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The influence of light on circadian rhythms

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Introduction

A fundamental property of living organisms, aside from the capacity of self-replication, is the ubiquitous phenomenon of rhythms. The time span of rhythmic processes ranges from seconds, i.e. the firing of an isolated neuron, to periods of one year (seasonal rhythms). Rhythms with a period of about 24 h in particular have aroused the curiosity of observers and scientists. Early observations of the movements of leaves and flowers suggested that light influences these processes[18]. The discovery that these rhythms persist in constant darkness was a fundamental contribution to the study of circadian rhythms[17]. Later, investigators found that rhythms were also present in complex structures, such as vertebrates; since then, a large body of evidence showing that they are generated and maintained within the organism has been accumulated. The prevailing light-dark (LD) cycle entrains the organism so that the overt rhythms are in phase with the environment and the organism is adapted to its surroundings.

This review will deal with the impact of light on 24-h rhythms, the so-called circadian rhythms, especially in man. When necessary, data on subhuman species will be used for the purpose of illustration. Several excellent reviews on (human) circadian rhythms have been published and they may be consulted for details not discussed in the present review[47, 52, 56, 57, 79, 103].

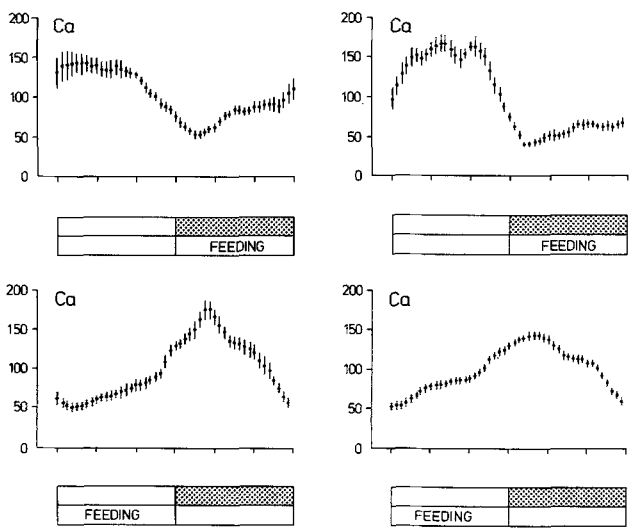

Figure 1. The 24-h variation in calcium excretion for rats fed during either the light part of the LD-cycle (lower panel), or during the dark phase (upper panel). During the first part of the experiments the rats were infused intravenously with a 2.5% glucose solution and the food contained calcium (left part of the fig.); during the second part calcium was excluded from the food but was infused i.v. continuously (right part of the fig.). Note that the acrophase of calcium occurred at the end of the feeding period, irrespective of whether calcium was administered via the food or intravenously. Data are expressed as the percentage of the mean 24-h excretory rate and shown as the mean ± SEM.

Methods of study

Many studies of man, animals and other forms of life have revealed that rhythmic phenomena persist under constant environmental conditions. Generally, the period does not last precisely 24 h so that shorter or longer periods can be observed, depending partly on the species. Therefore, these rhythms which should persist for many cycles are not caused by an external driving force but are generated within the organism. These rhythms are called endogenous rhythms in contrast to exogenous rhythms which are driven by cyclic changes in the environment. A sharp delineation between these two classes is sometimes difficult, and mixtures of both forms of rhythm may be observed. For instance, urinary calcium excretion is largely influenced by the intake of food. However, when calcium is given to rats solely via intravenous infusion, the excretory rhythm still persists (fig. 1). This example illustrates that for a correct interpretation of experiments the influence of other factors should be known. These factors include, among others, the wake-sleep cycle, body activity, temperature and intake of food; their influence on rhythms under both fixed LD cycle and constant environmental conditions should be known[75-78].

For statistical reasons it is necessary to collect a large amount of data on a single human being or animal in order to calculate with enough precision the period, other rhythmic parameters such as the acrophase (i.e. the time of maximal deviation from the mean level), the mean level, the maximal deviation (amplitude) and the range of oscillation when the rhythm is not symmetrical with respect to the time-axis. Many methods for the calculation of rhythm parameters, including cross-correlation techniques, spectral analysis, cosinor analysis and the periodogram method, have been described[9, 19, 21, 60, 70, 96, 108]. Simple techniques such as linear regression analysis of the onset of body activity or the calculated acrophases may also be used to obtain an estimation of the period of the rhythm.

The strategy of studies on circadian rhythms in man is to insulate the subject temporally from environmental and social time cues. This may be accomplished by carrying out such studies in deep caves, the polar region during summer or, more comfortably, one of the several specially constructed isolation units now in existence[52, 99, 103]. Under such conditions, particularly those in an isolation unit, various light regimes can be tested (e.g. abrupt shifts of the LD cycle to simulate transatlantic flights).

As outlined above, many data points are required for ultimate proof of the endogenous character of a rhythm and therefore it is hardly surprising that the rhythms studied in depth are those of the wake-sleep cycle, body temperature and urinary excretion[52, 103]. The study of blood constituents, for example hormones, is complicated by the limitation of acquiring sufficient samples, but some human data is available[99]. As a result of this problem, that also applies to animal studies, it is often only another rhythm that is described, which hardly contributes to our understanding of the organization and structure of the circadian system.

Studies of man in isolation

The first studies of human beings insulated from the environment were performed in caves; they have been summarized by Wever and Minors[52, 103]. Without exception all studies demonstrated that the free-running periods, i.e. rhythms deviating from 24 h under constant conditions, exceeded 24 h[2, 10, 27, 49, 85]. During the most prolonged studies the sleep-wake cycle resembled bicircadian cycles (rhythms of about 48 h)[40].

Most of our information, however, comes from the studies carried out in specially constructed isolation units. Aschoff and Wever made a major contribution to our knowledge of human circadian rhythms[5, 101–104]. In 135 subjects they found a free-running period for rectal temperature of 24.82–25.04 h (99% confidence interval) which is significantly longer than 24 h and is also longer than the apparent revolution of the moon. The free-running activity period (or sleep-wake cycle) was 25.11–25.26 h; similar differences with respect to the revolutions of the earth and moon were demonstrated. From the same studies it was concluded that there is no relation between the age of the subject and the free-running period.

For most of the subjects the period of the sleep-wake cycle and that of deep body temperature were identical, although the normal phase relationship between the two rhythms changed: there was a shift of the temperature maximum towards the beginning of the activity period and a shift of the minimum towards the start of the sleep period. After re-entrainment to the 24-h LD cycle the normal phase relationship was reestablished. In other subjects the constant phase relationship was lost, resulting in different periods for the two cycles (fig. 2). This loss of a constant phase relationship, called internal desynchronization, was demonstrated in about 30% of the subjects studied by Aschoff and Wever[5, 103]; it has also been found in other studies in a comparable number of subjects[99]. Internal desynchronization occurred more frequently in older persons, those with an underlying neurotic structure and those who could control illumination conditions[101–103]. Although no sex dependence was observed, when females exhibited internal desynchronization the activity rhythms appeared faster than the temperature rhythms while in males the opposite occurred. Many similarities with studies performed in animals are apparent. No sex difference was found for the free-running period, although very few studies on this aspect have been published[16, 113]. In our extensive studies on the free-running period of urinary excretory rhythms in the rat we

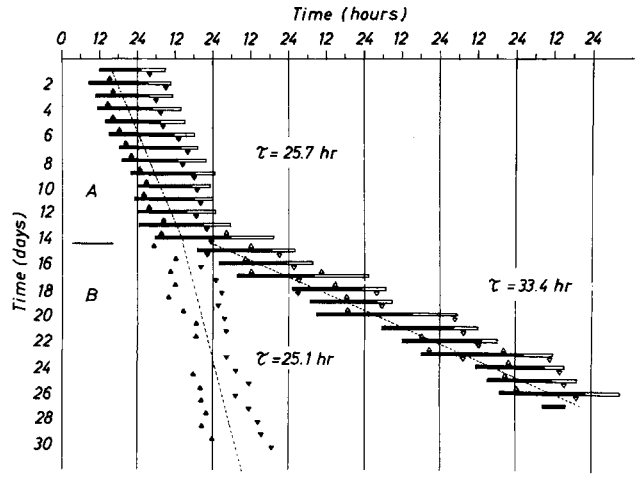

Figure 2. Circadian rhythms of wakefulness and sleep (black and white bars, respectively) and rectal temperature (maxima, minima) in a subject who lived alone for 30 days in an isolation unit with no indication of the time of day. Successive days are plotted from the top downwards. From days 1 through 14 the subject was internally synchronized with the two rhythms showing similar periods (25.7 h). On day 15 spontaneous desynchronization took place, i.e. the two rhythms showed different periods (rectal temperature 25.1 h, sleep-wakefulness 33.4 h). Open triangles show temporally corrected positions of temperature. (From Wever, with permission).

did not find differences between male and female rats, irrespective of the illumination conditions. In most animal studies light intensity was found to exert an influence on the circadian period: in general increased intensity caused an increase in the free-running period in night-active animals while the reverse was found for day-active species[3, 32, 64, 91]. However for the human being such an influence has not been found[101, 103]. Another difference between the results found for animals and man is the phenomenon of internal desynchronization, which has not been reported for any animals except the squirrel monkey[90]. In addition, constant bright light illumination may fractionate the circadian rhythms in animals into ultradian rhythms (rhythms with a period shorter than 20 h)[34]. Furthermore, in animals locomotor activity may split into two rhythms, which indicates the presence of two oscillators controlling activity[14, 64, 65].

The impact of the LD-cycle on the circadian system after a shift of the LD-cycle and during days of abnormal length has also been studied. After an air flight through many time zones resynchronization to the new LD-cycle does not occur immediately; it usually takes 3–8 days, depending upon the direction of the flight and the relative contribution of the endogenous component[20, 23, 28, 29, 41, 42, 83]. Rhythms with a large exogenous component, such as urinary volume and calcium excretion adapt faster than rhythms with a large endogenous component, e.g. potassium excretion, adrenal steroid excretion and deep body temperature[20, 52]. The different rates of re-entrainment cause a state of temporary dissociation, probably leading to the so-called jet lag syndrome. Jet lag is certainly not caused by travelling per se, because it also occurs after simulated flights. After north-south flights, rhythms and behavior are normal by the second post-flight day[30]. In human beings, resynchronization takes place sooner after west-bound flights than after east-bound flights[20, 42, 43].

This asymmetry effect has also been observed in animal studies[4, 6, 66, 100]. Resynchronization is faster after delay shifts (equivalent to a west-bound flight) than after advance shifts for day-active animals (with an autonomous period exceeding 24 h). Opposite results are obtained for animals with an autonomous period shorter than 24 h[4]. According to the oscillation theory these results are to be expected, because after a delay shift the phase of the rhythm has to come later each day which is easier to accomplish when the endogenous period is longer than 24 h. In our studies on urinary electrolyte excretion in rats we also found a directional asymmetry: delay shifts resulted in faster resynchronization than advance shifts, which was expected because the autonomous period in our rat strain is generally more than 24 h[67, 69]. This is illustrated in figure 3, which also shows that the asymmetry effect occurs in both male and female rats.

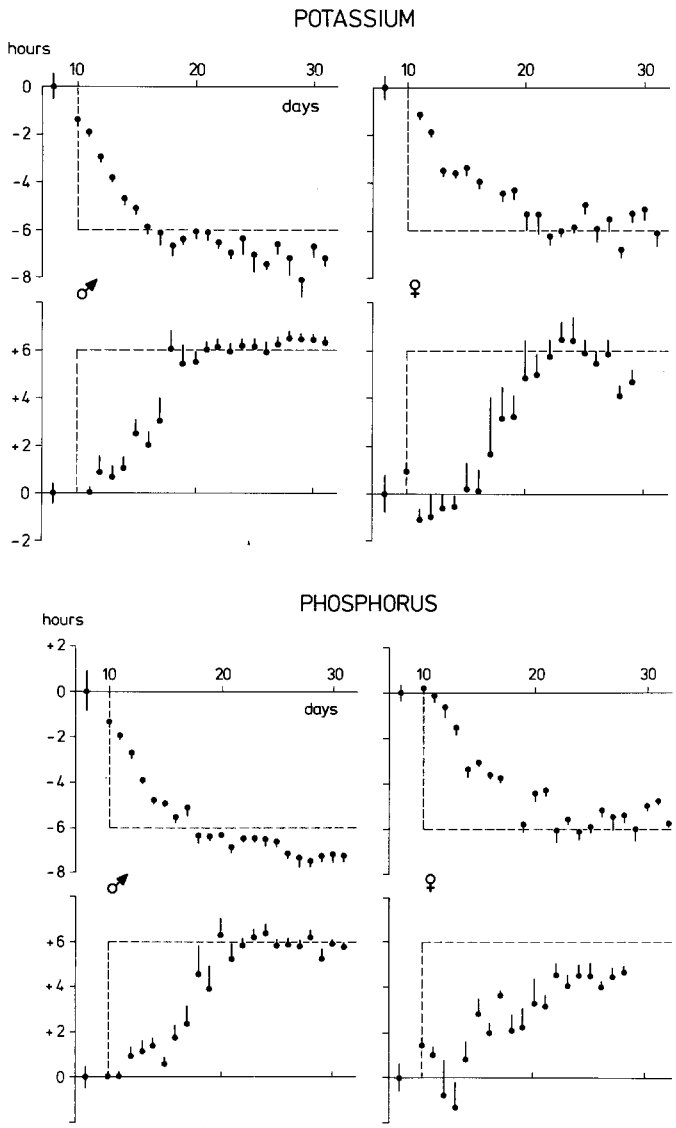

Figure 3. Resynchronization of the acrophases of urinary potassium and phosphate excretion. On day 10 the LD-cycle was shifted either −6 h (delay shift) or +6 h (advance shift). Note the differences in rate of resynchronization between male and female rats. The 90% resynchronization time for potassium and phosphate was 7 days for male rats after the delay shift and 11 days after the advance shift. The corresponding values for female rats were 15 and 18 days after the delay shift; after the advance shift the resynchronization time was 15 days for potassium, while for phosphate no resynchronization was obtained.

Although most studies point to a relation between the autonomous period and the directional asymmetry, a notable exception is Wever's study of human beings in an isolation unit[103, 104]. Their subjects showed faster resynchronization after advance shifts, while in the studies of Mills[51] and Hume[36] opposite results were obtained. No certain explanation for this discrepancy can be offered at the present time.

Another phenomenon which can be found in both man and animals is resynchronization by partitioning. Advance shifts of the LD-cycle may lead to a shift of the overt rhythm in the opposite direction[51]. This has only been found for rhythms with an autonomous period exceeding 24 h and a large endogenous component. In human beings the overt rhythms after advance shifts moved in the expected direction, but when days of constant routine were included (i.e. the subject did not sleep and took regular meals), the acrophases of the overt rhythms differed markedly with delay shifts appearing in a large proportion of the subjects[51]. These interesting results show that, at least in man, resynchronization by means of repartition is more common than generally realized, but is masked by external conditions and sleep. In our studies of the rat we found that male rats resynchronized faster and more efficiently than female rats, irrespective of the direction of the shift[69]. Surprisingly, no such data on human beings are available, at least they have not been analyzed in this respect.

Another aspect of rhythms to be discussed is the range of entrainment to an LD-cycle of days of abnormal length. Such studies have been carried out in the arctic and in the laboratory[44-46]. The results obtained in the laboratory were the most informative. In the studies of Mills et al.[50] the subjects were exposed to a 21-h or 27-h day. During the 21-h day most rhythms showed both a 21-h component and a component lasting (slightly) longer than 24 h, but the relative contributions of the two rhythms varied. Artificial 27-h days generally produced rhythms that could be described by a single period, usually between 23 and 27 h. In their studies Aschoff and Wever exposed the subjects to either a weak 'Zeitgeber' (i.e. the subjects had access to a reading lamp) or a strong zeitgeber (i.e. they were forced to adhere to the LD-cycle). During the first type of illumination the range of entrainment was 23–27 h for the activity rhythm and less for the temperature rhythm, so that a state of internal desynchronization developed. When a strong zeitgeber was used the range of entrainment for the sleep-wake cycle was larger (from 20 up to 48 h), while that for the temperature rhythm remained considerably smaller, lying within 2.3 h of the free-running period[103]. Similar ranges of entrainment have been reported for acoustic signals presented during constant illumination[106]. Therefore, in part the results may have been caused by the influence of light-dark transitions on behavior. However, bright light does influence human circadian rhythms: intensities of 4000 lux are able to entrain rhythms of at least 29 h, including those of rectal temperature and urinary potassium, calcium and sodium excretion[105].

Under most experimental conditions the LD-cycle is relatively unimportant in man. This may be illustrated by

observations of subjects exposed to a weak zeitgeber of 24 h who frequently exhibit free-running rhythms, although relative coordination to the LD-cycle is observed sometimes. When the zeitgeber is enriched by periodic acoustic signals or other forms of social contact, the rhythms are entrained more readily. Thus for man social contacts are more important than the LD-cycle per se. This hypothesis is further strengthened by the results obtained after time shifts. Resynchronization occurred faster in those subjects who had social contacts outside than in those who remained in their hotel room[43]. In addition, the rhythms of blind subjects in isolation could be synchronized readily by social contacts. However, others have reported free-running rhythms in blind subjects[48, 61], in whom the circadian rhythms have been found to be weakly developed[62, 94]. The conclusion therefore must be that more than one zeitgeber is used and that the relative importance of these zeitgebers will differ according to the individual and the circumstances[52].

Neuro-anatomical basis of the circadian clock(s)

As is often the case when a major advance takes place, the discovery of the fundamental role of the suprachiasmatic nuclei (SCN), located in the hypothalamus, in the circadian organization of vertebrates came from separate lines of investigation. The pioneering studies of Richter indicated that lesions in the ventral part of the hypothalamus caused the loss of behavioral rhythms in the rat[74]. Subsequently, it was demonstrated that in mammals a neuronal connection between the retina and the hypothalamus existed, and that the axons terminated in the suprachiasmatic nuclei[31, 53]. Destruction of these nuclei rendered the animals arrhythmic, pointing to a key role for these nuclei in the generation or expression of rhythms[15, 37, 38, 54, 59, 71, 82, 88, 89]. In vivo and in vitro studies have demonstrated conclusively that there is a self-sustaining rhythmicity in these nuclei but not in other parts of the hypothalamus or brain[24, 25, 39, 84]. Electrical stimulation of these nuclei causes either advance or delay phase shifts[81]. Because of this capacity for self-sustained oscillations, as well as the phase shifts after an appropriate stimulus and the neuronal connection with the retina, the SCN have all of the properties of an endogenous clock or pacemaker. Photic input into the SCN comes not only from the retinohypothalamic tract but also via the ventrolateral geniculate nuclei. Lesions of the optic tract or the ventrolateral geniculate nuclei cause an advance shift in the phase of the rhythms but otherwise the rhythms are not affected. These results indicate that the additional photic input via the geniculate nuclei plays a role in the entrainment process[80, 95]. Various other connections between the SCN and other parts of the hypothalamus have been described, but the functional significance of the afferent and efferent neurons is largely unknown at the present time[86]. In addition, an indirect connection exists between the SCN and the pineal gland. Pinealectomy in birds causes arrhythmicity of locomotor activity but in mammals circadian rhythms are not affected or only marginally altered[7]. Eventual differences in entrainment between intact and pinealectomized mammals are caused by the masking effect of light, which is enhanced by the absence of melatonin.

The SCN is not homogenous in structure, and different cell types occur[97]. Furthermore, the nucleus contains several hormones and neurotransmitters, including vasopressin, VIP, somatostatin, substance P, Gaba and 5-hydroxytryptamine[86]. The functional significance of these substances is not known. However, rats of the Brattleboro strain, who are genetically incapable of synthesizing vasopressin, do not show abnormalities of the circadian organization[63]. In humans the SCN can be visualized easily by immunostaining with antibodies against vasopressin[92].

Subhuman primates with lesions of the SCN show a loss of the drinking rhythm, while the temperature rhythm persists[22]. This observation suggests that at least two different pacemakers are present in primates, one for the temperature rhythm and one for drinking behavior. This interpretation is also in accordance with the observations of internal desynchronization, either spontaneous or forced, of the behavioral and vegetative rhythms. Similar data have been reported for other animals bearing SCN lesions, indicating that at least some of the pacemakers are outside the SCN[89].

Modulating factors on rhythms

The impact of the endocrine system on human circadian rhythms is largely unknown. However, studies in animals suggest that various hormones may affect rhythms. Estradiol has an influence on the period, inducing a shortening of the cycle in both hamsters and rats[1, 58, 113]. In addition, decreased estrogen levels are associated with a decrease in the total amount of activity, an increase in the variability of day-to-day onsets of activity and a change in the pattern of activity expressed. Progesterone alone does not influence the rhythm but can antagonize the effects of estradiol[93]. Castrated male mice show an increase in the period of wheel-running activity, which can be reversed by testosterone treatment[12]. In birds testosterone induces splitting of the activity rhythm[26].

Hypophysectomy in hamsters leads to an increased free-running period for the activity rhythm[112], while in rats the periods of the various excretory rhythms remained unchanged[68]. However, in the same experiments the acrophases of the electrolyte excretions altered markedly, with the exception of that for phosphate. During treatment with thyroxine and corticosterone normal acrophases were restored. Administration of either hormone resulted in differential shifts of the acrophases: thyroxine shifted the acrophase of sodium, corticosterone that of potassium, possibly indicating that these rhythms are controlled by separate oscillators. Under constant illumination a large proportion of the animals showed arrhythmicities of one or more urinary constituents, but none of the animals became totally arrhythmic. Another phenomenon which we observed in a high proportion of the hypophysectomized rats was the presence of ultradian rhythms, especially during constant illumination. The relative strength of the ultradian rhythms was greater than that found for the circadian component, which was still present in most instances. Thyroxine and corticosterone had no influence on the ultradian components. Whatever mechanism caused the emergence of ultradian rhythms, this result indicates that the hormonal system

contributes to the proper functioning of the pacemaker(s). Indeed, lesions of the SCN in hamsters cause disruption of the activity rhythm into unstable ultradian rhythms, a finding similar to our results which provides some support for our speculations.

Only a few data on the influence of thyroid hormones on the circadian system are available[8]. These studies indicate that the period increases in hypothyroid animals. Another hormonal system which must be considered in this context is the pituitary-adrenal axis. Under constant illumination adrenalectomized primates show circadian rhythms, although a prominent 12-h rhythm emerges when glucocorticoids are infused continuously[55]. In our rat studies we did not observe such a phenomenon. However, under a fixed LD-cycle, excretory and behavioral rhythms in both subhuman primates and rats can be phase-shifted by adrenal steroids[55]. Other studies have shown that the temperature rhythm for the rat can be phase-shifted by a large dose of dexamethasone[35], while in our studies it was found that a small dose of corticosterone (equivalent to the amount produced daily) is capable of inducing phase shifts, the amount and direction being dependent on the circadian phase at the time of administration. Therefore, a phase-response curve for glucocorticoids can be constructed for rats and the data point to a role for this system in entrainment. Considering the findings for subhuman species it would appear to be important to investigate patients with endocrine diseases in isolation units in order to clarify the impact of the hormonal system on the circadian organization.

Not only the hormonal system but also drugs can affect circadian rhythms. Deuterium oxide is well-known for its retarding effect on rhythms[13], while antidepressant drugs such as lithium, clorgyline and imipramine can also slow rhythms and even abolish them when infused directly into the SCN[109-111].

Concluding remarks on the implications of rhythms in man

It is clear from the observations described in this article that light plays an essential role in the functioning of the human body, and in particular in the physiological rhythms. For many blood and urinary constituents the diurnal rhythms are known, although the amplitudes may vary considerably. Precise knowledge of these phenomena is a prerequisite for clinical diagnosis and treatment. A well-known diagnostic criterion in Cushings' syndrome is the loss of diurnal rhythms for cortisol and ACTH, which return after successful surgical removal of the pituitary adenoma. Confusion may occur when the subjects are not in phase with the surroundings, as in the case of jet travellers, shift workers and students on unconventional sleep-wake schedules.

The effectiveness and toxicity of drugs have been shown to have a circadian rhythm caused by multiple factors, including absorption, excretory rate, metabolism and tissue susceptibility; this has been reviewed extensively[72, 73, 98].

Undoubtedly, the impact of working in shifts is important, in terms of both well-being and economical production. By applying the principles outlined above it is possible to construct a sound system of rotating shifts, as corroborated by a careful study by Czeisler[11].

These few examples demonstrate the implications which cyclic phenomena have for the daily life of man. A fundamental understanding of the basic mechanism of rhythms in man and animals, however, demands much more research.

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