

**Age Related Changes in Urinary
6-P hydroxycortisol in Normal
Infants**

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Drug metabolism in the liver is carried out by the hepatic drug metabolizing enzymes (HDME). The essential action of these enzymes is to convert lipophilic drugs into more water soluble metabolites which are easily excreted in the urine. Direct estimation of HDME activity in the infant cannot be undertaken as these enzymes are only present in the liver tissue which necessitates a liver biopsy. Urinary 6- β hydroxycortisol is reported to show a

very good correlation with HDME activity(1,2)

6- β hydroxycortisol is a polar metabolite of cortisol formed in the endoplasmic reticulum of hepatocytes by a cytochrome P 450 enzyme and is excreted in the urine. A simple and sensitive ELISA using penicillinase (β lactamase) as the enzyme marker for quantitation of 6- β hydroxycortisol in urine was developed by us(3). The 6 β hydroxycortisol levels thus obtained were used to prepare a normal pattern for infants from 15 to 195 days of age.

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Material and Methods

Twelve normal healthy one week old full term exclusively breastfeeding infants were recruited for the study. The infants weighed 2.5 ± 0.2 kg and all of them belonged to normal healthy mothers not on any medication.

Urine samples were collected from day 15 to 195 at fortnightly intervals. The 4 hours urine sample (8.00 a.m. to 12.00 noon) was collected in infants urine collection bags (B. Braun Melsungen A.G.) since an excellent correlation has been reported(4) between 3 hours and 24 hours urine samples. The urine samples were measured, aliquoted and preserved with sodium azide at -20°C until analysis. The urine samples were diluted 1:100 with the assay buffer just before analysis.

Internal quality control pools for the three different concentrations of 6- β hydroxycortisol were prepared as follows: High pool was prepared by pooling all the spot urine samples collected from normal healthy mothers not on any medication. Low pool was prepared by pooling all the spot urine samples collected from normal healthy infants not on any medication. Medium pool was prepared by mixing equal quantities of urine from low and high pools. All the three pools were aliquoted and preserved with sodium azide at -20°C . Samples from low and medium pools were diluted 1:100 times whereas those from high pool were diluted 1:200 times. The concentration of each pool was established by assaying each pool in replicates of 12. The 6-p hydroxycortisol in urine samples and internal quality control pools were estimated by the ELISA technique(3) using: (i) 6- β hydroxycortisol from Steraloids Inc. Wilton, USA; and (ii) Penicillinase (EC 3.5.2:6) specific activity 66000 n/mg protein and penicillin V from Hindustan Antibiotics Limited, Pune, India. The 6- β hydroxycortisol 21 - hemisuccinate and antisera to 6- β

hydroxycortisol 21- hemi-succinate-BSA were a gift from Dr. Hosoda, Pharmaceutical Institute of Tohoku University, Japan.

Results

The sensitivity of the assay was 2.5 pg/well (25 pg/ml). Intra-assay and inter-assay coefficient of variations were below 10.0% and 15.0%, respectively.

The 6- β hydroxycortisol levels (ng/ml) in urine of normal breastfed infants from 15 to 195 days of infant's age are shown in *Fig. 1*. The straight line running parallel to the abscissa, indicates the grand mean, *i.e.*, the mean of all the 6- β hydroxycortisol values from 15-195 days. The between infant variation in the 6- β hydroxycortisol levels for the same days of urine collection was not significant (ANOVA; $p=0.2532$). However, when the Analysis of Variance was used to compare the 6- β hydroxycortisol levels when the infants were between the ages of 15-90 days and when the same infants were between 105-195 days, a significant difference was observed ($p < 0.0123$). The mean \pm SE for 6- β hydroxycortisol at 15-90 days and 120-195 days were 31.67 ± 1.89 and 45.04 ± 3.82 , respectively. This probably indicates that with an increase in the age of the infant, 6-p hydroxycortisol levels increase or indirectly, the infant's capacity to metabolize drugs by HDME system improves.

ANOVA test coupled with Duncan's multiple range test showed significant differences between day 105 with days 15, 30, 45, 60, 75 and 90 at 0.05 level. Similar results were observed between days 120,135, 150,165,180 and 195 (each separately) with days 15, 30, 45, 60, 75 and 90.

Discussion

Drugs are known to induce, inhibit or have no effect on HDME(5). Induction of

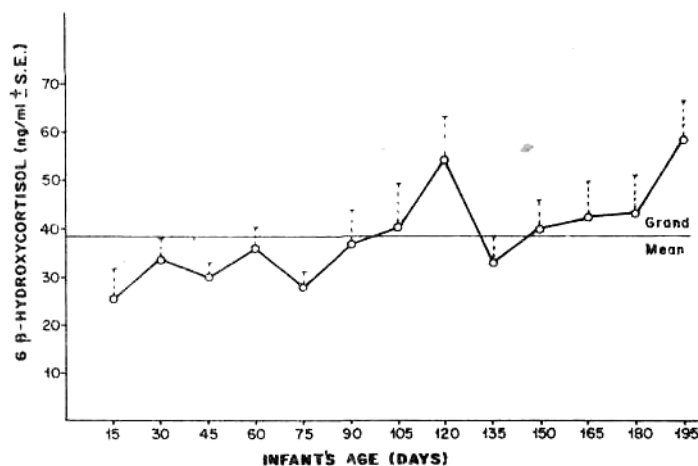


Fig. 1. 6- β hydroxycortisol levels (mean \pm SE) in urine of normal breastfed infants in relation to age.

the enzyme activity leads to reduced effects of the therapeutic drug due to inactivation by these enzymes. Inhibition of the enzyme activity results in an exaggerated and prolonged response with an increased risk of toxicity quantitative differences are known to exist between the rates of drug metabolism in children and adults(5-7). Hence, the data concerning the effects of drugs on the HDME levels in adults cannot automatically be applied to children. 6- β hydroxycortisol estimated serially in 12 infants from 15-195 days of infants age were used to plot a normal pattern. If 105 days of infants age are considered as a dividing point and all the 6- β hydroxycortisol values before and after 105 days are compared, it can be seen that all the first six values lie below the grand mean whereas of the later six, five lie above the grand mean. The normal pattern thus obtained can be used to compare it with the pattern obtained from infants who are exposed to maternal contraceptive or therapeutic drugs through their mother's milk.

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NOTES AND NEWS

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This event is to be held at Coimbatore on 6th and 7th July 1996. The Registration Fee is Rs. 400/- (for P.G. students Rs. 200/-). The last date for Registration is 15th June 1996. For further details please contact Dr. M. Ramaswamy, Organizing Secretary, Department of Pediatrics, P.S.G. Institute of Medical Sciences and Research, Peelamedu, Coimbatore 641 004.

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The IAP Bangalore Branch is organizing this event on 8th and 9th of June 1996. The Registration Fee is Rs. 200/- (for P.G. students Rs. 150/-). For further details please contact Dr. Radhakrishna Hegde S., Organizing Secretary, No. 64,1 Stage, 11th Cross, Indiranagar, Bangalore 560 038, Karnataka.

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This event is to be held between November 23-24, 1996 at Mumbai under the auspices of Pediatric Dermatology Unit, B.J. Wadia Hospital for Children, Indian Association of Dermatologists, Venereologists and Leprologists and Indian Academy of Pediatrics (Mumbai Branch). For further details please contact Dr. Deepak A. Parikh, Organizing Secretary, No. 1, Milan, Dixit Road, Vile-Parle (E), Mumbai 400 057. *Tel:* 6123410/9267: *Fax:* 00-91-22 6713580.
