ABSTRACT. Alterations in periodical functions are known to occur in aging and may be regarded as markers of the aging process itself. Melatonin and Thyroid Stimulating Hormone (TSH) circadian periodicities were studied in 22 aged subjects and in 13 adult controls. The study of rhythmicity was performed by the Cosinor analysis. Elderly subjects were hospitalized because of various concomitant diseases. Circadian periodicity of both hormones was disrupted in the aged group, and the deterioration of melatonin periodicity was significantly correlated with the decay in cognitive functions, quantified by the Mini Mental State evaluation. Diabetes was also found to affect, though not significantly, melatonin, but not TSH, periodicity. Melatonin and TSH nocturnal peaks were decreased in aged people. TSH oscillation amplitudes were inversely correlated with age. No correlation was found between melatonin and TSH secretory features both in adult and in aged subjects.

INTRODUCTION

The determination of the length of life span is probably related to genomic expressions via the homeostatic and integrative mechanisms of the body. A primary role has been attributed to neuroendocrine control (1). Degenerative lesions and functional impairments have been described to affect the central nervous system (CNS) in senescence, involving neurotransmission and neuroendocrine function (2, 3). The temporal organization of the neuroendocrine system reflects the integrative role of the CNS. Alterations in chronobiological features the occur in aging, so that changes in physiological periodicities may be taken as a marker of the senile decay of brain organization (4).

The pineal gland has been considered a transducer of environmental inputs to the endogenous timekeeping system, entraining both circadian and circannual periodicities. Pineal hormone melatonin is the main effector (5).

Much data suggest that melatonin and the hypothalamus-pituitary-thyroid axis are correlated in transducing signals at various levels and regulate the periodicity of functions, such as thermoregulation, reproduction and immune response. It was advanced that the impairment of this regulation is involved in the determination of the senescence processes (6).

This study evaluated the circadian periodicity of melatonin and Thyroid Stimulating Hormone (TSH) secretions in aged subjects with different health conditions. The secretory features of these two hormones physiologically reflect a circadian organization that is independent of synchronizing events, such as sleep (4).
years of age (mean age±SEM: 77.4±1.6 years) and in 13 adults (7 males, 6 females), aged 23 to 31 years (mean age±SEM: 25.0±0.9 years). The adult females had normal ovarian function, and were studied between the fifth and seventh day of their follicular phase.

The aged subjects were hospitalized at the Department of Endocrinological and Metabolic Sciences of the University of Genova. Thirteen subjects had moderate hypertension and/or chronic ischemic cardiopathy, that in 3 cases was associated with mild chronic renal failure. Three patients showed chronic obstructive respiratory failure. Five cases, aged 66 to 87 years, showed a well controlled non-insulin-dependent diabetes mellitus with no clinical evidence of autonomic neuropathy. In one case, Waldenström's disease was diagnosed. No drug known to interfere with pineal, pituitary and thyroid function was given during the study nor in the preceding week.

The Mini Mental State (MMS) (7) evaluation was used to identify cases with dementia. The scores obtained ranged between 15 and 30. Interference by depression was ruled out by the Hamilton Rating Scale (8). A condition of dementia, corresponding to an MMS score less than 24, was found in 3 males and 3 females, aged 66 to 90 (mean age±SEM: 80.2±3.7). These 6 patients had Senile Dementia of the Alzheimer type (SDAT) or a Mixed Alzheimer and Vascular Dementia (MAVD), according to the clinical findings, the CT data and the Hachinski test (9); 2 were diabetic.

Levels of serum T3 and T4 (mean±SEM) at 08:00 in the aged subjects were 0.85±0.04 ng/mL and 89.0±5.22 ng/mL, respectively (normal range for the laboratory: T3= 0.6-2.0 ng/mL; T4= 45-120 ng/mL).

All the subjects were accustomed to the ward routine for at least one week following their admission. They stayed and slept in a quiet ambience (lights out from 22.00 to 07.30, and temperature kept between 19 and 22°C) and received normocaloric meals at 07.30, 12.30 and 19.00. Control subjects were maintained under comparable conditions during the test.

The study was conducted according to the principles of the Helsinki Declaration, and was approved by the Department Ethics Committee.

**Sampling and assays procedures**

In all groups, blood samples were drawn every 120 minutes starting at 08.00, from an indwelling catether implanted in a forearm vein and maintained patent by a slow running saline drip. Nocturnal sampling was made with the aid of a shaded flashlight. Blood for melatonin assay was collected in glass vials containing EDTA, kept in ice-bath and then centrifuged to obtain plasma. Sera were concomitantly obtained for TSH determinations. All samples were stored at -20°C until use.

Plasma melatonin was assayed by an RIA method after methylene chloride extraction, according to Wetterberg et al. (10), using reagents purchased from Kalab (Danville, CA, USA). The final dilution of the employed antibody was 1:24,000.

Melatonin recovery after extraction was 84%; method sensitivity is 5 pMol/tube. The intra-assay and inter-assay percent coefficients of variation (CV%) were 17.0 and 25.4, respectively.

TSH was determined by an IRMA method, using reagents purchased from Ares Serono (Milan, Italy). The standard reference is the 2nd IRP 80/558 (1 mIU TSH MAIAclone = 1 mIU 2nd IRP 80/558). Sensitivity was 0.02 mIU/mL. The intra-assay and inter-assay CV% were less than 3.1 and 3.9, respectively.

**Analysis of the data**

The 24-hour melatonin and TSH values were analyzed by Single and Population Mean Cosinor procedures to assess the presence of statistically significant circadian rhythms (11, 12). Fitting of the hormone concentration data by the least squares method to a cosine function detects the rhythm and its parameters, namely the Percentage Rhythm (PR) (percentage of variability accounted for by the fitted model), the Mesor (the value midway between the highest and the lowest values of the cosine function approximating the rhythm), the amplitude of the oscillation around the mesor, and the acrophase (F) (timing of the peak of the best fitting cosine). A statistically significant rhythm occurs when the amplitude-zero hypothesis is rejected at the p<0.05 level.

Mesor and amplitude values are expressed as mean±SEM. Acrophases are expressed as neg-