Sedormid-Induced Porphyria in the Rat.* (20419)

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Duesberg suggested that prolonged ingestion of allylisopropyl-acetyl-carbamide (Sedormid)† may have played a role in the development of acute porphyria in one of his patients(1). Recently, Schmid and Schwartz administered this compound to rabbits and produced a syndrome resembling adult human porphyria(2). The liver of these animals appeared to be one of the principal organs involved.

We have administered Sedormid to another species (rat) and observed a similar effect. This is of particular interest because a vast amount of biochemical information is available on rat liver.

Materials and methods. Female Sprague-Dawley rats weighing approximately 200 g were used. Sedormid was suspended in propylene glycol (50 mg per ml) and given by stomach tube in single daily doses of 200 to 400 mg per kg. The animals were allowed an unlimited intake of food (Purina chow) and water. Daily observations were made of weight, food and water intake, number of fecal pellets, appearance, strength and alertness. Seven control animals received propylene glycol alone by stomach tube in daily doses of 1.2 to 2.0 ml per kg. Six rats received subcutaneous injections of Sedormid in daily doses of 200 to 400 mg per kg. One rat received 100 mg Sedormid per day intraperitoneally. Sedormid was suspended for these parenteral injections in isotonic glycerol containing 0.1% "Tween 80."‡ Twenty-four-hour urine specimens collected in metabolism cages (3) were subjected to 4 separate determinations. Coproporphyrin was estimated by the method of Schwartz *et al.*(4). "Unboiled" uroporphyrin concentration was determined by combining the aqueous phase from the coproporphyrin determination with the 1% sodium acetate washes. This solution was then made to 1.5 N with HCl and the uroporphyrin determined by fluorescence against a coproporphyrin standard.§ An aliquot of the native urine was analyzed for its "boiled" uroporphyrin content after the sample was made to pH 5.0 with buffered acetic acid and boiled for twenty minutes to convert any uroporphyrin precursors. It was then estimated in exactly the same manner as "unboiled" uroporphyrin. Finally the native urine sample was tested for porphobilinogen using the method of Watson and Schwartz(5). The ratio of coproporphyrin isomers I and III was determined when porphyrin excretion was maximum(6). Uroporphyrin methyl ester was crystallized and characterized by its physical properties(7,8).

Results. Table I presents data on urinary porphyrin of normal rats, animals receiving oral propylene glycol alone, and animals receiving parenteral Sedormid injections. Table II shows the quantitative urinary porphyrin values from 9 rats at the height of the Sedormid-induced disorder. The magnitude and variability of the response to the drug are illustrated. Fig. 1 depicts the chemical and clinical course of the Sedormid effect on a typical animal. The large amounts of uroporphyrin which appeared in the rat urine were predominantly in the form of a non-fluorescent precursor which converted to uroporphyrin on boiling. Chromatography and crystallization of this porphyrin as its methyl ester yielded crystals which resembled curved hairs and melted at 260 to 261°C (uncorrected). The isomer

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§ All uroporphyrin values reported herein are in terms of coproporphyrin.

TABLE I. Urinary Porphyrins of Normal Rats and Rats Receiving Oral Propylene Glycol or Parenteral Sedormid Alone. 200 g female Sprague-Dawley rats.

Description	No. of determinations	C	U	UB	Daily dosage	Days treated
13 normal rats	59*				—	—
Avg		12.2	5.6	5.4		
Range		3.2–25.8	0.5–13.0	1.6–13.9		
7 rats given oral propylene glycol	7				1.2–2.0 ml	11–21
Avg		10.6	5.8	5.4		
Range		6.9–13.1	4.1–8.8	4.0–8.0		
6 rats given subcut. Sedormid	6				200–400 mg/kg	35–37
Avg		18.3	8.6	8.6		
Range		8.5–24.0	3.0–14.1	3.1–14.7		
1 rat given intraper. Sedormid	5				100 mg/kg	11
Range		12.5–17.7	3.3–5.7	4.6–7.0		

Values expressed as μg of coproporphyrin/24 hr. C = coproporphyrin; U = "unboiled" uroporphyrin, UB = "boiled" uroporphyrin. Porphobilinogen tests all were negative.
* 59 24-hr urine samples from the 13 rats.

TABLE II. Maximum Urinary Porphyrin Values during Oral Sedormid Administration.* 9 rats.

Drug administered, days	C	U	U(B)	P
25	98	42.5	1550	++++
15	150	105	1575	++++
16	93	16.7	116	++++
29			3230	++++
11	189	98.3	1254	++++
20	108	51.3	634	++++
12	13	48	114	++
12	232	168	1130	+++
13	114	100	400	++

* Values expressed as μg of coproporphyrin/24 hr.
C = coproporphyrin; U = "unboiled" uroporphyrin; U(B) = "boiled" uroporphyrin; P = porphobilinogen qualitative test.

ratio of the purified coproporphyrin was 90% type III and 10% type I.

A striking clinical change occurred coincident with the appearance of large amounts of uroporphyrin and porphobilinogen in the urine. The animals showed marked weakness, most evident in the hind legs. Lethargy was also apparent, together with weight loss, decreased intake of food and water, and reduced numbers of fecal pellets. A few rats continued to eat relatively well and maintained their weight. If the drug was continued, progressive paralysis and death in coma ensued. However, if Sedormid administration was stopped, complete clinical and chemical recovery usually took place in 48 to 72 hours. Autopsy of animals dead from Sedormid under a Woods light revealed no red fluorescence in the liver or bile duct. On cut surface the kidney exhibited a brilliant band of red fluorescence at the cortico-medullary junction which was not seen in normal animals.

Discussion. The data in Table I indicate that the amount of coproporphyrin in normal rat urine is relatively constant, with a range in 59 determinations of 3.2 to 25.8 μg per day. Levels of uroporphyrin ranged from 0.5 to 13.9 μg per day, and were essentially unchanged by boiling. This last fact is important because it indicates that no uroporphyrin precursor is present in normal rat urine.

Fig. 1 shows that after Sedormid administration, there is a steady rise of urinary coproporphyrin The levels of both "unboiled" and "boiled" uroporphyrin remain relatively low until a high level of coproporphyrin is reached, during 1 to 2 weeks of drug administration. A sharp increase in uroporphyrin excretion is then observed; simultaneously a strong positive porphobilinogen test appears, and the animal becomes seriously ill. The finding of a non-fluorescent uroporphyrin precursor, as shown by the increase of "boiled" over "unboiled" uroporphyrin, has been observed by others in both human and experimental rabbit porphyria(9,2). The melting point and crystal morphology of the uropor-

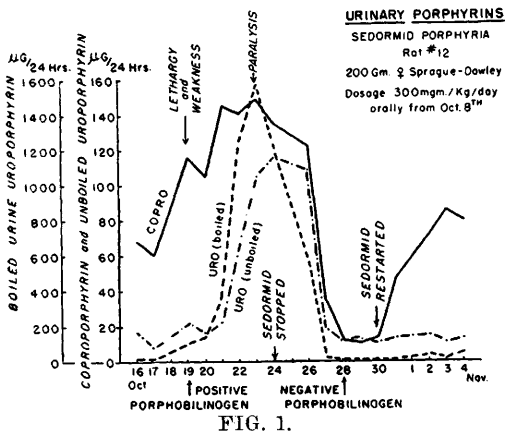

FIG. 1.

phyrin methyl ester suggest that it is probably uroporphyrin type III or the Waldenström uro-type porphyrin.

Subcutaneous injections of Sedormid in dosages that are effective orally caused no disorder of porphyrin excretion (Table I). Propylene glycol given alone by stomach tube likewise produced no change in urinary porphyrins.

The clinical features presented by Sedormid treated animals, the presence of porphobilinogen, increased amounts of predominantly type III coproporphyrin, and enormous amounts of uroporphyrin in the urine very closely approximate the criteria considered diagnostic of porphyria in human adults. The ready availability of extensive biochemical data on rat liver seems to make rats preferable to rabbits for the study of intermediary porphyrin metabolism. Furthermore, the similarity of our findings to those of the University of Minnesota investigators indicates that the effect of Sedormid is not limited to rabbits. The work is being continued.

Summary. Sedormid administered to rats by stomach tube has caused marked increases in urinary uroporphyrin, coproporphyrin, and porphobilinogen, and concurrent clinical changes of paralysis, lethargy and gastrointestinal dysfunction. These findings constitute a syndrome closely resembling human adult porphyria. The disorder thus produced resembles that recently reported in rabbits.

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Rat Plasma Aminopeptidase Activity in Hemorrhagic Shock.* (20420)

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As part of an investigation of the biologic significance of peptidases of blood and tissue, plasma peptidase activity was studied in rats subjected to hemorrhagic shock. The striking alterations which appeared are presented in this report.

Materials and methods. Shock was established in rats by bleeding from the cut tail as described previously(1). Serial samples of nonhemolyzed heparinized plasma were obtained by centrifugation of blood removed from

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