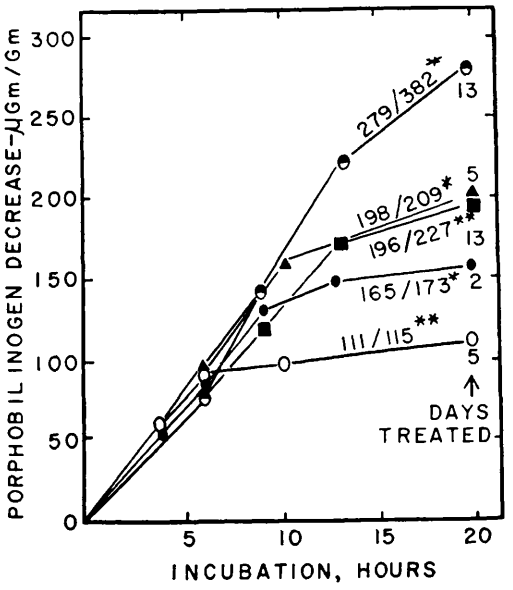




FIG. 2 (top). Disappearance of porphobilinogen from incubated liver homogenates from normal rats. Numerator = total μg of porphobilinogen disappearing from homogenate/g of liver during 20 hr incubation; denominator = μg of porphobilinogen added to homogenate/g of liver at 0 time.

FIG. 3 (bottom). Disappearance of porphobilinogen from incubated liver homogenates from Sedormid-treated rats. Homogenates incubated both with (*) and without (**) added porphobilinogen. Ratios again refer to μg porphobilinogen disappearing in 20 hr/μg present at 0 time/g of liver.

TABLE II. Porphyrin Content of Liver Homogenates Incubated with Porphobilinogen.

| Sedormid feeding (days) | Porphobilinogen, μg/g* | Porphyrin, μg/g Incubation period 6 hr "U" | 6 hr "C + P" | 13 hr "U" | 13 hr "C + P" | 21 hr "U" | 21 hr "C + P" |
|---|---|---|---|---|---|---|---|
| 0 | 91 | 9 | 33 | 4 | 29 | 13 | 14 |
| 0 | 185 | 7 | 17 | 26 | 45 | 9 | 20 |
| 0 | 383 | 4 | 20 | 23 | 24 | 34 | 27 |
| 2 | 173 | 7 | 27 | 1 | 29 | 1 | 14 |
| 5* | 115 | 4 | 25 | | | 6 | 19 |
| 13* | 227 | 9 | 29 | 13 | 33 | 17 | 16 |
| 13 | 382 | 8 | 27 | 16 | 21 | 23 | 19 |

* Porphobilinogen content of unincubated sample; solution of crystalline porphobilinogen added to all homogenates prior to incubation except for 5- and 13-day fed rats.
"U"—uroporphyrin fraction. "C + P"—copro- + protoporphyrin fraction.

pyl-acetyl-carbamide for 2, 5 and 13 days respectively. The livers from each group were combined for homogenization. The homogenates were incubated for various periods to 20 hours both with and without added PBG. With each group of treated rats, combined livers from 4 control untreated rats were analyzed similarly. In addition, varying amounts of PBG were added to 4 aliquots of the combined homogenate of a fourth group of normal rats. Data summarizing the decrease in PBG concentration of incubated homogenates of normal and Sedormid-treated rats are shown in Fig. 2 and 3. The same amount of PBG disappeared from the homogenate/g of liver during the first 6 hours, regardless of the initial PBG concentration. At this time interval 60 to 91 μg (average 73 μg) disappeared/g of liver in the 7 studies employing normal rats, while the corresponding values for Sedormid-treated rat livers ranged from 70 to 90 μg (average 81 μg).

Porphyrin analyses of several of the homogenates described in Fig. 2 and 3 are summarized in Table II. These data, too, indicate similar patterns of porphyrin accumulation in liver homogenates of normal(1) and Sedormid-treated rats; essentially similar concentrations of porphyrin were found during the first 6 hours in each sample. In most instances the recovered copro-plus protoporphyrin decreased in amount between 13th and 21st hours. In possible contrast to uroporphyrin(5), the latter porphyrins require enzymatic activity for formation from PBG and their presence is therefore especially significant. In agreement with the data reported in the companion paper, the major fraction of coproporphyrin behaved as the type III isomer on paper chromatographic analysis. After 25 hours incubation the uroporphyrin behaved chiefly as type I isomer.

In a final experiment, the effect of increased aeration on porphyrin content of incubated homogenates was investigated. Constant shaking resulted in the finding of increased amounts of uro-type porphyrin associated with a marked reduction in recovery of porphyrin in the coproporphyrin fraction. Shaking had no significant effect on the rate of porphobilinogen disappearance in at least 2 of the 3 studies. The data at 12 and 20 hours are summarized in Table III.

*Discussion.* It is evident that, under the conditions employed, the liver of Sedormid-treated rat is able to metabolize PBG at least as readily as the normal rat liver. The production of porphyrin from PBG was likewise similar in both. It would therefore appear that the excessive amounts of PBG in the liver and urine of these animals depends simply on an overproduction.

The nature of the primary abnormality in Sedormid-treated animals remains uncertain. Labbe and co-workers(6) have postulated a block in conversion of delta amino levulinic acid to purine metabolites(7), with resulting overproduction of pyrrol compounds. If this mechanism is significant it must involve only certain specific purines since we have found no significant alteration in concentration of ribo- or desoxyribonucleic acids in livers of Sedormid-treated rats, though there is a sig-

TABLE III. Effect of Aeration on Porphyrin Content of Liver Homogenates Incubated with Porphobilinogen.

| Sedormid feeding (days) | Sample* | Porphobilinogen, μg/g 0 time† | Porphobilinogen, μg/g 12 hr | Porphyrin, μg/g Incubation period 12 hr Uro | 12 hr Copro | 12 hr Proto | 20 hr Uro | 20 hr Copro | 20 hr Proto |
|---|---|---|---|---|---|---|---|---|---|
| 0‡ | A | 117 | 33 | 4 | 20 | 25 | 8 | 1 | 25 |
| | B | " | 41 | 17 | 0.4 | 23 | 21 | 2 | 3 |
| 6 | A | 98 | 16 | 5 | 22 | 40 | 8 | 2 | 51 |
| | B | " | 15 | 24 | 0 | 36 | 17 | 0 | 34 |
| 6‡ | A | 211 | 114 | 14 | 14 | 29 | 17 | 4 | 59 |
| | B | " | 78 | 20 | 0 | 37 | 33 | | |

Livers of 6 normal rats and 6 Sedormid-treated rats pooled.
* Sample A, no shaking. Sample B, continuous shaking.
† Porphobilinogen conc. in unincubated sample.
‡ Solution of crystalline porphobilinogen added to homogenate.

nificant decrease in concentration of total organic acid soluble phosphorus. It is also not clear how such a concept would explain the rapid decrease in liver catalase(8) and accumulation of large amounts of green porphyrins as recently described(9), the latter found within a few hours after feeding Sedormid or administering allyl-isopropyl-acetamide intraperitoneally to rats.

If the increased production of PBG is intimately related to or is the primary defect in (Sedormid) porphyria, one might expect to detect this phenomenon during the first few hours after Sedormid feeding. Thus far this has not been possible. Such detection would now seem to require either more sensitive analytical technics, or isotopic studies to determine the size of the hepatic PBG "pool." Meanwhile, the delayed appearance of PBG in liver and urine after Sedormid feeding, is consistent with either of the following concepts: (1) Production of PBG in the early period may actually be increased, but only to levels which can be completely metabolized, or (2) the increase in PBG production may occur only after several days as a secondary compensatory phenomenon, being stimulated by decreasing concentration of catalase and perhaps of other heme enzymes. This situation would be analogous to the stimulated synthesis of hemoglobin which follows excessive blood loss.

*Summary.* Liver homogenates of rats treated with allyl-isopropyl-acetyl-carbamide (Sedormid) converted PBG to porphyrin at approximately the same rate as homogenates of normal rat liver. It is concluded that the excess PBG in the liver and urine of the "porphyric" rats is due to overproduction and not to a block in its further metabolism.

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