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Experimental Hepatic Porphyria caused by Feeding 3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine

COMPARISON WITH SEDORMID PORPHYRIA

BY F. DE MATTEIS* AND BARBARA E. PRIOR
Department of Chemical Pathology, University College Hospital Medical School, London, W.C. 1

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Since Schmid & Schwartz (1952) described an experimental hepatic porphyria induced in rabbits by Sedormid (2-isopropylpent-4-enoylurea), several drugs chemically related to Sedormid (Goldberg & Rimington, 1955; Stich & Decker, 1955*a*, *b*; Talman, Labbe & Aldrich, 1957) and some barbiturates (Goldberg, 1954*a*) have been shown to cause excretion of both porphobilinogen and porphyrins in animals. Goldberg & Rimington (1955) and Rimington (1957) postulated that the following chemical structure was necessary to cause experimental porphyria:

$$CH_2{:}CH{\cdot}CH_2{\cdot}CH(R){\cdot}CO{\cdot}NH{\cdot}R^1$$

Stich & Decker (1955*b*) came to essentially similar conclusions.

Solomon & Figge (1959) have described in mice and guinea pigs an experimental hepatic porphyria caused by feeding with 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine, a substance chemically unrelated to the structure defined above. In porphyria induced by 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine Solomon & Figge found increased amounts of hepatic coproporphyrin and particularly protoporphyrin, as is also seen in Sedormid porphyria. It differed from the latter, however, in that the urine contained a large quantity of copro- but not of uro-porphyrin, and porphobilinogen was only occasionally present.

The purpose of this work was to study the 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine porphyria in more detail, to compare the effect of the new drug with that of Sedormid and of another, more effective member of the Sedormid group, 2-allyl-2-isopropylacetamide, and to pay particular attention to the excretion of porphyrin precursors in these experimental porphyrias. It seemed particularly interesting to investigate whether 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine is able, like Sedormid, to cause a decrease in liver-catalase activity, a phenomenon which was considered by Schmid, Figen & Schwartz (1955) to be of importance in the chemical picture and also in the pathogenesis of experimental hepatic porphyria.

Finally, since Labbe, Talman & Aldrich (1955) have described a disturbance of purine metabolism in Sedormid-poisoned chick embryos and rabbits, the effect of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine, 2-allyl-2-isopropylacetamide or Sedormid on purine metabolism in rats was studied, as estimated from the allantoin excretion in their urine. A preliminary report of these results has already appeared (De Matteis, 1960).

MATERIALS AND METHODS

Drugs

3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine was prepared by a method kindly provided by Dr U. Eisner (personal communication). Ethyl acetoacetate (26 ml.) and 6 ml. of acetaldehyde were dissolved in 500 ml. of aqueous 10% $(NH_4)_2CO_3$ solution. The mixture was left to stand for 24 hr. at 4° with occasional shaking. During this time yellow crystals appeared. These were filtered off under suction and mechanically shaken for 5 hr. with 200 ml. of 3 N-HCl to remove the basic yellow impurity. The crystals were again filtered, washed with a little water and sucked dry. The almost white product was purified by two successive recrystallizations from ethanol–water. The crystals were collected by filtration, washed with water and dried in a vacuum desiccator over NaOH; m.p. 128° (uncorr.).

Sedormid (2-isopropylpent-4-enoylurea) and 2-allyl-2-isopropylacetamide were obtained from Roche Products Ltd., Welwyn Garden City.

Animals

Male Wistar albino rats (average wt. 100 g.) were placed singly in metabolic cages that permitted separate collection of urine and faeces. They were fed on the M.R.C. cube diet, no. 41 B, without restriction. After a preliminary period of 4–5 days, the rats were given 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine, 2-allyl-2-isopropylacetamide or Sedormid (500 mg./kg./day) suspended in 1% Cellophas (Imperial Chemical Industries Ltd.), by gastric intubation, a blunt metal needle attached to a glass syringe being used. The collected urine was removed every 24 hr. and δ-aminolaevulic acid, porphobilinogen and

* Present address: Istituto di Clinica Medica, Policlinico, Bari, Italy.

allantoin were determined daily; urinary porphyrins were determined in a sample of the bulked 48 hr. collection. Ten rats were dosed with 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine, six with 2-allyl-2-isopropylacetamide and two with Sedormid.

In one experiment, three male rabbits (approximate wt. 2·5 kg.) were used. A rubber catheter was placed in the bladder, a single dose of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine or 2-allyl-2-isopropylacetamide (1·5 g.) was administered by gastric intubation and the urine collected at regular intervals before dosing and for some hours thereafter. Further samples of urine were obtained by catheterization during subsequent days.

Quantitative analyses

Urinary porphyrins. Ether-soluble porphyrins were determined by the method of Rimington (1958). The uro-type porphyrins were determined in the water phase of the urine, after extraction of the copro-type porphyrins into ether, together with the 3% sodium acetate washings of the ether, by the method described by Dresel & Tooth (1954).

Coproporphyrinogen was determined in samples of rabbit urine which had been collected with a catheter from the bladder in test tubes covered with black paper. The whole procedure for the estimation was carried out rapidly in a dark room under subdued red light. Ten vol. of 0·5N-sodium phthalate buffer, pH 3·6, was added to 2 vol. of urine. The mixture was quickly shaken twice with an equal volume of ether, the ether fractions were bulked, washed once with a little water and extracted with small amounts of 0·1N-HCl until the acid extract was no longer fluorescent. This acid extract represented the coproporphyrin present in the urine in oxidized form, the coproporphyrinogen being left behind in the ether phase. An equal volume of dilute (0·005%) iodine solution was then shaken with the ether to convert the porphyrinogen into porphyrin, which was then extracted with 0·1N-HCl. The whole procedure of iodine treatment and acid extraction of the ether was repeated two to four times, if necessary, to complete the extraction of coproporphyrinogen in the oxidized form.

Tissue porphyrins. These were determined according to the method of Schwartz (1955).

Porphobilinogen and δ-aminolaevulic acid. These were determined in urine by the method of Mauzerall & Granick (1956) with the only modification that elution of δ-aminolaevulic acid from the Dowex 50 resin was performed by running through 7·5 ml. of 0·5N-sodium acetate and utilizing all of this for condensation with acetylacetone. Tissue homogenates were tested for porphobilinogen and δ-aminolaevulic acid as follows: 6 ml. of 20% homogenate in water was mixed with 2 ml. of trichloroacetic acid–$HgCl_2$ solution [80 ml. of 20% (w/v) trichloroacetic acid–20 ml. of 0·25N-$HgCl_2$]. After vigorous shaking and centrifuging, 1·5 ml. of the supernatant was tested with an equal volume of modified Ehrlich reagent (Mauzerall & Granick, 1956) for the presence of porphobilinogen.

To a further 1 ml. of supernatant, 1 ml. of M-acetate buffer, pH 4·6, 0·1 ml. of 2·5N-NaOH and 0·05 ml. of acetylacetone were added. This mixture was heated in a boiling-water bath for 10 min., allowed to cool to room temperature and then mixed with an equal volume of the modified Ehrlich reagent. For the blank the tissue homogenate was treated in the same way but with the exclusion of acetylacetone before heating. The development of a pink colour in the test solution, but not in the blank, was considered indicative of the presence of δ-aminolaevulic acid in the tissue examined.

Allantoin. This was determined in the rat urine by the method of Young & Conway (1942).

Liver catalase. Liver homogenates were tested for catalase activity according to the method of Feinstein (1949). They were prepared in water and frozen and thawed twice before measurement of the enzyme activity. Nitrogen was determined by a micro-Kjeldahl method.

Blood examinations. Haemoglobin was estimated as oxyhaemoglobin (Dacie, 1956); the red and white blood cells were counted with a Bürker counting chamber.

Identification of compounds

Isomer analysis of porphyrins. This analysis was with a specimen of rabbit urine collected after dosage with 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine or 2-allyl-2-isopropylacetamide at the height of porphyrin excretion when it contained large quantities of both ether-soluble and -insoluble porphyrins.

The copro-type porphyrins were transferred from the ether into a minimal volume of 2·8N-HCl, the solution was concentrated to dryness *in vacuo* and the residue esterified by contact with methanolic (5%, v/v) H_2SO_4 for 20 hr. The porphyrin esters were transferred into $CHCl_3$, the $CHCl_3$ layer was washed twice with water, once with dilute aq. NH_3 solution and three times more with water. A portion of the ester was dried, hydrolysed with 7N-HCl for 42 hr. at room temperature and chromatographed on paper by the 2,6-lutidine method of Ericsen (1953).

The uro-type porphyrins were taken, after extraction of the ether-soluble porphyrins, into ethyl acetate at pH 3, transferred to HCl, evaporated to dryness and esterified as described above for the copro-type porphyrins. The porphyrin esters were chromatographed on paper by the method of Falk & Benson (1953).

Paper chromatography of porphobilinogen. The 1M-acetic acid eluate of the Dowex 2 (Mauzerall & Granick, 1956) was used as the source of purified porphobilinogen from urines. Chromatography was carried out at room temperature by the ascending method with Whatman no. 4 paper in butanol–acetic acid–water (63:11:26, by vol.). The chromatogram was dried and developed by spraying with Ehrlich reagent [*p*-dimethylaminobenzaldehyde, 10% in conc. HCl–acetone (1:4, v/v)].

Identification of δ-aminolaevulic acid. This was carried out by chromatography of the amino ketone on Amberlite IRC-50 (Elliot, 1960) and also by paper chromatography of the pyrrole resulting from condensation of δ-aminolaevulic acid with acetylacetone at pH 4·6 (Mauzerall & Granick, 1956).

RESULTS

Experimental porphyria in rats

Urinary porphyrins, porphyrin precursors and allantoin. Both 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine and 2-allyl-2-isopropylacetamide cause an immediate increase above the normal concentration of urinary coproporphyrin, porphobilinogen and δ-aminolaevulic acid (Fig. 1). Sedormid also caused a rise in the excretion of these

compounds, but its action was much weaker and slower. A good correlation was generally noted during the experiment between the concentrations of these three compounds and particularly between porphobilinogen and δ-aminolaevulic acid concentrations. No significant alteration of allantoin excretion in rat urine was observed after Sedormid, 2-allyl-2-isopropylacetamide or 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine treatment. The only obvious difference when comparing the pattern of excretion of porphyrins and porphyrin precursors of the 2-allyl-2-isopropylacetamide-treated rats with that of the 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine-treated rats was that the latter normally showed maximum excretion of all three compounds on the first 2 or 3 days of treatment whereas in the former this maximum was reached only towards the middle of the treatment (7–8 days); Sedormid resembled 2-allyl-2-isopropylacetamide from this point of view.

All groups of animals after about 10 days of treatment showed a gradual decrease in the quantity of compounds excreted, nearly normal values being reached in spite of the fact that the administration of the drugs was continued. This concerned not only coproporphyrin but also porphobilinogen and particularly δ-aminolaevulic acid. However, after withdrawal of the drugs for 1 day the animals responded again to their reinstitution with the excretion of large amounts of porphyrins and porphyrin precursors in their urine.

Urinary δ-aminolaevulic acid, which in rats, as in the human, is normally higher than porphobilinogen, increases after treatment but not to as great an extent as does the porphobilinogen, so that the δ-aminolaevulic acid/porphobilinogen ratio, which is more than unity in normal conditions, becomes less than 1 during treatment; thus ratios of 1/3 to 1/10 were usually observed.

Liver catalase and porphyrins. Rats were fed with either 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine or 2-allyl-2-isopropylacetamide at the same dose of 500 mg./kg. body wt. as in the previous experiment and killed after different periods of treatment. Two rats were studied at each time interval for each drug. The liver-catalase activity was determined and related to the tissue nitrogen content. Ether-soluble porphyrins were also estimated in the liver homogenates.

The two drugs caused a comparable fall in liver-catalase activity: 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine seemed to be a little slower in its action, taking 9 days to produce the same depression obtained after 5 days with 2-allyl-2-isopropylacetamide (Table 1).

A rise in hepatic ether-soluble porphyrins was also observed in both groups of rats and concerned both copro- and proto-porphyrin. The time relationship between this rise and the fall in liver-catalase activity is shown in Fig. 2. Whereas in the rats treated with 2-allyl-2-isopropylacetamide the increase in hepatic porphyrins seemed to coincide with the drop in enzyme activity, in the animals treated with 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine the two phenomena

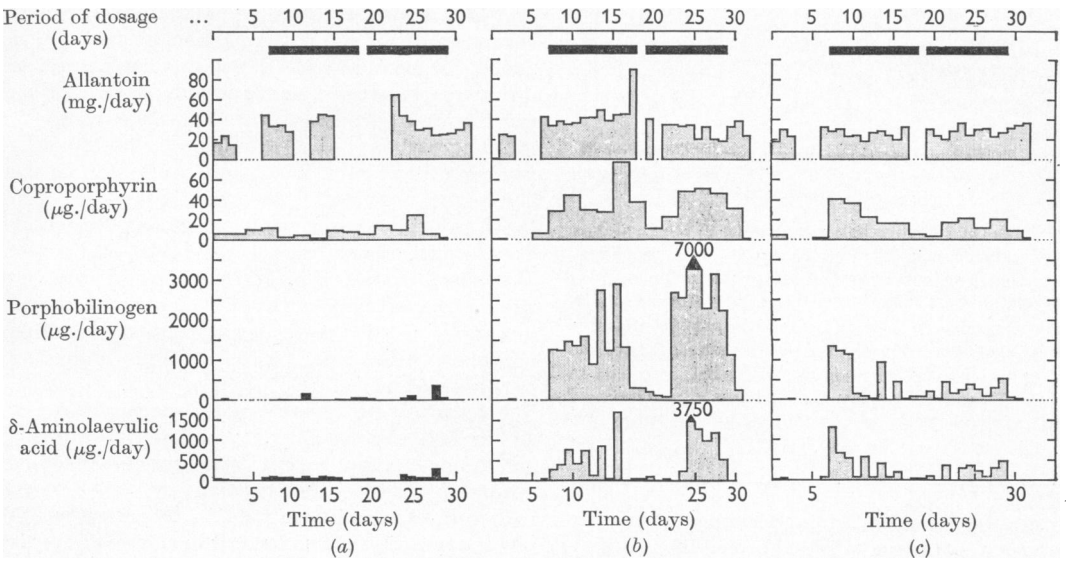

Fig. 1. Effect of administration of (*a*) Sedormid, (*b*) 2-allyl-2-isopropylacetamide or (*c*) 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine on the urinary excretion of coproporphyrin, porphobilinogen, δ-aminolaevulic acid and allantoin of the rat. The dose was 500 mg./kg. body wt./day.

Table 1. *Effect of the administration for various periods of time of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine or 2-allyl-2-isopropylacetamide on liver-catalase activity of the rat*

Results are expressed as m-equiv. of $NaBrO_3$ reduced in 5 min. at 37° and pH 6·8 by 1 mg. of nitrogen of liver homogenates and are given as arithmetic means of two concordant experiments. Dose of the drug administered was 500 mg./kg./day.

	Period of treatment (days)						
Drug	0 (control)	1	2	3	5	7	9
3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine	15·70	14·75	13·60	13·18	12·71	7·69	3·76
2-Allyl-2-isopropylacetamide	—	—	7·69	—	3·29	—	—

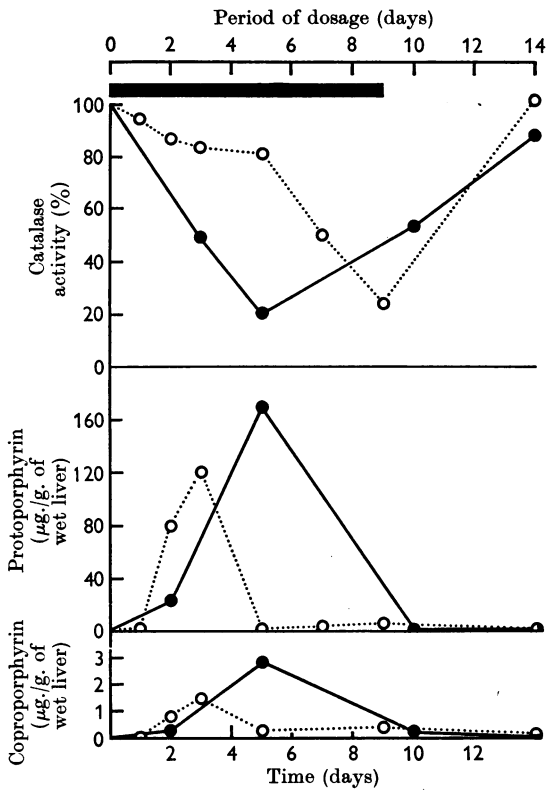

Fig. 2. Time-relationship between decline in liver-catalase activity and increase in hepatic copro- and proto-porphyrin concentrations after administration for various periods of time of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (○···○) or 2-allyl-2-isopropylacetamide (●—●). Catalase activity is expressed as percentage of the normal and estimated as m-equiv. of $NaBrO_3$ reduced in 5 min. at 37° and pH 6·8 by the addition of 0·07 mg. of nitrogen of liver homogenates. Dose of the drug administered was 500 mg./kg./day.

occurred independently. An early increase in hepatic porphyrins took place while the enzyme activity remained practically normal; later the concentration of both copro- and proto-porphyrin returned to normal values (in spite of the continued administration of the drug) and only then did catalase activity show a significant decrease.

Blood examination. Haemoglobin and red- and white-cell counts showed no appreciable change during administration of either 2-allyl-2-isopropylacetamide or 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine.

Experimental porphyria in rabbits

Experimental porphyria was also induced in rabbits with a single dose of 1·5 g. of either 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine or 2-allyl-2-isopropylacetamide and specimens of urine were collected at regular intervals for the estimation of uroporphyrin, coproporphyrin, porphobilinogen and δ-aminolaevulic acid. Two rabbits were dosed with 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine and one with 2-allyl-2-isopropylacetamide. The rise in the porphobilinogen and δ-aminolaevulic acid concentrations became evident in 8–14 hr. (Table 2). Uroporphyrin appeared or increased in the urine at the same time or a few hours later and immediately after; also coproporphyrin showed a significant rise. In the rabbit as in the rat there was no significant difference between the excretion pattern of porphyrins and porphyrin precursors induced by 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine or 2-allyl-2-isopropylacetamide respectively and a generally good correlation was noted between urinary concentrations of porphobilinogen and δ-aminolaevulic acid.

A specimen of rabbit urine collected freshly at the height of porphyrin excretion was carefully analysed in order to see what proportion of total coproporphyrin was present as non-fluorescent porphyrinogen.

As much as 51 % was found as porphyrinogen in the urine collected from the 2-allyl-2-isopropylacetamide-treated rabbit, whereas in the urine obtained after administration of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine all coproporphyrin was present in the reduced form. Chromatography of the porphobilinogen and δ-aminolaevulic acid excreted in abnormal amount in rabbit urine after feeding with 2-allyl-2-isopropyl-

acetamide or 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine showed them to behave similarly to authentic markers. Paper chromatography of porphyrin isomers revealed that both copro- and uro-porphyrin were present mainly as series III isomer in the urine obtained after treatment with either drug; small amounts of porphyrins behaving as pentacarboxylic and hexacarboxylic porphyrin in the 2,6-lutidine method were also found in the two cases.

After an initial dose of drug and after the excretion of porphyrins, δ-aminolaevulic acid and

Table 2. *Urinary excretion of porphyrins and porphyrin precursors before and after administration of a single dose* (1·5 *g.*) *of either* 2-*allyl*-2-*isopropylacetamide or* 3,5-*diethoxycarbonyl*-1,4-*dihydro*-2,4,6-*trimethylpyridine to the rabbit*

Time (hr.)	δ-Aminolaevulic acid (mg./l.)	Porphobilinogen (mg./l.)	Uroporphyrin (mg./l.)	Coproporphyrin (mg./l.)
	2-Allyl-2-isopropylacetamide			
Before dosing	4·2	1·6	0·09	0·47
After dosing				
4	3·6	3·2	0·11	0·45
8	2·7	8·6	0·045	0·16
10	19·7	48·8	0·15	0·68
12	41·7	124	0·56	1·1
14	20·6	113	0·63	1·4
27	60·4	194	6·1	3·2
48	17·6	26·8	6·7	2·7
67	3·3	1·3	0·086	0·36
118	3·7	0·7	0·009	0·42
	3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine			
Before dosing	1·7	Nil	Nil	0·16
After dosing				
4	1·2	0·74	Nil	0·07
6	2·4	Nil	Nil	0·16
14	1·2	5·8	Nil	0·78
16	0·84	10·6	0·06	0·99
18	5·9	50·7	0·10	2·7
54	26·2	54·7	0·29	1·7
102	41·3	219	4·9	0·80
126	5·3	66·3	1·0	4·5
174	0·42	Nil	0·03	0·23

Table 3. *Determination of porphyrins and test for porphyrin precursors on organs of two rabbits treated with either* 2-*allyl*-2-*isopropylacetamide or* 3,5-*diethoxycarbonyl*-1,4-*dihydro*-2,4,6-*trimethylpyridine*

Both rabbits were killed while excreting excessive amounts of porphyrins, porphobilinogen and δ-aminolaevulic acid in their urine. Figures represent μg./g. of tissue.

Treatment	Copro-porphyrin	Proto-porphyrin	Uroporphyrin	Porpho-bilinogen	δ-Aminolaevulic acid
			Liver		
Normal rabbit*	0·02–0·18	0·10–0·51	Nil	Nil	Nil
2-Allyl-2-isopropylacetamide	2·23	3·41	Nil	Very strong +	Very strong +
3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethyl-pyridine	20·10	406·70	Nil	Very strong +	Very strong +
			Kidney		
Normal rabbit*	0·03–0·24	0·02–0·07	Nil	Nil	Nil
2-Allyl-2-isopropylacetamide	0·27	0·25	0·15	Strong +	Strong +
3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethyl-pyridine	1·02	1·24	0·26	Strong +	Strong +
			Bone marrow		
Normal rabbit*	0·10–0·22	Nil–0·24	Nil	Nil	Nil
2-Allyl-2-isopropylacetamide	0·02	0·31	0·15	Nil	Nil
3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethyl-pyridine	0·01	0·01	Nil	Nil	Nil

* Goldberg & Rimington (1955).

porphobilinogen had returned to normal, two rabbits were given further doses of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine or 2-allyl-2-isopropylacetamide and killed while porphyric, and the concentration of porphyrins was determined in the liver, kidney and bone marrow; these were also tested for porphobilinogen and δ-aminolaevulic acid. As normal values for tissue porphyrins the figures given by Goldberg & Rimington (1955) were taken. In both the 2-allyl-2-isopropylacetamide- and 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine-treated animals there was an increase of ether-soluble porphyrins in the liver and, to a less degree, in the kidney with a strongly positive test for porphobilinogen and δ-aminolaevulic acid in these two tissues (Table 3). Moreover, the liver from the 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine-treated rabbit showed spontaneous red fluorescence in u.v. light and chemical analysis revealed massive deposition of copro- and proto-porphyrin. In neither of the two animals did the liver contain porphyrins with a higher HCl number, such as the so-called 'green porphyrins' described by Schwartz & Ikeda (1955) in experimental porphyria. The porphyrin concentration of the bone marrow did not exceed normal values in either animal.

DISCUSSION

This work has shown that, in spite of being chemically quite unrelated, 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine and 2-allyl-2-isopropylacetamide are able to induce in rats and rabbits a very similar, if not indistinguishable, picture of experimental hepatic porphyria. Post-mortem examination of the organs of rabbits treated with either drug showed a similar distribution of porphyrins and porphyrin precursors with a rise in the concentration of all these compounds in the liver but not in the bone marrow. There was no significant difference in the excretion pattern of urinary porphyrins and their precursors between 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine- and 2-allyl-2-isopropylacetamide-treated animals; both rats and rabbits responded to the administration of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine with excretion of porphobilinogen and uroporphyrin as well as coproporphyrin and this was also seen in the 2-allyl-2-isopropylacetamide-treated animals. We were therefore unable to confirm Solomon & Figge's (1959) findings of lower urinary uroporphyrin and only occasional presence of porphobilinogen in 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine porphyria as compared with Sedormid porphyria. A single dose of either drug to rabbits showed an increase in the urinary concentrations of δ-aminolaevulic acid and porphobilinogen preceding that of porphyrins themselves; this suggests that over-production of these precursors may be the primary abnormality in these experimental conditions. However, as the time interval between the two observed increases was only small and as an earlier slight increase of coproporphyrin was also seen, we feel that the observed data are not sufficient to draw a definitive conclusion on this point.

Coproporphyrin excreted in abnormal amount in rabbit urine after 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine or 2-allyl-2-isopropylacetamide administration was found to consist largely if not entirely of coproporphyrinogen. According to Heikel, Knight, Rimington, Ritchie & Williams (1960) it seems very likely that only coproporphyrinogen can pass through the glomeruli, the coproporphyrin found in fluorescent form in the urine being secondarily formed by oxidation. Our present findings in experimental porphyria confirm this view. Solomon & Figge (1959) found no characteristic red fluorescence in frozen sections of liver from porphyric animals examined, immediately after death, under an ultraviolet microscope. They found, however, that about 4 min. after preparation red fluorescence appeared in the form of numerous small areas scattered throughout the unstained preparation and gradually increased until it filled the whole microscopic field. This agrees with our findings of increased amounts of coproporphyrin excreted in the urine in non-fluorescent form and may suggest that the porphyrins accumulate in the liver in the form of reduced porphyrinogen.

Our detailed study of the urinary excretion of porphyrin precursors in both these experimentally produced porphyrias has revealed that not only porphobilinogen but also δ-aminolaevulic acid increases during treatment, a results which does not confirm Stich's (1958) finding of very high porphobilinogen but quite normal δ-aminolaevulic acid in experimental Sedormid porphyria. The rise in urinary δ-aminolaevulic acid coincided with that of porphobilinogen throughout these experiments. Post-mortem examination revealed in both rabbits the presence of abnormally high amounts of δ-aminolaevulic acid in the liver. Gibson (1955) has described an increase of δ-aminolaevulic acid dehydrase activity in the liver of a rabbit poisoned with Sedormid and this may account for our observed decrease in the urinary δ-aminolaevulic acid/porphobilinogen ratio in porphyric animals. Comparison have already been made between human acute porphyria and experimental hepatic porphyria (Schmid & Schwartz, 1952; Goldberg, 1954*b*; Goldberg & Rimington, 1955; De Matteis, 1960). In addition to the similarities already described we would like to lay emphasis on our find-

ings of increased δ-aminolaevulic acid excretion and decreased δ-aminolaevulic acid/porphobilinogen ratio in experimental porphyrias which parallel exactly the findings in human acute porphyria (Granick & Vanden Schrieck, 1955; Ackner, Cooper, Gray, Kelly & Nicholson, 1961).

No significant alteration of allantoin level in rat urine was observed after 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine, 2-allyl-2-isopropylacetamide or Sedormid treatment. Labbe *et al.* (1955) have described a disturbance of purine synthesis in Sedormid- and 2-allyl-2-isopropylacetamide-poisoned chick embryos with reduced excretion of uric acid in their allantoic fluid and reduced incorporation of [α-^{14}C]glycine into uric acid. Impaired utilization of 4-amino-5-imidazolecarboxamide by the livers of rabbits with experimental porphyria was also observed. On the basis of these results they postulated that in experimental porphyria there is a block in conversion of δ-aminolaevulic acid into purines with a resulting over-production of pyrrole compounds. However, Merchante, Wajchenberg & Schwartz (1957) found no significant alteration in the concentration of ribo- or deoxyribo-nucleic acid in the liver of Sedormid-treated rats, and also from our own results there is no evidence of any impairment of purine metabolism in rats with experimental porphyria.

A fall in liver-catalase activity was noted in both the 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine- and 2-allyl-2-isopropylacetamide-treated rats. No significant difference in activity was observed in this respect between the two drugs except that in the rats dosed with 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine the drop in enzyme activity and rise in hepatic porphyrins occurred at different times rather than at the same time. The meaning of the decline in liver catalase in experimental porphyria remains unsolved. The strict relation existing between increased excretion of porphyrins and porphyrin precursors on the one hand, and decrease of liver catalase on the other hand, in these differently produced experimental conditions, seems to suggest that the fall in catalase is not merely an incidental phenomenon. We are, however, unable to provide a satisfactory explanation for the observed dissociation in point of time between the rise in hepatic porphyrins and fall in hepatic catalase in the 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine-poisoned animals, a dissociation somewhat unexpected if the two phenomena are causally related.

The nature of the primary abnormality in experimental hepatic porphyria remains uncertain. It is believed that this abnormality in porphyrin metabolism is related primarily to a disturbance in liver metabolism, and there are two possibilities which might account for the excessive porphyrin, porphobilinogen and δ-aminolaevulic acid excretion, namely their under-utilization or their over-production. Under-utilization alone seems to be insufficient to account for the quantity of compounds accumulated and excreted (Goldberg & Rimington, 1955; Schmid, 1955); there is little doubt that over-production actually does occur. Further study is required to clarify these problems, and particular attention should be paid to the initial steps in porphyrin biosynthesis in the liver and their relation to other metabolic pathways (De Matteis, 1961).

SUMMARY

1. The effect of the administration of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine on the urinary excretion of porphyrins, porphobilinogen and δ-aminolaevulic acid has been studied in rats and rabbits and compared with the results obtained after administration of Sedormid (2-isopropylpent-4-enoylurea) or 2-allyl-2-isopropylacetamide. The influence of these drugs on liver catalase and urinary excretion of allantoin in rats has also been investigated.

2. In spite of being chemically quite unrelated 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine and the drugs of the Sedormid group have been found to induce in both species studied a very similar, if not indistinguishable, picture of experimental hepatic porphyria.

3. No significant alteration of the urinary concentration of allantoin in rats was observed after dosing with any of these drugs.

4. A fall in liver-catalase activity was obtained after administration of both types of drug with the difference that 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine was a little slower than 2-allyl-2-isopropylacetamide in causing depression of the enzyme activity.

5. The significance of these experimental findings and their relevance to human acute porphyria and to the pathogenesis of the experimental porphyria have been discussed.

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Biochem. J. (1962) **83**, 8

The Biochemical Preparation of D-Xylulose and L-Ribulose

DETAILS OF THE ACTION OF *ACETOBACTER SUBOXYDANS* ON D-ARABITOL, RIBITOL AND OTHER POLYHYDROXY COMPOUNDS

BY V. MOSES AND R. J. FERRIER*
Bio-organic Chemistry Group, Lawrence Radiation Laboratory, University of California, Berkeley 4, Calif., U.S.A.

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Bacteria of the genus *Acetobacter* have long been known to oxidize polyols with particular steric configurations to ketoses (Bertrand, 1898*a*, *b*). *Acetobacter suboxydans* (Kluyver & de Leeuw, 1924), in particular, is characterized by many very specific quantitative oxidations of this type (Butlin, 1936; Fulmer & Underkofler, 1947; Hann, Tilden & Hudson, 1938), and has been used on a preparative scale to obtain previously unknown or rare substances; e.g. in the oxidation of D-*gluco*-D-*ido*-heptitol to D-*ido*-heptulose (Pratt, Richtmeyer & Hudson, 1952), D-arabitol to D-xylulose (Prince & Reichstein, 1937), ribitol to L-ribulose (Reichstein, 1934), β-sedoheptitol to L-*gulo*-heptulose (Stewart, Richtmeyer & Hudson, 1952) and erythritol to L-erythrulose (Whistler & Underkofler, 1938). Other acyclic sugar derivatives have recently been used successfully as substrates (Jones, Perry & Turner, 1961).

The steric requirements for oxidation of polyols have been defined (Hann *et al.* 1938; Fulmer & Underkofler, 1947): two contiguous D-secondary hydroxyl groups must be adjacent to the primary hydroxyl group at the bottom of the Fischer projection formulae. Cyclitols have also been found to be susceptible to oxidations of this type and rules governing the stereospecificity of the reaction have been put forward (Magasanik, Franzl & Chargaff, 1952; Anderson, Tomita, Kussi & Kirkwood, 1953). Posternak & Ravenna (1947) showed that *A. suboxydans* attacked cyclohexane-

* Permanent address: Birkbeck College, University of London.