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Urinary Excretion and Circadian Rhythm of Cyclic-AMP and Cyclic-GMP in Childhood

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Normal urine values of cyclic-AMP and cyclic-GMP were determined in healthy children, using a competitive protein binding and a radioimmunoassay technique respectively. Neither sex nor age affected the cyclic nucleotides excretion.

A circadian rhythm in the urinary excretion of cyclic-AMP was observed throughout a 20 h period. The peak excretion occurred at 16.00 h, while minimal excretion was at 24.00 h. The differences observed between the periods of highest and lowest excretion of cyclic-GMP were not statistically significant.

Adenosine 3',5'-monophosphate (cyclic-AMP) and guanosine 3',5'-monophosphate (cyclic-GMP) are important components of the metabolic process in the human body. They are excreted in urine and their estimation therein has been used to asses some abnormal states including allergic diseases, in which the relation between both nucleotides and histamine release is now well established (4, 6). The levels of cyclic nucleotides have often been measured in the adult population, but there is not as much childhood information (7).

The aim of the present study was to determine the normal urinary excretion of both cyclic nucleotides in healthy children and to observe the relationship between ages and sexes, in order to evaluate which diseases might influence their urinary excretion.

This study was also carried out to provide evidence of a rhythmic pattern in the urinary excretion of cyclic-AMP and cyclic-GMP and to observe the possible fluctuations induced by treatment in some allergic diseases.

Materials and Methods

Studies were conducted in 50 healthy children of both sexes (males and females), aged 3-12 years; prior to the experiment, the subjects underwent medical examina-

tions and were found to be physically normal.

To determine a rhythmic pattern in the urinary excretion of cyclic-AMP and cyclic-GMP, 12 healthy children under normal physic activity were studied. Four-hourly urine speciments were collected by voluntary excretion over a period of 20 h, starting at 8.00 a.m. Each specimen of urine was centrifuged and aliquots were frozen at —20° C until analyzed. Aliquots of the frozen specimens were assayed for their cyclic-AMP, cyclic-GMP and creatinine contents.

Cyclic-AMP was measured by a competitive protein binding assay and cyclic-GMP by a radioimmunoassay, with reagents obtained from the Radiochemical Centre, Amersham. The cyclic-AMP assay is based on the competition between the unlabelled cyclic-AMP present in the speciments and a fixed quantity of tritium labelled cyclic-AMP to bind a protein which has a high specificity and affinity for cyclic-AMP. Separation of the protein bound cyclic-AMP from the unbound nucleotide is acheived by adsorption of the free nucleotide on charcoal, followed by centrifugation. The cyclic-GMP radioimmunoassay is based on the competition between unlabelled cyclic-GMP and a fixed quantity of tritrium labelled compound to bind a specific antiserum. Separation of the antibody-bound cyclic-GMP from the unbound nucleotide is achieved by ammonium sulphate precipitation of the former, followed by centrifugation. Incubation time for cyclic-AMP was 12 h and for cyclic-GMP 4 h. Using a refrigerated centrifuge at 2-4° C, a good separation of bound from free cyclic nucleotide was obtained after 10 minutes at 4,500 *g*.

The standard curves were constructed plotting Co/Cx (Co c.p.m. bound in the absence of unlabelled cyclic nucleotide and Cx c.p.m. bound in the presence of standard or unknown unlabelled cyclic nucleotide), against pmoles/tube of standard cyclic nucleotides on linear graph paper. The amount of cyclic nucleotides in the unknown were then read of these lines using the measured Co/Cx values.

Urinary cyclic-AMP and cyclic-GMP were determined in diluted samples (1:25 in distilled water) and expressed in terms of μmol/g creatinine, using this compound as a reference of glomerular filtration. Creatinine was measured by the method of BARTELS (2).

Distributions were gaussian for both cyclic nucleotides. The level of significance was calculated using Student's t-test for two means or the paired t-test as judged appropriate. The regression line was calculated by the method of least squares.

Sensitivity, under the conditions of estimation, was 0.25 pmole cyclic-AMP/tube and 0.125 pmole cyclic-GMP/tube. Intra-assay variations, inter-assay variations and affinity are shown in table I.

Results

The mean value of urinary cyclic-AMP expressed as the excretion per gram creatinine was 5.18 ± 1.84 μmol/g creat. (mean value $\pm$ S.D.) with a range 2.16-8.74. The mean value of urinary excretion of cyclic-GMP was 0.61 ± 0.26 μmol/g creat. with a normal range of 0.175-1.059. Neither sex nor age affected the cyclic nucleotides excretion (table II). There was no correlation between urinary cyclic-AMP excretion and cyclic-GMP excretion in the same subject ($r = 0.4135$).

Normal limits were established between 3.34-7.02 μmol per gram creatinine. When these limits were applied to the subjects,

Table I. *Characteristics of the cyclic nucleotide assays.*

	Intra-assay variation (%)	Inter-assay variation (%)	Affinity (%)
Cyclic-AMP	3.71	13.28	55.04
Cyclic-GMP	8.44	10.50	42.94

Table II. *Urinary cyclic nucleotides excretion (μmol/g creatinine) in the group children studied.*
Results are expressed as mean ± S.D. Number of subjects in brackets.

Males	Females	Age (years) < 7	Age (years) 8-9	Age (years) > 10
		cyclic-AMP		
5.09 ± 1.8 (34)	5.33 ± 1.9 (19)	5.19 ± 1.6 (22)	5.26 ± 2.1 (17)	5.04 ± 2.0 (14)
		cyclic-GMP		
0.61 ± 0.26 (32)	0.61 ± 0.27 (18)	0.68 ± 0.27 (23)	0.55 ± 0.24 (14)	0.54 ± 0.25 (13)

25 % of them were found to have lower or higher cyclic-AMP values. For cyclic-GMP the normal limits were established between 0.35-0.87 μmol per gram creatinine. 20 % of the children showed values outside this range.

The mean values of the serial specimens of urine indicate a clear diurnal rhythm with the peak daytime excretion at 16 h (6.49 ± 2.01 μmol cyclic-AMP/g creat.), while the minimal excretion was at midnight (3.48 ± 1.26 μmol cyclic-AMP/g creat.). As represented graphically in figure 1, the differences observed between the periods of highest and lowest excretion for cyclic-AMP are statistically significant ($P < 0.01$).

The fluctuations observed in daytime cyclic-GMP excretion were 0.601 ± 0.34 μmol/g creat. at midnight and 0.540 ± 0.39 μmol/g creat. at 20.00 h (fig. 2). No significant difference was observed.

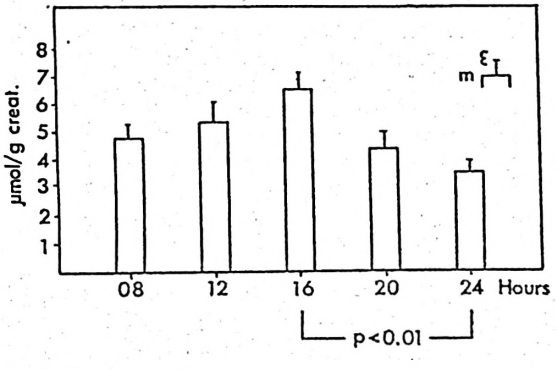

Fig. 1. *Cyclic-AMP excretion.*

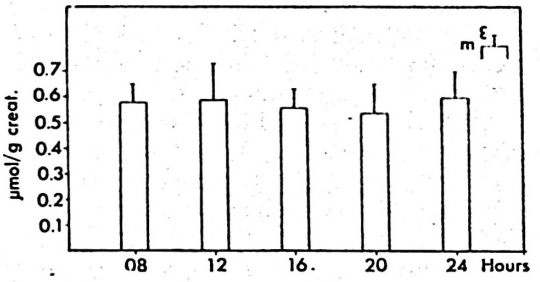

Fig. 2. *Cyclic-GMP excretion.*

Discussion

The urinary cyclic-AMP values for normal children are in agreement with results published by COFFEY and MIDDLETON (4) and MAXWELL *et al.* (7) and comparable to those reported by KOPP *et al.* (5) and BARBERÁ *et al.* (1) in healthy adult subjects. Our data differ from those observed by other authors (9, 10, 12). It is to be noted, however, that the former ones used a competitive protein binding similar to ours, while the latter used a radioimmunoassay. Our values for cyclic-GMP are similar to those reported by MURAD and PAK (9), but higher than those indicated by KOPP *et al.* (5) and BARBERÁ *et al.* (1).

Previously published papers have indi-

cated the absence of a circadian rhythm in cyclic-AMP excretion (4). The present data confirms the existence of this circadian rhythm in urinary cyclic-AMP excretion, in agreement with others (5, 9, 12), although our study was the first carried out in children.

There are two main sources of urinary cyclic-AMP; from about one-half to two-thirds are extra-renal and dependent on glomerular filtration for excretion. The remaining one-third originates from the renal parenchyma (3). Changes resulting from alteration in glomerular filtration rate have been minimized by relating the cyclic nucleotides excretion to that of creatinine; however, this does not eliminate the possible fluctuation in plasma levels of these nucleotides which may occur throughout the day. The data observed by PUJOL-AMAT *et al.* (11) and MIKUNI *et al.* (8) show a circadian variation in plasma cyclic-AMP. Our results are in agreement with MIKUNI *et al.* (8), being the peak and minimal excretions from 2 to 4 hours after the highest and lowest plasma concentrations found by the aforementioned author (8). However, our results are not consistent with those observed by PUJOL-AMAT *et al.* (11).

MURAD and PAK (9) suggested that a circadian rhythm in cyclic-GMP might exist, but no indications of the statistical significance of the observation was given. KOPP *et al.* (5) reported a marked diurnal rhythm in this cyclic nucleotide excretion which was clearly distinct from cyclic-AMP excretion. Our findings show no significant difference between the fluctuations in daytime cyclic-GMP excretion observed. The basis for the discrepance in timing between MURAD and PAK (9), our study and KOPP *et al.* (5) is not clear. It is possible that differences in analytic method might account for the differences.

It appears that unless it is desirable to study circadian rhythms in cyclic nucleotide excretion for clinical applications, the effects of fluctuations in their excretion should be eliminated by standardization of time collection.

Resumen

Se han determinado los valores normales en la excreción urinaria de AMP-cíclico y GMP-cíclico, en población infantil sana, utilizando un método de análisis competitivo y radioinmunoensayo respectivamente. No se observan diferencias de excreción en función de la edad o el sexo.

Asimismo, se han determinado las variaciones en la excreción urinaria de ambos nucleótidos cíclicos a lo largo de un período de 20 horas. Se observa un ritmo circadiano para el AMP-cíclico con un máximo a las 16 p.m. y un mínimo a las 24 p.m. No se han observado diferencias significativas en las variaciones halladas en el GMP-cíclico.

References

1. BARBERÁ, G., BONNIN, R., BUENDÍA, E. NAVARRO, M. A. and FERNÁNDEZ-SIMÓ, E.: *Biométrica*, **2**, 61-67, 1977.
2. BARTELS, H.: *Clin. Chim. Acta*, **32**, 81-89, 1971.
3. BROADUS, A. E., KAMINSKY, N. I., HARDMAN, J. G., SUTHERLAND, E. W. and LIDDLEG, W.: *J. Clin. Invest.*, **49**, 2222-2227, 1970.
4. COFFEY, R. G. and MIDDLETON, E.: *J. Allergy Clin. Immunol.*, **54**, 41-53, 1974.
5. KOPP, L., LIN, T. and TUCCI, J. R.: *J. Clin. Endocr. Metab.*, **44**, 673-680, 1977.
6. LINDGREN, B. R., ANDERSON, R. G. G. and BRUNDIN, A.: *Scand. J. Resp. Dis.*, **59**, 333-339, 1978.
7. MAXWELL, G. M., DAHLENBURG, G. W. and RENCIS, V.: *Biol. Neonate*, **33**, 55-61, 1978.
8. MIKUNI, M., SAITO, Y., KOYAMA, T., DAIGURI, M.: YAMASHITA, I., YAMAZAKI, K., HONMA, M. and UI, M.: *Life Sci.*, **22**, 667-672. 1978.
9. MURAD, F. and PAK, C. Y. C.: *N. Engl. J. Med.*, **286**, 1382-1388, 1972.
10. O'DONNELL, J. M.: *J. Physiology*, **273**, 81-82, 1977.
11. PUJOL-AMAT, P., DAVI, E., MARTÍN-COMÍN, J. and MONFORT, R.: *Lancet*, **1**, 489, 1977.
12. SAGEL, J., COLWELL, J. A., LOADHOLT, C. B. and LIZARRALDE, G.: *J. Clin. Endocr. Metab.*, **37**, 570-573, 1973.