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## DISRUPTIONS IN THE SECRETION OF CORTISOL, PROLACTIN, AND CERTAIN CYTOKINES IN HUMAN AFRICAN TRYPANOSOMIASIS PATIENTS

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### Ruptures de la sécrétion de cortisol, de prolactine et de certaines cytokines dans la trypanosomose humaine africaine.

**Résumé :** *Il a été montré récemment que la maladie du sommeil au stade de méningoencéphalite se caractérise par une perturbation importante du rythme circadien du cycle veille-sommeil. Le but de cette étude a été d'examiner l'importance de cette dysrythmie circadienne chez de tels patients en analysant : le profil de 24 heures du cortisol plasmatique, pris comme exemple d'un rythme circadien classique relativement indépendant du sommeil; celui de la prolactine plasmatique, dont le rythme de sécrétion est principalement lié au cycle veille-sommeil. Le taux plasmatique de certaines cytokines a également été analysé pour tenter de mieux comprendre l'immunopathogénie de la maladie. Nous avons tenté de lier les perturbations des rythmes circadiens au degré de sévérité de l'atteinte méningoencéphalitique. Les trois patients les plus atteints ont présenté une dysrythmie circadienne du cycle veille-sommeil, du cortisol et de la prolactine. Chez nos patients, l'interféron-gamma est la cytokine la mieux corrélée avec le degré d'évolution de la maladie, avec des niveaux 7 à 12 fois plus élevés chez les patients affectés par une disparition des rythmes circadiens. Ces résultats sont en faveur de l'hypothèse selon laquelle des modifications sélectives du noyau suprachiasmatique, principal oscillateur de l'horloge biologique, sont responsables de l'induction des perturbations circadiennes chez l'homme infesté par le trypanosome.*

**Summary:** *It has been shown previously that sleeping sickness at the stage of meningoencephalitis manifests itself as a significant disturbance in the circadian rhythm of sleep-wakefulness. The objective of the current study was to examine the extent of circadian disruption in infected patients by measuring 24 hours patterns of plasma cortisol, an example of a classical circadian rhythm relatively independent of sleep, and prolactin, a primarily sleep-related rhythm. Plasma levels of certain cytokines were also measured to examine the immunopathogenesis of human African trypanosomiasis. An attempt was made to relate any circadian disruptions to the severity of the disease. The three most advanced patients demonstrated circadian disruptions in cortisol, prolactin and sleep-wake rhythms. The prime cytokine factor that correlated with the progression of the disease in humans was interferon-gamma, levels being 7- to 12- fold higher in the patients without any circadian rhythms. Our findings support the hypothesis that human African trypanosomiasis induces selective changes in the suprachiasmatic nucleus, important as a pacemaker for biological rhythms, resulting in disruptions of circadian rhythmicity in advanced stages of the disease.*

## INTRODUCTION

Evidence has accumulated that human African trypanosomiasis (HAT) disrupts endogenous circadian rhythms. Fragmentation of sleep-wake cycles has been reported in humans, the degree of fragmentation appearing to be related to the progression of the disease (3). More recently, disruptions in the circa-

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dian rhythms of cortisol and prolactin have been reported in patients in the advanced meningoencephalitic stages of the disease (9). Furthermore, selective changes in the suprachiasmatic nucleus (SCN), important as a pacemaker for biological rhythms, have been found in trypanosome-infected rat brains (2). Thus, alterations in endogenous rhythms due to disruptions in neurohumoral functions may play an important role in the pathogenesis of HAT.

Functional relationships between the hypothalamic-pituitary-adrenal (HPA) axis hormones which modulate host responses, and cytokine mediators of fever, inflammation, and immunity, have been demonstrated (1). It has been shown in rats that trypanosomes trigger a class of lymphocytes (CD8<sup>+</sup> T-cells) to produce interferon-gamma (IFN- $\gamma$ ) which stimulates the further growth of the organism (8). Increased concentrations of plasma tumour necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6) associated with impaired adrenocortical function have been reported in HAT (10). There is also strong evidence that the immune system is involved in the regulation of sleep-wakefulness (4).

The identification of factors that are related to the progression of the disease in humans is important for understanding the pathogenesis of sleeping sickness and its management. The specific aims of this paper were: to examine 24-hourly plasma concentrations of two HPA hormones, cortisol and prolactin, and selected cytokines; and to investigate their relationship with established markers of disease progression.

## PATIENTS AND METHODS

Eight volunteer patients (5 men and 3 women, age 17-58 years) with sleeping sickness were selected during a medical prospection in two villages from an endemic area near Daloa and examined at the Clinical Research Project on Trypanosomiasis at Daloa on the Ivory Coast (3). The diagnosis of sleeping sickness was confirmed by the presence of *Trypanosoma brucei gambiense* in the blood or a lymph gland puncture and in the cerebrospinal fluid (CSF), and was further confirmed by a serologic immunofluorescence test. The patients were then ranked by two neurologists according to the clinical and biological severity of their illness (3) (presence of lymph nodes, daytime somnolence, intermittent fever, headaches, hyperesthesia, presence of primitive reflexes, deep tendon reflexes, psychiatric disorders, pruritus and tremor, and very high number of cells in the CSF). Of the eight patients, six had very high cell counts of over 50 cells/mm<sup>3</sup>, and two had counts between 5-10 cells/mm<sup>3</sup>. All patients were at the stage of meningoencephalitis, the most severely ill patient being identified as P1 and the least severe one as P8. No cases were seropositive for HIV or HTLV-1. Subsequently, one of the patients (P5) was excluded from the study due to the

additional presence of severe diabetes. On completion of the study, all patients received immediate specific treatment.

In order not to disturb the sleep of the patients during the study, they were kept in an air-conditioned room (24° C) adjacent to the recording room to which equipment leads and catheters were passed through an opening in the adjoining wall. Patients and subjects remained at bed rest during the 24 hours experimental period and all blood samples were taken while patients were in a supine position to eliminate any variations in plasma concentrations due to changes in posture.

Polysomnographic recordings were taken continuously throughout the 24 hours period (3). Sleep traces were scored at 20 seconds intervals and the temporal distribution of sleep and wakefulness throughout the 24 hours expressed as the hourly percent of wakefulness, non-rapid eye movement sleep (NREM) (stages 1 to 4), and rapid-eye movement (REM) sleep. The sleep patterns of the patients have been reported in detail elsewhere (3).

The patients were catheterized in the median basilic vein with the end of the catheter accessible from the other side of the wall via a three-way valve where a syringe could be attached for withdrawal of blood. Blood samples (10 ml) were removed each hour over a 24 hours period and transferred to tubes containing ethylenediaminetetraacetate (10.5 mg of EDTA per tube). Each sample was centrifuged immediately in a refrigerated centrifuge and the separated plasma frozen and stored at -60° C.

Hormones were measured in duplicate by radioimmunoassay (RIA), the 24 samples of each patient being analyzed at the same time in order to minimize any effect of interassay variability. Plasma cortisol was analyzed by the GammaCoatTM[125I] Cortisol RIA kit (INCSTAR Corporation, Stillwater, MN) and prolactin by a double antibody assay (Diagnostic Products Corp., Los Angeles, CA). The cytokine assays consisted of: IL-1 $\beta$  and IL-2 (RIA [125I] kit, Amersham, Ont., Canada), TNF- $\alpha$  (RIA [125I] Genzyme Corp., Ont., Canada), and IFN- $\gamma$  (ELISA assay, Endogen Inc., Boston, MA, USA).

To facilitate comparisons between the patients, the 24 hourly plasma values were subjected to a Z-statistical transformation. The Z-values for the 24 hours plasma cortisol and prolactin levels were tested for the presence of potential circadian rhythmicity by applying the cosinor analysis technique, which models the data through the best fitting cosine function with an admitted period of 24 hours (7). Statistical comparisons were made using a one or two factor analysis of variance and the Student's t test. All results are shown as means  $\pm$  SEM.

## RESULTS

A cosinor analysis of the 24 hours cortisol and prolactin variations revealed that the three most severely

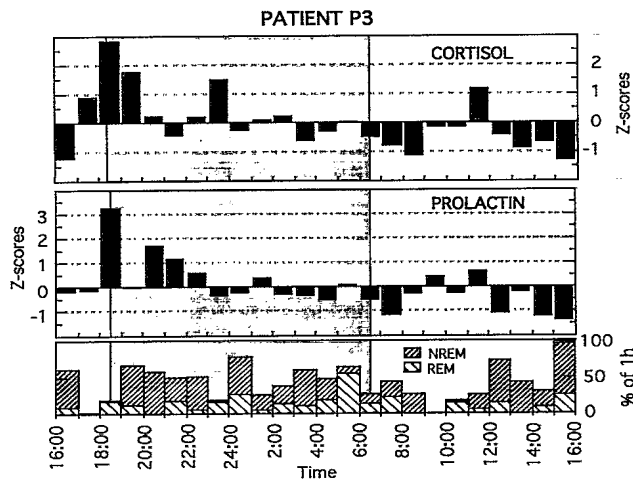


Fig. 1. — Cortisol, prolactin and NREM-REM variations over 24 hours for Patient P3. Plasma levels of cortisol and prolactin are shown as Z-transformed scores.

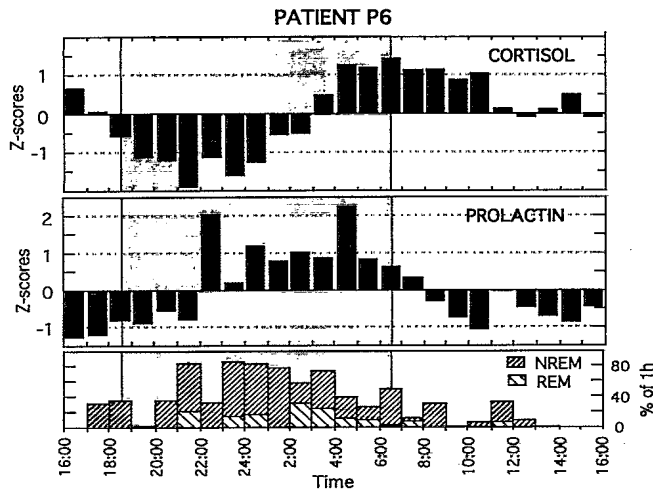


Fig. 2. — Cortisol, prolactin and NREM-REM variations over 24 hours for Patient P6. Plasma levels of cortisol and prolactin are shown as Z-transformed scores.

ill patients (P1, P2, P3) did not show any significant circadian rhythm in cortisol, prolactin, or sleep-wake cycles. Figure 1 shows the disruptions in the 24 hours patterns of cortisol and prolactin plasma levels and the sleep-wake cycle for patient P3, patients P1 and P2 demonstrating similar patterns. The less severely sick patients (P4, P6, P7, P8) demonstrated significant circadian rhythms in these parameters (patterns for a representative patient, P6, are shown in Fig. 2). There were no significant correlations between the 24 hours average plasma hormone levels with the level of severity of the disease (data not shown).

Due to the insufficient volume of remaining blood samples, it was not possible to carry out a circadian analysis of cytokine variations over the 24 hours period. Rather, the 24 hours average value were calculated for each patient. Figure 3 shows the mean

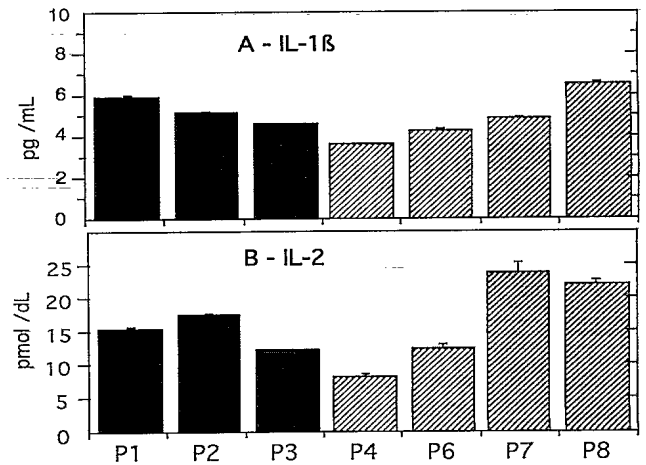


Fig. 3. — Mean 24-hours concentrations for interleukin-1 beta (IL-1 $\beta$ ) (3A) and interleukin-2 (IL-2) (3B) in all seven patients ranked in severity from P1 (most severe) to P8 (least severe). Solid bars represent the patients with no circadian rhythmicity in cortisol or prolactin. Values shown are the means  $\pm$  standard error of the mean.

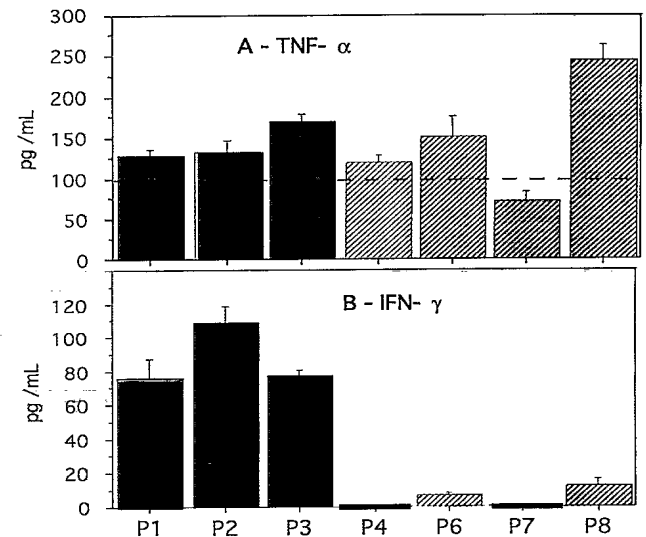


Fig. 4. — Mean 24-hours concentrations for tumour necrosis factor-alpha (TNF- $\alpha$ ) (4A) and interferon-gamma (IFN- $\gamma$ ) (4B) in all seven patients ranked in severity from P1 (most severe) to P8 (least severe). Solid bars represent the patients with no circadian rhythmicity in cortisol or prolactin. Values shown are the means  $\pm$  standard error of the mean.

concentrations of IL-1 $\beta$  and IL-2 for the 7 patients. The distributions of IL-1 $\beta$  between patients showed a U-distribution, with the least severe (P7, P8) and the most severe (P1, P2, P3) patients exhibiting levels significantly higher than normal (Fig. 3A). A similar pattern was observed with IL-2 levels (Fig. 3B).

With the exception of P7, TNF- $\alpha$  concentrations were significantly higher than normal values by this assay ( $> 100$  pg/ml) (Fig. 4A). However, no rela-

tionship to the severity of the disease was apparent. On the other hand, the three most severely ill patients (P1, P2, P3) demonstrated levels of IFN- $\gamma$  7 to 12 fold higher than normal ( $> 10$  pg/mL) (Fig. 4B). These are the same three patients who had no circadian rhythm in cortisol, prolactin, or sleep-wake cycles (see Fig. 1).

## DISCUSSION

This paper examined the circadian rhythms of cortisol and prolactin in a group of patients infected with HAT to determine to what extent the circadian system may be disrupted by sleeping sickness. BUGUET *et al.* (3) had already demonstrated that the disappearance of the circadian variation of the sleep-wake cycle was related to the severity of the clinical symptoms of the disease. The three patients judged to be most severely ill in this study exhibited virtually no circadian rhythmicity in either cortisol or prolactin secretion. Cortisol secretion represents an endogenous circadian rhythm that is relatively independent of the sleep-wake cycle, whereas prolactin secretion is primarily a sleep-related rhythm (12). Our findings suggest that disruptions in the mechanisms controlling the sleep-wake cycle also extend to other circadian rhythms. These findings would support the hypothesis that the networks of the biological clock (SCN, pineal gland) are altered in the course of the disease. Cortisol and prolactin output must be viewed as a reflection of underlying circadian mechanisms, much like the hands of the clock. Alterations in such indicators may or may not point to changes in the pacemaker itself.

Such disappearances of circadian rhythms have been observed in animals after a lesion of the SCN (11). Recently, dysregulation of gene expression in the SCN has been demonstrated in trypanosome-infected rat brains (2). Thus, the pathogenesis of the disease, particularly in its advanced state, appears to be related to dysfunctions of the circadian pacemaker.

Altered levels of cytokine mediators of fever, inflammation, and immunity may play a role in the immunopathogenesis of HAT. Experimental studies on trypanosome-infected rodents have shown that parasitic components stimulate production of TNF- $\alpha$  by macrophages (6) and of IFN- $\gamma$  by CD8<sup>+</sup> T-cells (8). TNF- $\alpha$  is trypanostatic whereas IFN- $\gamma$  stimulates parasite growth. We found in our patients that all of the cytokines examined in this study were elevated in the plasmas of the infected patients, but that IFN- $\gamma$  was highest in the three most severe patients who also demonstrated disrupted circadian periodicity in cortisol, prolactin, and the sleep-wake cycle. Since a variety of cytokines have been reported to affect sleep (5), elevations of IFN- $\gamma$  in patients P1, P2 and P3 may be related to the severe disruptions observed in their sleep-wake patterns, as well as to the advancement of the disease.

Evidence that the parasite has been shown to localize near brain areas as the pineal gland and the SCN indicates that the biological clock may indeed be affected by this infection, particularly in its advanced stages. Our findings on the loss of circadian rhythmicity in both cortisol and prolactin support this hypothesis. Furthermore, our data indicate that both the elevations in circulating cytokines and the suppression of circadian rhythmicity are indices of disease activity, and may be reflective of the severity of the disease.

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