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Amniotic fluid maturation defect
coproporhydin premature infants

Coproporphyrin Excretion in Amniotic Fluid and Urine from Premature Infants: A Possible Maturation Defect

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Extract

Coproporphyrin analysis was performed on urine from 17 male and 7 female premature infants (birth wt 1.5–4.5 kg, average 1.7 kg). Six specimens of amniotic fluid obtained before elective abortion induced by saline solution infusion were also examined. Normal adult control subjects excreted 24.6% ± 5.6% of total coproporphyrin as coproporphyrin I. Twenty-three premature infants excreted 59.4% ± 17.3% as coproporphyrin I, significantly higher amounts than control subjects ($P < 0.001$). Coproporphyrin I excretion in six specimens of amniotic fluid was 84.9% ± 10.4%, significantly higher than in urine from adults and premature infants ($P < 0.001$).

Speculation

These results raise the possibility of a similar hepatic excretory defect in porphyrin metabolism in the developing fetus and in the Dubin-Johnson syndrome. In the former, the defect is developmental; in the latter, it is lifelong and is present in obligate heterozygotes. Uroporphyrinogen III cosynthetase normally catalyzes conversion of porphobilinogen to isomer III rather than isomer I porphyrins. Developmental deficiency of this enzyme may be responsible for the observed pattern of coproporphyrin isomers seen in the fetus and neonate.

Introduction

Porphyrins exist in nature as I and III isomers. Isomer I porphyrins are excretory products without known function. Isomer III porphyrins are precursors of heme. Both isomers are produced primarily in liver and bone marrow and are found normally in urine and feces [12]. Coproporphyrin excretion in urine has been studied in adults [2, 3, 8–11, 18], but not in infants or amniotic fluid.

Methods

Total coproporphyrin excretion was determined spectrophotometrically in random urine specimens according to the method of Rimington [14, 15] as modified by Ben-Ezzer *et al.* [3, 18]. Creatinine concentration in urine was determined by the alkaline picrate method, and micrograms of coproporphyrin per gram of creatinine were determined. Assuming excretion in urine of 8 mg of creatinine/kg/24 hr in infants [1, 19] and 20 mg/kg/24 hr in adults [4], micrograms of coproporphyrin excreted per kilogram per day were calculated. From standard tables [5], using height and weight of each individual, surface area (in square meters) was estimated, and micrograms of coproporphyrin per square meter per day were calculated. On repeated assay of a single specimen, total coproporphyrin excretion in urine was within 14% of the mean.

Coproporphyrin isomers I and III were separated by thin layer chromatography (Uniplate Silica Gel G-250) by a modification of the method of Jensen [3, 6, 18] and the relative percentage of the two isomers was calculated [3]. The average R_F was 0.17 for coproporphyrin I, and 0.23 for coproporphyrin III. Repeated coproporphyrin determinations of single specimens were within 1.4% of the mean. Statistical analysis was performed with the use of Student's t test [17].

Coproporphyrin analysis was performed on urine from 17 male and 7 female premature infants (birth wt 1.5–4.5 kg, average 1.7 kg). Six specimens of amniotic fluid obtained before elective abortion induced by saline solution infusion were also examined. These results were compared with those obtained from studying 16 adults with the Dubin-Johnson syndrome (D-JS), 25 individuals classified as obligate heterozygotes [18] for the D-JS (parents and children of affected individuals), and 20 normal control subjects.

Results

Results of coproporphyrin studies are summarized in Table I and are expressed as mean ± SD.

Normal adult control subjects excreted 24.6% ± 5.6% of total coproporphyrin as coproporphyrin I. Twenty-three premature infants excreted 59.4% ± 17.3% as coproporphyrin I, an amount significantly higher than control subjects ($P < .001$). The proportion of coproporphyrin I in six specimens of amniotic fluid was 84.9 ± 10.4%, significantly higher than in urine from adults and premature infants ($P < 0.001$). As reported previously [18], patients with D-JS excreted 88.9% ± 5.0% as coproporphyrin I, an amount significantly higher than control subjects ($P < 0.001$). Obligate heterozygotes excreted 31.6% ± 5.9% as coproporphyrin I, intermediate between control subjects and D-JS patients ($P < 0.001$).

Total coproporphyrin excretion in urine in control subjects was 37.8 ± 14.1 $\mu g/m^2/24$ hr, and was significantly lower ($P < 0.001$) in 14 premature infants (16.9 ± 11.9 $\mu g/m^2/24$ hr) and in 22 obligate D-JS heterozygotes (21.3 ± 9.6 $\mu g/m^2/24$ hr). Sixteen D-JS patients excreted 41.5 ± 16.9 $\mu g/m^2/24$ hr, not significantly different from control subjects ($P > 0.40$).

Coproporphyrin I excretion in control subjects was 9.3 ± 4.5 $\mu g/m^2/24$ hr. This was not significantly different in premature infants (11.2 ± 8.4 $\mu g/m^2/24$ hr, $P > 0.2$) or in obligate heterozygotes (6.9 ± 3.3 $\mu g/m^2/24$ hr, $P > 0.05$). Coproporphyrin I excretion was significantly higher in D-JS patients (37.0 ± 15.4 $\mu g/m^2/24$ hr) than in control subjects ($P < 0.001$).

Coproporphyrin III excretion in control subjects was 28.5 ± 10.8 $\mu g/m^2/24$ hr. This was significantly lower in premature infants (6.3 ± 5.3 $\mu g/m^2/24$ hr, $P < 0.001$), in D-JS patients (4.5 ± 2.6 $\mu g/m^2/24$ hr, $P < 0.001$), and in obligate D-JS heterozygotes (15.4 ± 9.8 $\mu g/m^2/24$ hr, $P < 0.001$).

Discussion

Amniotic fluid and urine from premature infants contain a large proportion of coproporphyrin I when compared with urine from normal adults. These findings could result from alterations in metabolic clearance or production of porphyrins. The coproporphyrin excretion pattern present in amniotic fluid as well as urine from premature infants may be secondary to circulating estrogens or other inhibitory hormones which increase coproporphyrin I excretion in urine by reduction of hepatic excretory function [7, 13, 16]. Increased coproporphyrin I in amniotic fluid and urine from neonates may also result from immaturity of porphyrin metabolism producing a developmental defect similar to that seen as a life-long abnormality in patients with the D-JS [3, 11, 18]. This syndrome results from defect in hepatic excretion of certain organic anions, and is characterized uniquely by

Table I. Coproporphyrin excretion in urine and amniotic fluid[1]

	No.	Coproporphyrin I, %	No.	Total coproporphyrin in urine, $\mu g/m^2/24$ hr	No.	Coproporphyrin I in urine, $\mu g/m^2/24$ hr	No.	Coproporphyrin III in urine, $\mu g/m^2/24$ hr
Amniotic fluid	6	84.9 ± 10.4[2]						
Premature infants	23	59.4 ± 17.3[2]	14	16.9 ± 11.9[2]	13	11.2 ± 8.4	13	6.3 ± 5.3[2]
Dubin-Johnson syndrome	16	88.9 ± 5.0[2]	16	41.5 ± 16.9	16	37.0 ± 15.4[2]	16	4.5 ± 2.6[2]
Dubin-Johnson syndrome obligate heterozygotes	25	31.6 ± 5.9[2]	22	21.3 ± 9.6[2]	22	6.9 ± 3.3	22	15.4 ± 9.8[2]
Normal adults	20	24.6 ± 5.6	20	37.8 ± 14.1	20	9.3 ± 4.5	20	28.5 ± 10.8

[1] All results are expressed as mean ± SD.

[2] Differs from normal adults, $P < 0.001$.

normal total coproporphyrin excretion in urine with approximately 80% or greater as coproporphyrin I [2, 3, 9–11, 18]. Genetically obligate heterozygotes for the D-JS have intermediate coproporphyrin I excretion in urine [18]. The observations in amniotic fluid and urine from neonates are similar and may result from developmental immaturity of an enzyme (or enzymes) such as uroporphyrinogen III cosynthetase which regulates conversion of porphobilinogen to the III rather than the I isomer series. Developmental deficiency of this enzyme may be responsible for the observed pattern of coproporphyrin isomers seen in the fetus and neonate.

References and Notes

1. Amberg, S., and Morrill, W. P.: On the excretion of creatinin in the new-born. J. Biol. Chem., *3:* 311 (1907).
2. Aziz, M. A., Schwartz, S., and Watson, C. J.: Studies of coproporphyrin. Reinvestigation of the isomer distribution in jaundice and liver disease. J. Lab. Clin. Med., *63:* 596 (1964).
3. Ben-Ezzer, J., Rimington, C., Shani, E. M., Seligsohn, U., Sheba, C., and Szeinberg, A.: Abnormal excretion of the isomers of urinary coproporphyrin by patients with Dubin-Johnson syndrome in Israel. Clin. Sci., *40:* 17 (1971).
4. Case Records of the Massachusetts General Hospital. Normal laboratory values. New Engl. J. Med., *283:* 1276 (1970).
5. Diem, K., editor: Documenta Geigy Scientific Tables, Ed. 6, p. 632 (Geigy Pharmaceuticals, Ardsley, N.Y., 1962).
6. Jensen, J.: Separation of the coproporphyrin isomers I and III by thin layer chromatography. J. Chromatogr., *10:* 236 (1963).
7. Kappas, A.: Estrogens and the liver. Gastroenterology, *52:* 113 (1967).
8. Koskelo, P., Eisalo, A., and Toivonen, I. Urinary excretion of prophyrin precursors and coproporphyrin in healthy females on oral contraceptives. Brit. Med. J., *1:* 652 (1966).
9. Koskelo, P. and Toivonen, I.: Separation of urinary coproporphrin isomers I and III by thin-layer chromatography: Studies in healthy subjects and patients with myocardial infarction. Scand. J. Clin. Lab. Invest., *18:* 543 (1966).
10. Koskelo, P., and Toivonen, I.: Urinary excretion of coproporphyrin isomers I and III and δ-aminolaevulic acid in normal pregnancy and obstetric hepatosis. Acta Obstet. Gynecol. Scand., *47:* 292 (1968).
11. Koskelo, P., Toivonen, I., and Adlercreutz, H.: Urinary coproporphyrin isomer distribution in the Dubin-Johnson syndrome. Clin. Chem., *13:* 1006 (1967).
12. Marver, H. S., and Schmid, R.: In: J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson: The Porphyrias, The Metabolic Basis of Inherited Disease, Ed. 3, p. 1087 (McGraw-Hill Book Company, New York, 1972).
13. Mowat, A. P., and Arias, I. M.: Liver function and oral contraceptives. J. Reprod. Med., *3:* 19 (1969).
14. Rimington, C.: Quantitative determination of porphobilinogen and porphyrins in urine and feces (Broadsheet No. 36, new series) (Association of Clinical Pathologists, London, 1961).
15. Rimington, C., and Sveinsson, S. L.: The spectrophotometric determination of uroporphyrin. Scand. J. Clin. Lab. Invest., *2:* 209 (1950).
16. Roy, E. J., and Brown, J. B.: A method for the estimation of oestriol, oestrone and oestradiol-17β in the blood of the pregnant woman and of the foetus. J. Endocrinol., *21:* 9 (1960).
17. Snedecor, G. W., and Cochran, W. G.: Statistical Methods, Ed. 6 (Iowa State University Press, Ames, Iowa, 1967).
18. Wolkoff, A. W., Cohen, L. E., and Arias, I. M.: Inheritance of the Dubin-Johnson syndrome. New Engl. J. Med., *288:* 113 (1973).
19. Younoszai, M. K., and Haworth, J. C.: Excretion of hydroxyproline in urine by premature and normal full-term infants and those with intrauterine growth retardation during the first three days of life. Pediat. Res., *2:* 17 (1969).

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