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THE OCCURRENCE AND METABOLISM OF MELATONIN

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S U M M A R Y

This thesis concerns a study of the production of melatonin, a putative pineal gland hormone, in man and sheep. In particular it concerns the potential use of measurement of the unique urinary melatonin metabolite, 6-sulphatoxymelatonin (6SM) as an index of melatonin production.

A method for urinary 6SM analysis was developed based on isotope dilution gas chromatography-mass spectrometry. Of 2 mass spectrometers employed, repeated analysis of a control urine gave interbatch coefficients of variation of 9.8% (n=7) and 7.9% (n=17) for the AEIMS-30 and Hewlett-Packard 5992B respectively.

Studies in intact sheep revealed urinary 6SM excretion of 3.0-17.3 $\mu\text{g } 24 \text{ h}^{-1}$ (n=7), with approximately 80% (n=4) occurring during darkness. An estimate of the urinary production rate (UPR) of melatonin of ewes (39.1-153.5 $\mu\text{g } 24 \text{ h}^{-1}$, n=10) was obtained by measurement of the specific incorporation of deuterium unto urinary 6SM following intravenous administration of deuterated melatonin; incorporation of deuterium into urinary 6SM was complete within 24 hours of injection. Two ewes were studied before and after successful pinealectomy. Pre-operatively their melatonin UPRs were 39.1 and 84.1 $\mu\text{g } 24 \text{ h}^{-1}$ and their urinary 6SM levels 3.4 and 4.7 $\mu\text{g } 24 \text{ h}^{-1}$, indicating that only 4.2 and 3.3% respectively of the administered $^2\text{H}_3$ -melatonin was excreted as $^2\text{H}_3$ -6SM. Post-operatively no endogenous urinary 6SM was detected.

Urinary 6SM excretion for man was 9.5-29.1 $\mu\text{g } 24 \text{ h}^{-1}$ (n=10) and for menstruating women in the follicular and luteal phases was 5.3-28.9 (n=5) and 9.9-30.9 (n=5) $\mu\text{g } 24 \text{ h}^{-1}$ respectively. In each case 24 hour urinary 6SM excretion profiles for a man and a menstruating

woman revealed that approximately 80% of the daily output was associated with the nocturnal sleep phase. Over five consecutive days urinary 6SM excretion for a man was $25.7 \pm 3.6 \mu\text{g } 24 \text{ h}^{-1}$.

Following intravenous administration of $^2\text{H}_3$ -melatonin to a man 99.7% of the $^2\text{H}_3$ -6SM was excreted within 24 hours. For that subject melatonin UPR was $23.7\text{-}34.5 \mu\text{g } 24 \text{ h}^{-1}$ (n=4) while the pre- and post-dosage urinary 6SM excretion was $15.1\text{-}22.3$ (n=4) and $16.5\text{-}22.5$ (n=3) $\mu\text{g } 24 \text{ h}^{-1}$, respectively. The percentage of $^2\text{H}_3$ -melatonin excreted as $^2\text{H}_3$ -6SM by that subject was 40.7 ± 2.3 (n=4). For that subject melatonin UPR determined after injection of $^2\text{H}_3$ -melatonin at 1300 and 2230 h was 33.0 and $29.8 \mu\text{g } 24 \text{ h}^{-1}$, respectively. In another male melatonin UPR was 29.1 and $28.2 \mu\text{g } 24 \text{ h}^{-1}$.

Urinary 6SM excretion at monthly intervals throughout one year for 4 men at 35°S was 15.9 ± 4.0 (n=13), 28.0 ± 9.6 (n=13), 30.4 ± 6.3 (n=12) and 39.4 ± 7.9 (n=12) $\mu\text{g } 24 \text{ h}^{-1}$, with the percentage contribution of the nocturnal sleep component to the daily output at 73.0 ± 7.2 (n=13), 75.6 ± 9.3 (n=13), 72.6 ± 11.4 (n=12) and 74.2 ± 11.6 (n=12), respectively; no clearly defined seasonal changes in excretion pattern were evident.

Daily urinary 6SM excretion for 2 women throughout a menstrual cycle was 19.3 ± 2.2 (n=30) and 24.2 ± 3.1 (n=27) $\mu\text{g } 24 \text{ h}^{-1}$ with the percentage contribution of the nocturnal sleep component to the daily output at 75.5 ± 5.8 (n=30) and 81.5 ± 4.2 (n=27), respectively; no discernible rhythms in excretion pattern were detected throughout either cycle.

Urinary 6SM excretion for women in the first, second and third trimesters of pregnancy was 20.5 ± 4.5 (n=5), 22.7 ± 7.5 (n=6) and 18.3 ± 6.5 (n=7) $\mu\text{g } 24 \text{ h}^{-1}$ with the percentage contribution of the

nocturnal sleep component to the daily output at 80.1 ± 7.7 (n=5), 76.1 ± 7.6 (n=4) and 75.2 ± 6.2 (n=7), respectively. No marked changes in excretion pattern of women repeatedly sampled during the course of pregnancy, parturition and early lactation were revealed.

No 6SM was detected in either full term amniotic fluid or neonatal urine in man. Urinary 6SM excretion (approximately $0.3 \mu\text{g}$ for the nocturnal period) was first observed in a 16 week old boy. In a 12 month old boy urinary 6SM output was $17.4 \mu\text{g}$ from 1930-0800 h and $0.7 \mu\text{g}$ from 0830-1000 h. Adult levels and pattern of urinary 6SM excretion were also observed in children aged 2-6 years.