lower or half doses. In our study, all patients were given the same dosage of intermediate-dose melatonin, so their individual rates of melatonin clearance determined their relative systemic exposure to this drug. It is interesting that the serum melatonin concentrations achieved in patients with fast clearance were consistently below 20 μmol/l, the extracellular concentration which has been reported to be necessary for substantial passive uptake of melatonin by human lymphoblastoid cells.

Although it is conceivable that differences in the clearances of all drugs, and not simply the clearance of melatonin, caused the different clinical outcomes in our patients, this possibility is unlikely since the doses of the other post-induction chemotherapy (daily mercaptopurine and low-dose weekly methotrexate, both given orally), were adjusted upwards (in 4 slow-clearance patients, 6 medium-clearance patients, and 7 fast-clearance patients) or downwards (in 1 slow-clearance patient only) to maintain the white-blood-cell count between 2000 and 4000/µl. Since the doses of these drugs were adjusted to biological tolerance, the main difference in the intensity of chemotherapy was determined by the rate of intermediate-dose-methotrexate systemic clearance. Moreover, doses of intermediate-dose methotrexate cannot readily be adjusted on the basis of a biological measure of drug effect, such as white-blood-cell count, since bone-marrow suppression and other toxic effects are generally not seen with a wide range of methotrexate doses (1-25 µg/m²) when leucovorin rescue is given.

Since these patients received intermediate-dose methotrexate for only the first 75 weeks of therapy and the median follow-up was 26 months, the long-term effect of variations in the rate of methotrexate clearance was not assessed and could prove to be different from its early importance. Also, it is possible that the clinical significance of methotrexate clearance would be less in a treatment regimen that included more aggressive therapy with additional drugs. Nonetheless, this study has demonstrated the potential clinical importance of the interindividual variability in the rate of drug clearance in children with ALL. Since this should be easy to overcome with methotrexate because doses much higher than those used in this and previous studies can be given with leucovorin rescue, clinical trials should be undertaken in children with ALL to establish the dosage of methotrexate that would ensure adequate exposure even in patients with fast clearance.

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occurs at night when it is inconvenient to obtain blood samples from children who have shown the investigation of a postulated relationship between pineal secretion and puberty.

We have measured serum melatonin in daytime and nighttime blood samples obtained from 89 children, adolescents, and young adults, by a modification of existing serum melatonin assays\textsuperscript{14,15} that increases its sensitivity and that differentiates between the authentic hormone and drugs (eg, aminopyrine) that cross-react with a melatonin antiserum.

Subjects and Methods

Serum samples were obtained from 58 children and adolescents (38 males and 20 females), aged 1–18 years, with no evidence of endocrine diseases who were inpatients in the paediatric or laryngological units of a general hospital near Vienna, and from 31 young adult control subjects (17 males and 14 females), aged 20–35 years, who were in hospital only on the day of sample collection. It seems unlikely that admission to hospital per se or the diseases prompting the admission significantly affected melatonin secretion. Stresses as severe as pneumonoclephalography or electroconvulsive therapy did not affect secretion of the hormone.\textsuperscript{5}

Studies were conducted throughout the year. The degree of sexual maturation was staged\textsuperscript{16} by the same investigator. The study protocol was explained to subjects or their parents, and informed consent was obtained. Blood samples (8 ml) were taken by venepuncture between 7:30 AM and 10:00 AM, after exposure to a lighted environment for at least one hour, and again between 11 PM and 1 AM, after exposure to darkness for at least one hour. Sera were separated by centrifugation and stored at −20°C until assayed for melatonin (in one of three assay runs); nighttime specimens were also assayed for LH.

Assay of Serum Melatonin

We used a highly specific anti-melatonin serum\textsuperscript{15} provided by Dr G. Brown (McMaster University, Hamilton, Ontario). Sodium hydroxide (0.5 ml of 1 mol/L) for the serum melatonin radioimmunoassay was added to 1 ml serum or an appropriate dilution of serum, and melatonin was extracted into 5 ml chloroform. The aqueous phase was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.55 ml 0.05% gelatin "triz" buffer (1·21 g 'Trizma' base, 8·16 g sodium chloride, and 1·10 g sodium azide, in 100 ml of double-distilled water), and this buffer extract was then washed with 1 ml petroleum ether. 500 µl of the extract (or 500 µl samples of melatonin standards) was mixed with 100 µl of the anti-serum solution (diluted with 2·15% gelatin "triz" buffer to a dilution of 1:18 000) and 100 µl 3H-melatonin (New England Nuclear, Boston) diluted with 2·15% gelatin "triz" buffer to yield 1570 cpml/100 µl. The mixture was shaken and then incubated at 35°C for 60 min. Saturated ammonium sulphate (1 ml) was then added, after which the mixture was incubated overnight at 4°C. After centrifugation the precipitate was dissolved in 200 µl 0·1 mol/L sodium hydroxide; a scintillation fluid ('Biofluor'; New England Nuclear) was then added, and radioactivity was counted.

The sensitivity of the assay (85% displacement) varied between 4 and 6·6 pg/ml serum. Water blanks were lower than the detection limit. The recovery of authentic melatonin added to serum samples was 86·9±2·1% (SEM). At melatonin concentrations of 20·8 and 100·7 pg/ml of serum, the intra-assay coefficients of variance were 7·9% (n=7) and 6·1% (n=9). The melatonin concentration curve produced by serial dilution of pooled serum samples collected nocturnally from prepubertal children paralleled the melatonin standard curve (fig 1).

The daytime and nighttime serum melatonin levels that we observed in our young healthy adults (fig 2) were within the ranges reported by others.\textsuperscript{17–20} Aminopyrine (10 µg) added to 1 ml human serum did not cross-react with the melanotin antiserum used; this antipyrexic did cross-react with the melatonin antiserum that we used previously.\textsuperscript{21}

![Graph showing inhibition curves for a serial dilution of authentic melatonin and pooled nighttime sera from prepubertal children.](image)

B/B, (%) percentage of 3H-melatonin bound at a particular melatonin concentration to that bound in absence of unlabelled melatonin.

pg/ml = picograms per milliliter.

![Graph showing daytime and nighttime serum melatonin levels in children, adolescents, and young adults.](image)

Daytime levels did not differ significantly among groups. Nighttime levels varied significantly (p<0·001), with children in stage 1 (<7 yr) having significantly higher values than subjects in groups II–V plus adults (p<0·05), and children in stage I (<7 yr) having lower values than those younger than 7 yr (p<0·05), but higher values than individuals in groups II–V plus adults (p<0·05). Solid line indicates mean value.
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