Porphyrin Metabolism in Mice Following Quantitative Administration of Collidine Compounds and Oxidation Products. (26149)

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In a study involving the fluorescence of the forestomach of mice, diethyl-1, 4-dihydro-2, 4,6 - trimethylpyridine - 3, 5 - dicarboxylate (hereafter called "collidine derivative") was added to a white bread diet for the mice(1). The "collidine derivative" was blue fluorescent but it caused the gall bladder and part of the intestines to become bright red fluorescent. It was concluded that the "collidine derivative" probably disturbed the porphyrin metabolism of the mice to such a degree that the bile became red fluorescent(1). This hypothesis was later tested and validated(2). It was thus established that the "collidine derivative," when fed to mice, produced a disturbance in porphyrin metabolism characterized by marked increases in hepatic proto- and coproporphyrin. The oral method of administration made a precise and uniform dosage of the agent impossible. In the present study, the "collidine derivative" was injected intraperitoneally daily to achieve a more accurate method of drug administration. In addition, the effects of chemical alteration of the "collidine derivative" molecule have been studied and the hepatic coproporphyrins were subjected to isomer analysis.

Materials and methods. Twenty-five male mice of the ZBC strain weighing 20-25 g were housed in the dark and exposed to tungsten light only during the time of the daily injection. "Collidine derivative" dissolved in Mazola corn oil 20 mg/ml was injected but it was necessary to warm the solution slowly before use to dissolve completely the "collidine derivative." Daily injections of 0.25 ml/mouse were administered intraperitoneally. Liver samples were prepared as described previously(2) and subjected to porphyrin analysis using the method of Schwartz and Wikoff(3). Qualitative analysis for green porphyrins was carried out according to the method of Schwartz and Ikeda(4). Qualitative isomer determinations of hepatic coproporphyrins were made employing ascending paper chromatography (method of Dr. Mohammed Azziz, to be published).

The following compounds were prepared according to the method of Hantzch(5): 1) diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate. 2) 2,4,6-trimethylpyridine-3, 5-dicarboxylic acid. 3) Dipotassium-2,4,6-trimethylpyridine-3,5-dicarboxylate. Since the first compound is an oil, it was tested by intraperitoneal injection. The remaining 2 compounds were added to a ground food diet (1:18). Thirty male mice of the A strain weighing 20-25 g were used to test these substances.

Results. Animals receiving "collidine derivative" *via* the intraperitoneal route showed a disturbance in porphyrin metabolism similar to those receiving the drug in their diets (2). Hepatic protoporphyrin was present in much higher concentration than hepatic coproporphyrin. No green porphyrins could be detected in livers of animals treated for 10 days. Isomer analysis revealed the presence of only coproporphyrin III in hepatic tissue of animals receiving "collidine derivative" for 10 days. Examination of livers from animals treated with diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate; 2,4,6-trimethylpyridine-3,5-dicarboxylic acid; and dipotassium-2,4,6-trimethylpyridine-3,5-dicarboxylate revealed no increases in hepatic porphyrin concentrations within a 10-day period of treatment.

Discussion. The disturbance in porphyrin metabolism caused by "collidine derivative" has been contrasted with Sedormid porphyria (2). Both agents cause increases in hepatic proto- and coproporphyrin. The high concentrations of urinary porphobilinogen and uroporphyrin in Sedormid porphyria are not found following administration of "collidine derivative." Large quantities of green por-

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phyrins were noted in hepatic tissue of Sedormid-treated animals by Schwartz and Ikeda (4). Accumulation of these compounds in Sedormid porphyria and their absence in porphyrin induced by "collidine derivative" is further evidence of dissimilar mechanisms of the action of these two agents. Examination of sections of mouse liver (stained with H & E) from animals receiving "collidine derivative" for 7 days revealed a marked proliferation of bile ducts and periportal fibrosis. The portal areas were infiltrated with inflammatory cells. This picture of liver injury was associated with increased hepatic coproporphyrin III levels. No coproporphyrin I could be detected in livers of treated animals.

Mild oxidation of "collidine derivative" yields diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate. The latter compound has no effect on the porphyrin content of mouse liver. Williams(6) states that pyridine is oxidized *in vivo* to methylpyridinium hydroxide and excreted as an ethereal sulphate and a conjugated glucuronide. Similarly, picoline is oxidized *in vivo* to picolinic acid and excreted as the glycine conjugate, pyridinuric acid. The metabolism of "collidine derivative" has not been described; however, its activity may be related to an inability of liver to convert it to the oxidized, inactive compound: diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate.

Conclusions. Parenteral administration of 5 mg/mouse/day of diethyl-1, 4-dihydro-2, 4,6-trimethylpyridine-3,5-dicarboxylate ("collidine derivative") in corn oil causes increased hepatic and biliary proto- and coproporphyrin levels. Hepatic coproporphyrin was found to be entirely Type III isomer. No green porphyrins are found in livers of animals receiving "collidine derivative." Oxidation products of "collidine derivative" did not produce abnormalities in porphyrin metabolism within a 10-day observation period.

1. Figge, F. H. J., *J. Histochem. Cytochem.*, 1959, v7, 257.

2. Solomon, H. M., Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 583.

3. Schwartz, S., Wikoff, H. J., *J. Biol. Chem.*, 1952, v194, 564.

4. Schwartz, S., Ikeda, K., Ciba Foundation Symposium Porphyrin Biosynthesis and Metabolism. Boston, Little, Brown and Co., 1955, p. 209.

5. Hantzch, A., *Annalen*, 1882, v215, 21.

6. Williams, R. T., *Detoxication Mechanisms*. New York, John Wiley & Sons, Inc., 1949, p. 199.

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Glucose-6-Phosphatase Activity in Various Sheep Tissues.* (26150)

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The significance of carbohydrate in ruminant metabolism differs in some ways from that in other mammals(1). Of special interest are the chemical precursors and sites of formation of glucose, because little glucose appears to be absorbed from the ruminal digestive tract(1). Arteriovenous differences have suggested that glucose may be contributed to the blood of sheep not only by the liver but also by extra-hepatic tissues(2). A similar result on ruminant-like marsupials has been reported(3). It is usually considered that tissues which can release glucose into the blood have glucose-6-phosphatase activity, and in the rat this is largely restricted to liver, kidney and intestine(4). To investigate the possibility that ruminants differ in this respect from rats we have estimated the glucose-6-phosphatase activity of various sheep tissues.

Methods. The technic has been described by Hers and de Duve(4,5). We repeated their work on rats, then continued with adult sheep. About 5 g samples of liver, kidney, intestine, salivary gland, brain, muscle, rumen epithelium and mammary gland were

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