3 Iwakawa, M., Pinto, B. M., and Szarek, W. A., Can. J. Chem. *56* (1978) 326.
4 Sheehan, J. C., and Yang, D.-D. H., J. Am. chem. Soc. *80* (1958) 1158.
5 Takata, T., Kuo, M., Tamura, Y., Kabe, Y., and Ando, W., Chem. Lett. *1985*, 939.
6 Uhle, F. C., J. Am. chem. Soc. *83* (1961) 1460.
7 Kuivila, H. G., Synthesis *10* (1970) 449.
8 Arnstein, H. R. V., and Morris, D., Biochem. J. *76* (1960) 318.
9 Mimura, T., Kohama, Y., Kuwahara, S., Yamamoto, K., Komiyama, Y., Satake, M., Chiba, Y., Miyashita, K., Tanaka, T., Imanishi, T., and Iwata, C., Chem. pharm. Bull. *36* (1988) 1110.
10 Born, G. V. R., and Cross, M. J., J. Physiol. *168* (1963) 178.
11 Kohama, Y., Iwabuchi, K., Okabe, M., and Mimura, T., J. Pharmacobio-Dyn. *9* (1986) 182.
12 Griffett, E. M., Kinnon, S. M., Kumar, A., Lecker, D., Smith, G. M., and Tomich, T. G., Br. J. Pharmac. *72* (1981) 697.
13 Uchiyama, T., Tanihata, S., Ohta, S., and Ito, R., Sulfur Amino Acids *7* (1984) 61.
14 Holmsem, H., and Karpatkim, S., in: Hematology, p. 1149. Eds W. L. Williams, E. Bentler, A. J. Esleu and M. A. Lichmann. McGraw-Hill Book Company, New York 1983.
15 Owen, N. C., and Le Breton, B. C., Am. J. Physiol. *241* (1981) H613.
16 O'Rourke, F. A., Harenda, S. P., Zavoico, G. B., and Feinstein, M. B., J. biol. Chem. *260* (1985) 956.
17 Lands, W. E., A. Rev. Physiol. *41* (1979) 633.

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Splitting of the circadian activity rhythm in common marmosets (*Callithrix j. jacchus*; Primates)

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Summary. Long-term recordings of the free-running circadian activity rhythm in common marmosets, *Callithrix j. jacchus*, living under constant environmental conditions (LL 200–470 lx) provided evidence of rhythm splitting in nonhuman primates. In two out of ten test animals two different types of splitting occurred; spontaneous persistent splitting and temporary splitting. Neither a reduction of the illumination intensity nor the application of dark pulses had any effect on the two activity components of the persistently split rhythm.
Key words. Circadian rhythm; activity; splitting; Primates; *Callithrix*.

In the continuing discussion about whether one, two, or more, central nervous oscillators or pacemakers regulate the numerous circadian rhythms of the vertebrate organism, two phenomena are thought to point to the existence of a two- or multi-oscillator system; internal desynchronization and splitting. Spontaneous and/or forced internal desynchronization, in which various circadian functions display different period lengths for a long time, has only been observed thus far in man and in the diurnal squirrel monkey (*Saimiri sciureus*). Splitting has been found in several diurnal and nocturnal rodents, in tree shrews, and in certain species of birds, reptiles and fishes[1]. While free-running under constant environmental conditions, individual circadian rhythms dissociate (split) into two (or more) distinct components which either continually or only temporarily free-run with different spontaneous periods.

Splitting has been observed most frequently in locomotor activity. In a few cases, however, it has been reported that along with the splitting in the locomotor activity splitting may also occur in the free-running circadian rhythms of feeding, drinking and electrical brain self-stimulation[2] as well as in the serum concentration of luteinizing hormone[3]. Indications of splitting in the core temperature rhythm have only been observed in a few squirrel monkeys[4]. In these cases, however, the test animals had been restrained in a chair throughout the whole of a constant conditions experiment (LL 600 lx; 28 ± 1 °C) which lasted less than three weeks. Therefore it is not known whether splitting can occur in the activity rhythm as well, either in this diurnal Cebid species or in other nonhuman primates. We carried out two long-term constant conditions experiments to examine the possibility of social entrainment, and to test the effect of estrogen application on the circadian spontaneous period in free ranging common marmosets (*Callithrix j. jacchus*). During these experiments, we found individual cases of temporary and continuous splitting of the free-running circadian activity rhythm.

During the experiments, a total of 10 adult common marmosets (2 males and 8 females) aged 2–8 years were kept for 3–6 months in constant light (LL) of 200–470 lx at an ambient temperature of 25 ± 1 °C and 60 ± 5% relative humidity. The small diurnal monkeys lived individually housed in wire mesh cages (75 × 105 × 95 cm) which were placed in isolation boxes with thick (10 cm) sound-attenuating walls. The boxes were placed in the same room. Food, consisting of a protein-rich rice-flake porridge and mixed fruit, was provided ad libitum at irregular intervals once or twice per circadian cycle.

The locomotory activity of the test animals was recorded by a PC-controlled electroacoustic device. The vibrations (sounds in solids) of the wire mesh of the cage generated by the various activities of the animals (e.g., moving around, intense scratching, scent marking) were picked up by a transducer-like special microphone (Merula

TVJ-XL, Holthausen) that is highly sensitive to substrate conducted sounds and insensitive to sounds propagated by air. The electrical signals of the microphone were then used to trigger a series of constant-frequency square wave pulses that were added by electronic counters (Demel V224). The count was sampled at 1-min intervals by an Apple II$^+$ PC, totaled every 5 min, and the sum was then stored on a disk. For further analysis the data were transferred, via the Kermit program, to an IBM-compatible Olivetti PC M-24 with two floppy disks, a 10 MB hard disk, and attached plotter (HP 7475 A). The period length of the free-running circadian activity rhythm was determined both by periodogram analysis of the consecutive 30-min values according to Dörrscheidt and Beck [5] and by eye-fitting regression lines to the double plots of these data. For further details see Erkert et al.[6, 7].

Under the experimental conditions used, all the marmosets displayed free-running circadian activity rhythms (CAR). Their period lengths varied between 23.8 and 22.8 h ($\bar{x} = 23.2 \pm 0.3$ h) and for 30–40 days experienced distinct after-effects of the preceding entrainment to an LD 12:12 (cf. fig. 2 and Erkert [7]). After living in LL for about 40 and 75 days, respectively, a four-year-old male and an eight-year-old female showed splitting of their CAR. As can be seen from figure 1, the spontaneous period in the male lengthened from 23.3 h on LL-days 1–20, to 23.9 h from days 40 to 56. Between the 30th and 40th day of constant conditions, a very weak and slightly slower activity component split from the end of the main activity time. Finally two distinct weak activity components evolved which free-ran in antiphase with identical period lengths of 24.6 h from days 85 to 115.

In tree shrews and hamsters it has been shown that changes in the constant lighting conditions and dark pulses during LL may induce changes in the mode of splitting, or a re-fusion of the split activity rhythm, and different phase responses of the two split components, respectively [8–11]. Therefore we tested the effect of a reduction of the LL-intensity from 470 to 52 lx (from days 83 to 117) and of 12-h dark pulses (applied on days 61 and 95 at different circadian phases) on the male's split activity rhythm. However, none of these alterations in the environmental conditions resulted in a reunion or discernible phase shifts of the two split activity components. From days 121 to 142 the male was re-united with his twin sister, who displayed an unsplit free-running CAR of 23.2 h, obviously dominating throughout this whole period of social contact. The male's activity rhythm shown thereafter, when kept isolated again from its partner for a few days, suggests that the circadian periodic social contact, which in the other test animals led to social entrainment of the free-running circadian rhythms, did not result in a termination of splitting.

In the female marmoset, a temporary splitting of the free-running circadian activity rhythm occurred immediately after an i.m. control injection of 0.1 ml ricinus oil

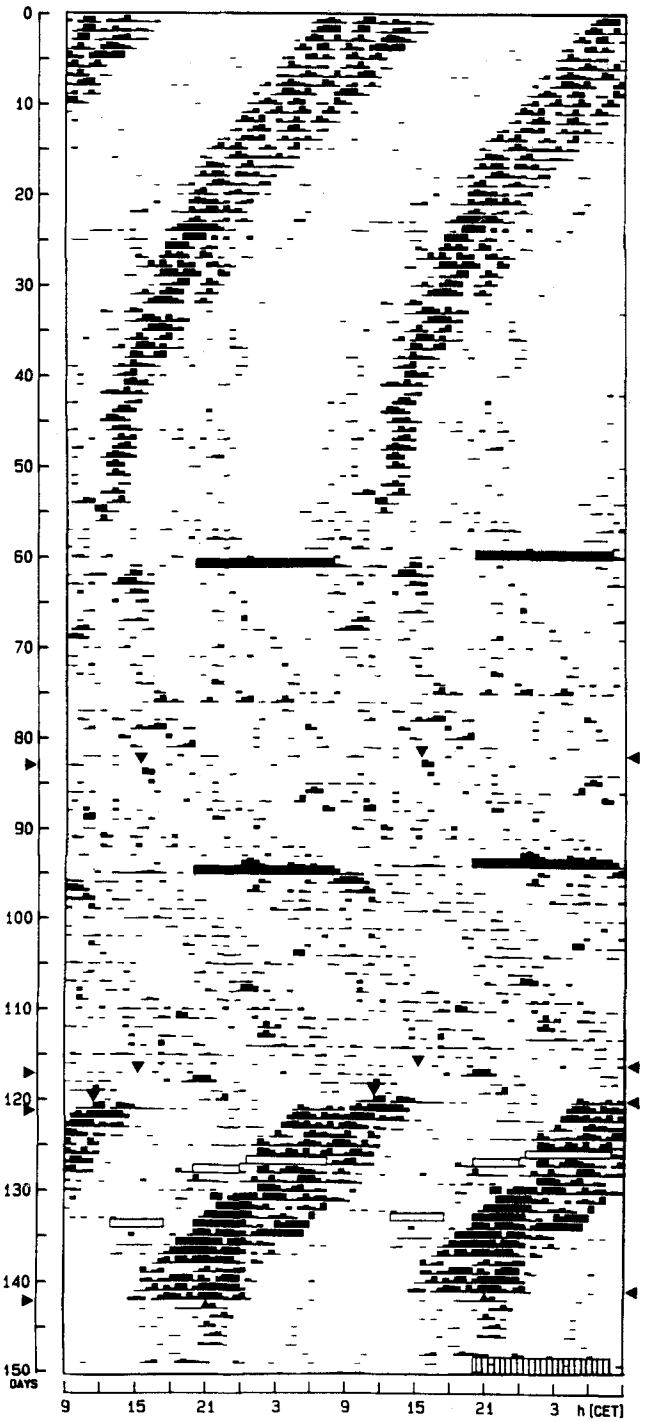

Figure 1. Spontaneous splitting of the free-running circadian activity rhythm in a four-year-old male common marmoset living in constant light of 470 lx (days 1–83 and 117–150) and of 52 lx (days 83–117). The plot starts on the sixth day of constant conditions. Black bars indicate 12-h dark pulses applied at different circadian phases; white bars represent failures of the recording device. To test the effect of circadian periodic social contact on the split rhythm, from days 121–142 the male was reunited with its former female social-partner whose activity rhythm free-ran unsplit with a spontaneous period of 23.2 h. During this period the locomotor activity of both animals was recorded together. The black triangles mark the exact times of the respective changes in the environmental conditions.

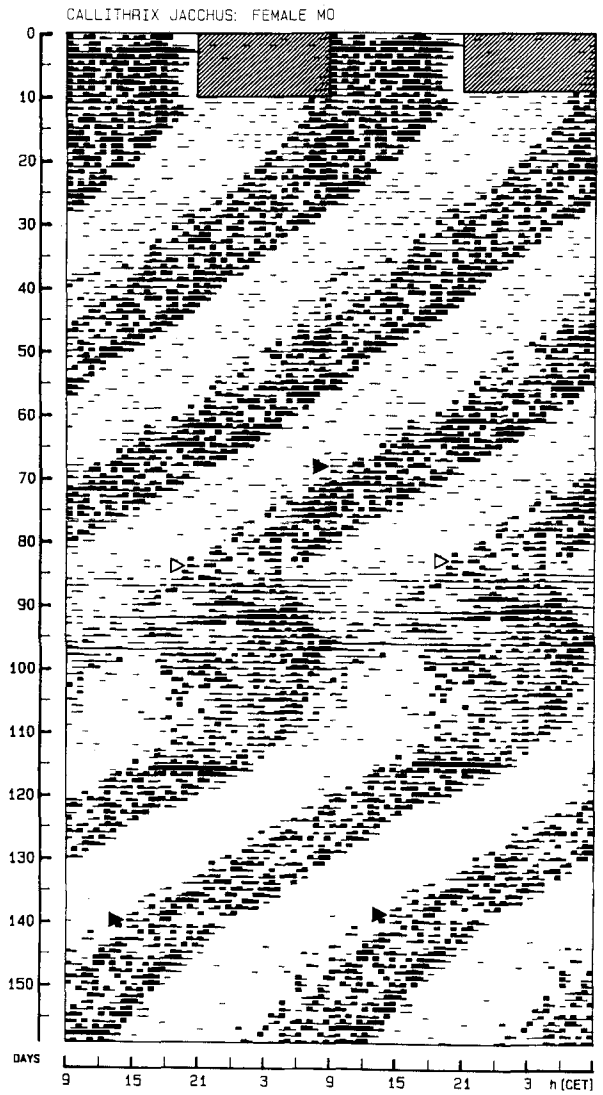

Figure 2. Temporary splitting of the free-running circadian activity rhythm in an eight-year-old female marmoset (LL 350 lx) treated with estradiol valerate dissolved in ricinus oil (black triangles) and with pure ricinus oil (open triangle) as control.

applied 15 days after an i.m. injection of 2.5 mg/kg estradiol valerate dissolved in ricinus oil (fig. 2, open and black triangles, respectively). Here the two split activity rhythms free-ran for about two weeks with different period lengths; 23.1 h, which coincides with the pre-split period of 23.0 h, and 24.4 h. Then they became synchronized again and exhibited a short spontaneous period of 23.1 h. After a second application of estradiol valerate (5 mg/kg i.m. on day 139) the rhythm slowed down, and the activity time lengthened steadily. Since this marmoset had to be removed from the experiment in order to avoid risking its health, it cannot be ascertained whether this might have been the beginning of another period of splitting or not. It also remains unclear whether or not the (first) period of splitting had been induced by the injection of estradiol valerate, by its pure solvent used as a control, or by another unknown factor. In none of the five other (much younger) female marmosets, treated in this experiment similarly with estradiol valerate and ricinus oil, did the free-running circadian activity rhythm show any indication of splitting.

The ultimate causes, i.e. the physiological mechanisms underlying the splitting of free-running circadian rhythms are still unknown. Nowadays, however, this phenomenon is generally assumed to be based on a temporal uncoupling of two different circadian pacemakers or pacemaker systems which are located in the central nervous system and generate different spontaneous periods [1]. According to the basic hypotheses of Pittendrigh's two-oscillator-model [12, 13], these pacemakers are often termed morning (M) and evening or night (E or N) oscillators [9, 14, 15]. In cases of temporary splitting, as observed here in the female marmoset, the two oscillators are thought to become uncoupled and to free-run with different period lengths until reaching their original phase relationship, at which point they again couple.

Persistent splitting, as shown by the male *Callithrix*, is also consistent with this two-oscillator hypothesis. It would occur when a stable mutual coupling is achieved at another distinct phase relation (mostly at 180 °) between the two oscillators. Compared to the unsplit circadian rhythm, such synchronized split rhythm components often do free-run with a markedly altered common period length (cf. fig. 1). This observation led to the hypothesis that environmental factors influencing the spontaneous period of unsplit circadian rhythms (e.g. light and ambient temperature) do this at least in part by modifying the coupling strength of the two circadian oscillators or oscillator systems. Correspondingly, the exogenous factors thus far proven to induce splitting i.e. a) relatively low light intensities in certain diurnal mammals such as tupaias and squirrels, or b) relatively high LL intensities in several nocturnal rodents such as hamsters, rats and mice, and c) testosterone application in starlings and rats have been thought to do this by a weakening of the mutual coupling of these oscillators [1].

The occurrence of spontaneous splitting of the free-running activity rhythm, as observed in numerous hamsters and rats as well as in the male marmoset (fig. 1), may be explained in a similar way by assuming a spontaneous (endogenously induced) reduction in the coupling strength of the oscillatory control mechanisms involved. Whether estrogens, which thus far have not been shown to induce splitting, may have similar effects on the internal coupling of the circadian pacemaker system cannot be deduced from the temporary splitting as observed in the female marmoset (fig. 2), because the causes of this episode of splitting are unclear.

To explain a splitting of overt circadian rhythms one must not necessarily assume the existence of two or more central nervous pacemakers. As recently pointed out by Carpenter and Grossberg [16], several modes of splitting can be obtained by computer simulations based on a more physiologically oriented neural model of circadian

rhythms. This model starts by assuming the existence of one circadian pacemaker (the SCN) consisting of gated pacemaker circuits in which 'on-cells and off-cells excite themselves via positive feedback, inhibit each other via negative feedback, and are tonically aroused'. However, experimental data exclusively favoring this and/or other one-oscillator models [17–19] are still lacking. Results obtained from experimental manipulations of split activity rhythms in hamsters, such as differing phase responses of the two activity components to dark pulses [11], more likely support a two-oscillator or multi-oscillator hypothesis.

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1 Rosenwasser, A. M., and Adler, N. T., Neurosci. Biobehav. Rev. *10* (1986) 431.
2 Boulos, Z., and Terman, M., J. comp. Physiol. *134* (1979) 75.
3 Swann, J. M., and Turek, F. W., Science *228* (1985) 898.
4 Fuller, Ch. A., Sulzman, F. M., and Moore-Ede, M., Am. J. Physiol. *236* (1979) R153.
5 Dörrscheidt, G. J., and Beck, L., J. math. Biol. *2* (1975) 107.
6 Erkert, H. G., Nagel, B., and Stephani, I., Behav. Ecol. Sociobiol. *18* (1986) 443.
7 Erkert, H. G., Am. J. Primat. (1989) in press.
8 Hoffmann, K., in: Biochronometry, p. 134. Ed. M. Menaker. Natl Acad. Sci. Washington 1971.
9 Earnest, D. J., and Turek, F. W., J. comp. Physiol. *145* (1982) 405.
10 Ellis, G. B., McKlveen, R. E., and Turek, F. W., Am. J. Physiol. *242* (1982) R44.
11 Lees, J. G., Hallonquist, J. D., and Mrosovsky, N., J. comp. Physiol. *153* (1983) 123.
12 Pittendrigh, C. S., in: The Neurosciences, Third Study Program, p. 437. Eds F. O. Schmidt and W. G. Worden. MIT Press, Cambridge 1974.
13 Pittendrigh, C. S., and Daan, S., J. comp. Physiol. *106* (1976) 333.
14 Morin, L. P., and Cummings, L. A., Physiol. Behav. *29* (1982) 665.
15 Swann, J., and Turek, F. W., Am. J. Physiol. *243* (1982) R112.
16 Carpenter, G. A., and Grossberg, S., J. theor. Biol. *113* (1985) 163.
17 Daan, S., and Berde, C., J. theor. Biol. *70* (1978) 297.
18 Enright, J. T., The Timing of Sleep and Wakefulness. Springer-Verlag, Berlin 1980.
19 Borbély, A. A., Human Neurobiol. *1* (1982) 195.

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Number of glucocorticoid receptors in lymphocytes and their sensitivity to hormone action

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Summary. The study demonstrated a decreased level of glucocorticoid receptors (GR) in peripheral blood lymphocytes from hypercholesterolemic subjects, and an elevated level in patients with acute myocardial infarction. In the lymphocytes with a high GR number, dexamethasone inhibited [^{3}H]-thymidine and [^{3}H]-acetate incorporation into DNA and cholesterol, respectively, in the same manner as in the control cells. On the other hand, a decreased GR number resulted in a less efficient dexamethasone inhibition of the incorporation of labeled compounds. These data showed that the sensitivity of lymphocytes to glucocorticoids changed only with a decrease of GR level.
Key words. Glucocorticoid receptors; lymphocytes; hypercholesterolemia; acute myocardial infarction.

The cellular receptor system provides for high efficiency and selective action of glucocorticoid hormones in target tissues. Opinions as to whether the response of somatic cells to hormone action depends on the number of receptors are contradictory [1–4]. Human peripheral blood lympocytes are well-known target cells for glucocorticoids. It has been demonstrated that glucocorticoids have a direct, specific inhibiting effect on metabolic processes in these cells [3]. The aim of this work is to find out whether the number of lymphocyte glucocorticoid receptors (GR) on a cell determines its sensitivity to hormone action.

Materials and methods

Lymphocytes were separated from the blood of 40–55-year-old men: (1) normolipidemic healthy volunteers (Ch $\leq$ 270 mg/dl, Tg $\leq$ 200 mg/dl, HDL Ch > 35 mg/dl); (2) normolipidemic patients with acute myocardial infarction (no more than 24 h since the onset of anginal attack and appearance of abnormal Q wave) (3) hypercholesterolemic subjects (Ch > 270 mg/dl, with coronary atherosclerosis angiographically verified). Patients with diabetes mellitus and other endocrine pathologies were excluded from the study.

Fifteen ml of blood was drawn from the ulnar vein into dry test-tubes with EDTA (1 mg/ml) and separated over a Ficoll-Pague [5]. The mononuclear cell layer was removed and suspended in medium 199. This cell suspension was incubated in plastic tissue culture flasks at 37 °C for 45 min to remove monocytes. After this procedure differential cell counts showed that the number of monocytes or polymorphonuclear leukocytes was less than 3% of the cells remaining after adherence. The lymphocytes were washed with phosphate buffer saline (PBS) pH 7.2 and resuspended in medium 199 at a density of $5-6 \times 10^6$ cells/ml.