# Coproporphyrin Studies in Children. 2. Erythrocyte Coproporphyrin and Protoporphyrin Levels in Normal Infants and Children.* (21017)

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Although the presence of free protoporphyrin in the circulating red blood cells was described by van den Bergh and Hyman(1) in 1928, it is only with the advent of improved fluorimetry that coproporphyrin has also been found in the red blood cells by Schwartz and Wikoff(2). In studies on normal adults, Watson(3) has reported the mean erythrocyte protoporphyrin (EP) to be 28.5 ± 4.5 μg/100 cc of packed erythrocytes for males and 41.7 ± 5.9 μg/100 cc for females. The mean erythrocyte coproporphyrin (ECP) was found to be 0.3 ± 0.39 μg/100 cc of packed erythrocytes for males and 0.7 ± 0.6 μg/100 cc for females. Krammer and his coworkers(4) have shown essentially the same values for EP, but their ECP values were 0.76 ± 0.26 μg/100 cc of packed erythrocytes for males and 0.71 ± 0.23 μg/100 cc for females. They attribute this difference of ECP concentration to the greater sensitivity of the fluorimeter used.

Since EP and ECP are known to be altered in many childhood diseases(3-8), the present study was undertaken to define the range of EP and ECP in normal infants and children.

*Methods and materials.* A total of 24 newborn infants and 19 older children of various ages were selected for this study. All were healthy and free from anemia and other hematologic disturbances. The following studies were done on samples of venous blood collected in potassium oxalate tubes: 1) hemoglobin, 2) reticulocyte count, 3) total plasma bilirubin(9), 4) EP and ECP of the packed erythrocytes using the method of Schwartz and Wikoff(2) with certain minor modifications. The coproporphyrin was measured on a Calectron fluorimeter† employing a photomultiplier tube and using the Corning No. 5543 as primary filter and Corning No. 2418 as the secondary filter. Values were determined by comparison with standard coproporphyrin solutions in 0.3 N HCl.‡ The protoporphyrin concentration determined by absorption analysis in an Evelyn colorimeter using a calibration curve as shown by Grinstein and Watson(10).

*Results.* The EP and ECP for normal children are given in Table I. The mean EP was 66 ± 37 μg/100 cc packed erythrocytes and the mean ECP was 1.1 ± 0.57 μg/100 cc packed erythrocytes. The EP and ECP did not appear to be related to either the age or sex of the children.

The EP and ECP for normal newborn infants are given in Table II. The mean EP was 86 ± 37 μg/100 cc packed erythrocytes and the mean ECP was 1.5 ± 0.70 μg/100 cc packed erythrocytes. The EP and ECP did not appear to be related to the sex, weight, or

TABLE I. Erythrocyte Coproporphyrin and Protoporphyrins in Normal Children.

| Sex | Age | Hgb (g %) | Retic. (%) | EP (μg/100 cc) | ECP (μg/100 cc) |
|---|---|---|---|---|---|
| ♀ | 3 wk | 12.0 | .3 | 54 | 1.3 |
| ♀ | 3 yr | 13.2 | .4 | 43 | .6 |
| ♂ | 3 | 12.0 | .2 | 150 | 1.8 |
| ♀ | 5 | 16.6 | .3 | 175 | 2.7 |
| ♂ | 5 | 14.2 | .3 | 92 | .7 |
| ♀ | 5½ | 12.1 | .5 | 47 | .7 |
| ♂ | 6 | 11.6 | .6 | 78 | .5 |
| ♂ | 6 | 13.8 | .5 | 59 | .8 |
| ♀ | 6 | 13.4 | .8 | 55 | 1.7 |
| ♂ | 7 | 13.4 | .5 | 55 | .8 |
| ♂ | 8 | 13.1 | .6 | 69 | 1.8 |
| ♂ | 10 | 12.9 | .4 | 33 | .7 |
| ♂ | 10 | 13.1 | .3 | 30 | .7 |
| ♀ | 12 | 12.9 | .3 | 58 | 1.8 |
| ♂ | 12 | 12.5 | .5 | 38 | 1.1 |
| ♀ | 12 | 14.2 | .3 | 22 | .7 |
| ♀ | 13 | 13.1 | .3 | 68 | .8 |
| ♀ | 15 | 13.4 | .4 | 58 | 1.6 |
| ♀ | 15 | 15.7 | .2 | 62 | 1.3 |
| | | | Mean | 66 | 1.1 |
| | | | S.D. | ±37 | ±.57 |

Plasma bilirubin was less than .8 mg % in all instances.

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† Manufactured by Calectron, Inc., St. Paul, Minn.

‡ Kindly supplied by Dr. Samuel Schwartz.

TABLE II. Erythrocyte Coproporphyrin and Protoporphyrin in Newborn Infants.

| Sex | Wt (lb) | Hgb (g %) | Bili. (mg %) | Retic. (%) | EP (μg/100 cc) | ECP |
|---|---|---|---|---|---|---|
| | | | Cord blood | | | |
| ♂ | 7'11 | 16.6 | 1.3 | 2.7 | 125 | 1.8 |
| ♂ | 7'3 | 16.0 | 4.3 | 2.8 | 203 | 2.8 |
| ♂ | 6'14 | 16.6 | 1.9 | 1.8 | 167 | .8 |
| ♂ | 7'2 | 17.1 | .8 | 2.1 | 101 | .6 |
| ♂ | 8'1 | 18.1 | .6 | 1.6 | 63 | 1.4 |
| ♀ | 7' | 20.1 | 3.7 | 1.8 | 114 | 1.4 |
| | | | | Mean | 125 | 1.5 |
| | | | First day (0-24 hr) | | | |
| ♀ | 7'14 | 18.9 | .9 | 3.1 | 63 | 1.3 |
| ♂ | 8'8 | 23.8 | 1.6 | 2.3 | 102 | 1.9 |
| ♂ | 5'12 | 18.1 | 3.2 | 2.6 | 46 | 1.3 |
| ♂ | 6'11 | 17.1 | 3.6 | 2.4 | 96 | 3.1 |
| ♀ | 7'11 | 22.0 | 1.6 | 2.1 | 69 | 1.6 |
| ♂ | 6'11 | 22.0 | 4.3 | 2.9 | 54 | 2.4 |
| | | | | Mean | 71 | 1.9 |
| | | | Second and third day (24-72 hr) | | | |
| ♂ | 7'13 | 17.0 | 1.4 | 1.0 | 55 | .9 |
| ♀ | 7'7 | 17.1 | 3.2 | 1.8 | 50 | 1.6 |
| ♀ | 6'14 | 19.9 | 0 | 2.6 | 107 | 1.6 |
| ♀ | 6'14 | 19.6 | 5.0 | 1.4 | 95 | 2.1 |
| ♂ | 9'2 | 14.9 | 5.8 | 3.6 | 97 | 2.6 |
| ♀ | 8'5 | 20.2 | 1.9 | 2.1 | 70 | .9 |
| | | | | Mean | 79 | 1.6 |
| | | | Fourth and fifth day (72-120 hr) | | | |
| ♂ | 8'7 | 17.0 | 3.6 | .8 | 96 | 1.7 |
| ♀ | 6'6 | 21.0 | 1.0 | 1.4 | 68 | 1.1 |
| ♀ | 7'10 | 19.6 | 1.6 | 1.3 | 54 | 1.7 |
| ♂ | 8'6 | 19.3 | 2.6 | 1.0 | 48 | .2 |
| ♂ | 6'10 | 13.4 | .9 | .8 | 54 | .8 |
| ♂ | 8'4 | 19.9 | .5 | .5 | 67 | .7 |
| | | | | Mean | 64 | 1.0 |
| | | | Mean for infants | | 86 | 1.6 |
| | | | S.D. | | ±37 | ±.7 |

hemoglobin determination. The correlation coefficient between bilirubin and ECP was $r = +0.62$ and between bilirubin and EP was $r = +0.22$. Although no significant difference could be found between the EP and ECP values from the cord blood to the fourth and fifth day, there appeared to be a decreasing trend with the values on the fourth, and fifth day close to normal.

The correlation coefficient in the entire series between ECP and reticulocyte percentage was $r = +0.43$ and between EP and reticulocyte percentage was $r = +0.27$. The correlation between EP and ECP was $r = +0.40$.

*Discussion.* The results show a general increase of both EP and ECP among the newborn infants and older children as compared with normal adults(5,6). The increase in EP in the older children is highly significant ($P < 0.01$) when compared with normal adults, but the difference between the newborn infants and older children is not significant. The increase in ECP in children is not significant when compared with normal adults, but the ECP in newborn infants shows a value greater than 2 standard deviations above the mean for the older children and is highly significant ($P < 0.01$) when compared with normal adults.

The increase of EP in infants and children probably reflects the iron deficient state in general. Cartwright(11) showed in adults that iron deficiency is manifested by decrease in serum iron, a reciprocal rise in serum copper, and moderate elevation of the erythrocyte protoporphyrin. Sturgeon(12) has suggested in infants and young children that the hypoferremia with reduction in the per cent saturation of the total iron-binding capacity, hypercupremia, and elevated erythrocyte protoporphyrin represents a general state of iron deficiency. The data here confirm his observations.

The increase of ECP in the early newborn period probably reflects increased erythropoiesis *in utero*. Schwartz and Wikoff(2) have shown that a close correlation exists between ECP and reticulocyte percentage. By removing large volumes of blood at regular intervals from dogs, they were able to stimulate erythropoiesis and cause simultaneous increase of reticulocytes and ECP levels. They believe that ECP is therefore a reflection of increased erythopoiesis in the body. Goldbloom and Gottlieb(13,14) first suggested that the fetus acquires an overabundance of red cells as a response to the relatively anoxic conditions *in utero*. At birth, these extra cells are no longer needed and are destroyed. Mollison(15) has shown that the rate of breakdown of the newborn infant's own erythrocytes may be increased during the first 10 days of life. Hsia(16) has found that the mechanical fragility of erythrocytes is elevated at birth and falls to normal levels by the fifth and sixth days of life. He found a direct correlation between the height of the mechanical fragility of erythrocytes on the first day

and the subsequent degree of hyperbilirubinemia suggesting an increased breakdown of "abnormal" erythrocytes during that period.

In the present study, the correlation between reticulocyte percentage and ECP described by Schwartz and Wikoff(2) could only be partially demonstrated. This is not surprising since very few of the reticulocyte counts exceeded 2% and it is difficult to demonstrate real differences at this low level. However, in the infants with high ECP, where presumably there is an increased degree of erythropoiesis prior to birth, there is a greater destruction of erythrocytes and subsequently more jaundice. In those with low ECP, the converse could be true. Sturgeon(12) has pointed out the extreme range and variability of EP in children as compared with normal adults. This is confirmed for both EP and ECP in this study. From the practical standpoint, it would appear as has been previously suggested in connection with urinary coproporphyrin excretion(17), that values higher than twice the standard deviation above the mean for either EP or ECP or both should be regarded as abnormal.

*Summary.* 1. Erythrocyte coproporphyrin and protoporphyrin levels have been determined in normal newborn infants and older children. 2. There is a significant elevation of EP in children as compared with normal adults. This increase probably reflects the general iron deficient state in children. 3. There is a significant elevation of ECP in newborn infants as compared with normal adults. This increase probably reflects increased erythropoiesis *in utero* and rapidly disappears by the fourth and fifth day of life. 4. The EP and ECP should be regarded as abnormal if they exceed twice the standard deviation above the mean.

1. van den Bergh, A. A. H., and Hyman, A. J., *Deutsche med. Wchnschr.,* 1928, v54, 1492.

2. Schwartz, S., and Wikoff, H. M., *J. Biol. Chem.,* 1952, v194, 563.

3. Watson, C. J., *Arch. Int. Med.,* 1950, v86, 797.

4. Krammer, A., Cartwright, G. E., and Wintrobe, M. M., *Blood,* 1954, v9, 183.

5. Dunsky, J., Freeman, S., and Gibson, S., *Am. J. Dis. Ch.,* 1947, v74, 305.

6. Watson, C. J., *J. Clin. Invest.,* 1936, v15, 332.

7. Kench, J. E., Gilliam, A. E., and Lane, R. E., *Biochem. J.,* 1952, v36, 384.

8. Watson, C. J., Hawkinson, V., Capps, R. B., and Rappaport, E. M., *J. Clin. Invest.,* 1949, v28, 621.

9. Hsia, D. Y. Y., Hsia, H. H., and Gellis, S. S., *J. Lab. and Clin. Med.,* 1952, v40, 610.

10. Grinstein, M., and Watson, C. J., *J. Biol. Chem.,* 1943, v147, 675.

11. Cartwright, G. E., and others. *Blood,* 1948, v3, 501.

12. Sturgeon, P., *Pediatrics,* 1954, v13, 107.

13. Goldbloom, A., and Gottlieb, R., *Am. J. Dis. Ch.,* 1929, v38, 57.

14. ———, *J. Clin. Invest.,* 1930, v8, 375.

15. Mollison, P. L., *Lancet,* 1948, v1, 513.

16. Hsia, D. Y. Y., Goldbloom, R. B., and Gellis, S. S., *Pediatrics,* 1954, v13, 24.

17. Hsia, D. Y. Y., and Page, M., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 86.

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