CONTROVERSIES IN MEDICINE

This paper and the one on pages 569-578 are invited contributions to *Clinical Science* presenting opposing views on the restorative role of sleep

Protein synthesis, bodily renewal and the sleep-wake cycle

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Introduction

Clinicians with interests in endocrinology, and in nutrition, have been among those with a concern for how the body builds and renews itself. Growth and renewal depend upon protein synthesis, for which food is necessary, for food provides amino acids. The biochemical controls for cellular protein synthesis, however, are similar for all organisms, and not immediately dependent on diet.

We ourselves have an interest in sleep and share the common man's belief that sleep is in some way restorative. If the belief is correct, then one must ask what the link with restoration or renewal may be. Studies of cell and tissue kinetics confirm that there are circadian (i.e. about 24 h) variations in protein synthesis and in cellular proliferation [1]. Indeed, endogenous 'clocks' govern the function of every living tissue and in the whole body the clocks are co-ordinated by the brain, directly or indirectly.

The chief human circadian rhythm is that of sleep-wakefulness. Entrained to it is, for example, a rhythm of plasma amino acids, the timing of the peaks and troughs being independent of feeding, and there are also 24 h rhythms in intracellular amino acid levels that bear no simple relation to feeding or to plasma amino acid levels, which is not surprising, for amino acids are actively transported. As a feature of the sleep-wakefulness rhythm there are 24 h variations too in bodily activity and in the concomitant degradation of body fuels. Since amino acids provide one source of fuel, it will be realized that if activity during part of the 24 h were associated with conditions promoting greater degradation of protein and amino acids, and if synthesis meanwhile remained unchanged, there would be net degradation of protein. If the phase of activity were simultaneously associated with inhibition of protein synthesis, then there would be even greater net degradation. The converse, of greater net synthesis, would apply when activity was diminished, other things being equal.

A human example of one among many controls of protein synthesis can be provided by cortisol, which inhibits protein synthesis, and the secretion of which is governed by an endogenous clock that causes plasma cortisol concentration to be very low during the usual early hours of sleep and highest after the usual breakfast time. Although the rhythm is one that persists even during continuous wakefulness by day and night, the fall of cortisol is to lower levels at night if sleep supervenes than if wakefulness continues. The rhythm will only be changed in its phase by adaptation during weeks to a new sleep-wakefulness schedule, as during residence after trans-meridian jet travel.

More protein synthesis and cellular proliferation occur during sleep

Most tissues grow and renew themselves by cellular division, with its accompanying protein synthesis, but others, such as adult brain or muscle, are renewed simply by the replacement of their constituent protein molecules. Direct study is difficult to pursue in man, and in animals there have tended to be more studies of accessible epithelial tissues.

In recently reviewing the published reports of diurnal variations we noted the clock time for

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peak rate of cell division or peak rate of protein synthesis when given, and omitted two papers that failed to detect a rhythm (and in which the number of time points had been inadequate). In common with other authors we have taken the mid-point of any period of metaphase arrest induced by colchicine, where the latter had been used and a precise peak hour not specified. We have plotted the peak times of cell division (Fig. 1) and the peak times for protein synthesis and related measures (Fig. 2) in a wide variety of tissues [2–79]. The peak rates throughout the body generally coincide with the time of sleep in nocturnal animals (most rodents are nocturnal). The scatter might be attributable in part to uncontrolled lighting regimens, animal house routines intruding on the natural sleep period, or simultaneously measuring labelled thymidine incorporation and mitoses, for the former can disturb the latter [23]. In reviewing the literature care must be taken to distinguish thymidine studies, sometimes confused with the study of mitoses.

A sequence of events leads to cell division: DNA replication, followed by increased RNA transcription and increased protein synthesis. Protein synthesis is maximal just before division. DNA replication, which in human skin takes 6 h [80], and the copying of DNA to make RNA (transcription), are mutually exclusive; e.g. in rat skin, thymidine incorporation reaches a peak many hours before the peak in uridine incorporation [62], whereas the peaks in protein synthesis and in cell division are closely associated in time [8]. So cells are primed for possible cell division many hours in advance of the usual sleeping period, but sleep may affect whether or not these primed

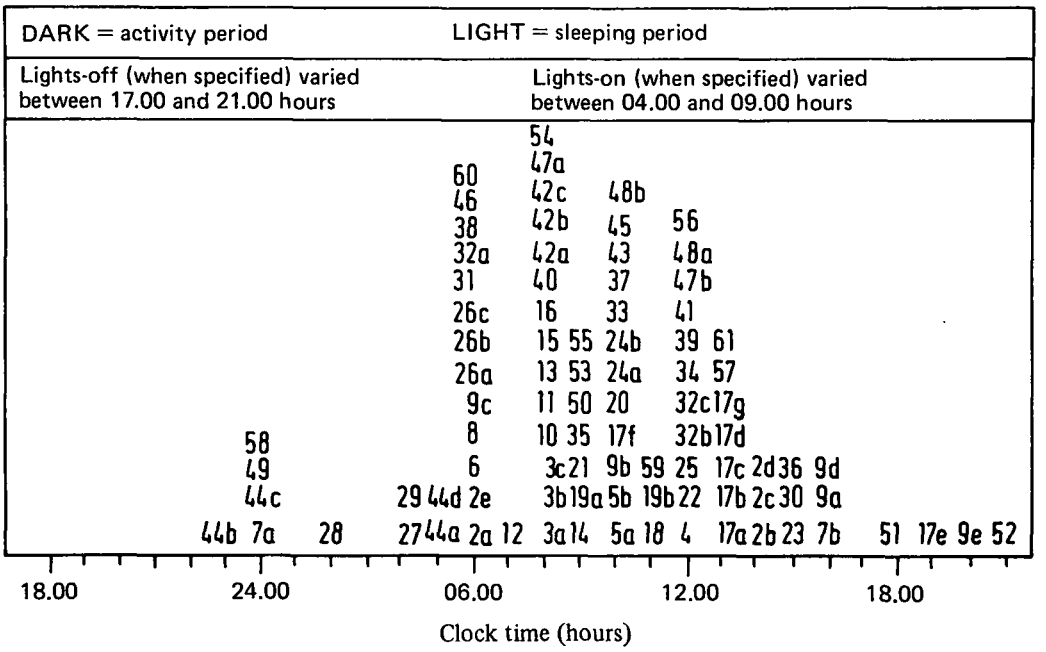

FIG. 1. In nocturnal animals peak number/rate of mitoses occur at the time when sleep predominates (i.e. during the light period) in a wide variety of tissues. Numbers in brackets correspond to those in the reference list. Letters a, b etc. denote different tissues reported in a paper. Key to tissues: epidermis [2]a, [3]a, [4], [5]a, [6], [7]a & b, [8], [9]a, [10]; corneal epithelium [11–16]; digestive tract: lip [17]a; tongue [17]b, [18], [19]a; mouth [3]b, [17]c, [20]; cheek pouch [21–23]; oesophagus [2]b, [17]d & e, [19]b; stomach [17]f, [24]a & b; duodenum [2]c, [25], [26]a; jejunum [26]b, [27], [28]; intestine [26]c, [29–31], [32]a; caecum [32]b; colon [32]c, [33], [34]; rectum [35]; anus [17]g; kidney [36–39]; liver [40], [41], [42]a & b, [43]. [44]a; lung [45]; spleen ♂ [9]b, ♀ [9]c; thymus [46], [47]a; bone marrow ♂ [9]d, ♀ [9]e; [47]b, [48]a & b, [49], [50]; salivary glands [42]c, [44]b, c & d; connective tissue [3]c, [5]b; cartilage [51], [52]; miscel. epithelia: tooth inner enamel [53]; mammary gland [54]; tympanic membrane [55]; crystalline lens [56]; epididymis [2]d; seminiferous tubules [2]e; sebaceous glands [57]; adrenals [58]; pineal [59]; pituitary [60]; thyroid [61].

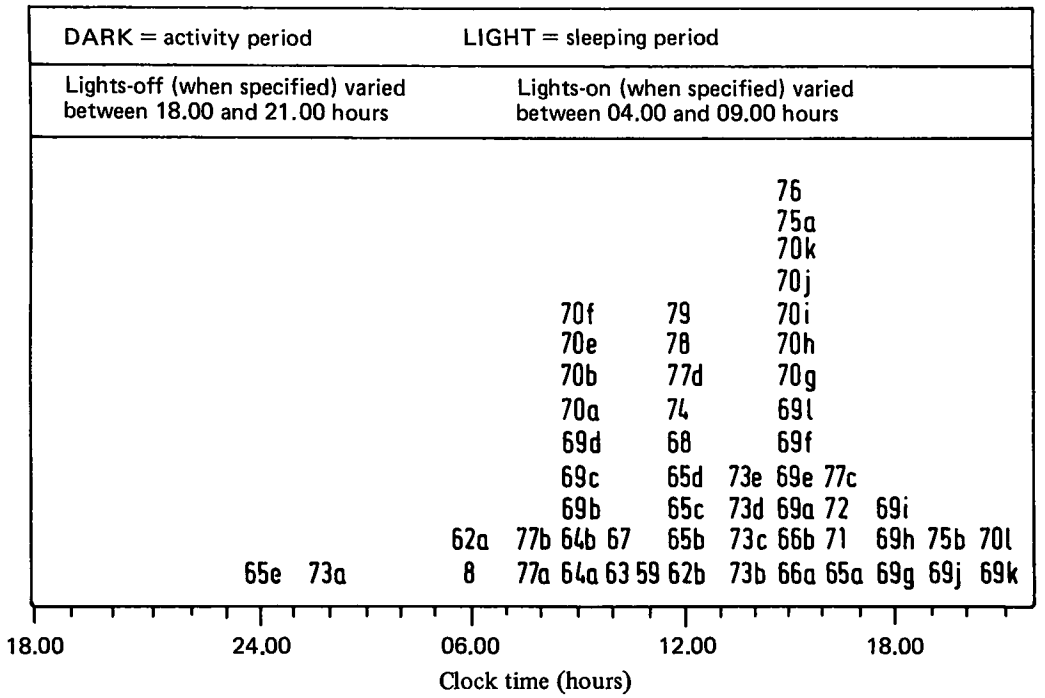

FIG. 2. In nocturnal animals the peak rates of RNA and protein synthesis, RNA and protein content, number of cells and growth rates occur at the time when sleep predominates, i.e. during the light period. References [73]a, b, c, d, e: estimated clock times, as reversed lighting schedule was used; reference [74]: no clock time was reported, but higher in daylight. Key to tissues: protein synthesis in: epidermis [8]; suprachiasmatic and paraventricular nuclei [64]a & b; visual and motor cortices [65]a & b; lateral geniculate nucleus [65]c; retina [65]d; cerebellum and cerebrum [66]a & b; whole brain [67], [68]; putamen ♂ [69]a, [70]a, ♀ [69]g, [70]g; thalamus ♂ [69]b, [70]b, ♀ [69]h, [70]h; amygdala ♂ [69]c, ♀ [69]i, [70]i; pre-optic area ♂ [69]d, ♀ [69]j, [70]j; median eminence ♂ [69]e, [70]e, ♀ [69]k, [70]k; anterior pituitary ♂ [69]f, [70]f, ♀ [69]l, [70]l; muscle [71], [72]; liver [65]e; collagen [73]a, b, c, d, e; pineal [74] (RNA synthesis); cartilage [75]a & b, [76], [77]a, b, c, d (content), cell size [59], number [63]; epidermis [62]a & b (RNA synthesis); bone growth [78], [79].

cells actually divide. Keeping mice awake when they normally sleep reduces the expected rate of mitoses [81], and if the time for sleep is shifted the peak in mitoses shifts too [54, 81].

Repair and growth are faster at the time of sleep

Not only are more cells dividing but also it takes a cell less than half the time to divide during the sleep period [10, 19, 46]. The replacement of epithelia is faster at the time of sleep [2–6, 8–26, 30–35]. In connection with such variation it may be remembered that within any 24 h there is complete turnover of the cells lining the gut [82]. After damage to tissue the reparative processes rhythmically rise to peaks that coincide with the sleep period, e.g. epidermal wound healing [6], repair of rectal mucosa [35] and tympanic membrane [55]. Cell replacement after partial nephrectomy [39] and hepatectomy [43], and post-fracture linear bone growth [78] all peak around noon (when rats sleep). Bone growth, too, is faster at the time of sleep in animals [77, 79] and boys [83].

What of man?

The reports summarized in Figs. 1 and 2 refer to nocturnal creatures, and less information is available for animals that sleep by night. Mitoses peak in the evening in the ruminal epithelium of sheep [84], and at midnight in a day-active species of mouse [85]. Four reports record peak rates at

night for human skin mitoses (e.g. [86, 87]), one for proliferative activity in human bone marrow [88] and one for human bone growth [83], as mentioned.

The information for man is thus thin but there is also some indirect evidence. During the day there are high levels of human catecholamines and cortisol, hormones that inhibit protein synthesis [89]. Even standing up causes a threefold increase in urinary catecholamines. In sleep there occurs the principal secretion of growth hormone, dependent upon the appearance of EEG slow-wave sleep in the early night. Growth hormone rapidly stimulates protein and RNA synthesis and amino acid uptake [90] and is widely used clinically to enhance growth. When it is, for example, coupled with haemopoietin, growth hormone accelerates the formation of human erythrocytes *in vitro* [91]. If nitrogen retention is taken as an index, then there is a greater anabolic response in humans deficient in growth hormone if an injection of growth hormone is given just before sleep than if the same injection is given before breakfast, when cortisol is high [92]. It has been reported, too, that during constant protein intake, sleep deprivation and disrupted sleep schedules led to negative nitrogen balance, after an initial lag compatible with the inertia in urea excretion [93]. Apart from growth hormone, secretions of prolactin (an obscure hormone, often held to be anabolic), luteinizing hormone and testosterone are also linked to sleep.

Food

Starving children grow badly and over a period of weeks it may appear legitimate to suppose that:

Dietary protein intake + (B), unknown
= Bodily protein synthesis, unknown
+ (O), unknown

Protein may be represented by a chosen amino acid, unknown B as internal provision of the amino acid by protein breakdown, and unknown O as the oxidation of the amino acid. Attempts to determine the three unknowns have rested upon a series of disputable assumptions.

Were it hypothesized that such an equation might be applicable to short time periods, and if B and O were small or constant, there could then appear to be an instantaneous and direct relation between dietary intake and protein synthesis. Garlick *et al.* [94] made just such use of this equation and wrote that "protein synthesis fell immediately the hourly food intake ceased": a conclusion hardly surprising when one looks at the equation. They had applied it to the human day/night situation and were inevitably led to suppose that, because feeding did not take place at night, whole body protein synthesis was dramatically diminished at night. It seems to us that a rough-and-ready equation, that may have appealed to commonsense when the time scale was one of weeks, should not be taken for granted across a period of hours. We have elsewhere set out more detailed criticisms [95] and would here also recall that food takes some hours to pass through the gut, where it may be mixed with protein released by intestinal secretions and cells shed from the mucosal lining of the gut each day, and in man variously estimated as up to 300 g of protein/day. Although liver protein synthesis correlates with food intake, giving rise to the peak in global liver protein synthesis during the feeding period [65], structural regeneration in liver through mitoses is maximal at the time of sleep [40–44].

Man's cellular biochemistry is the same as that of other organisms and we would suppose that future studies of synthesis in most human tissues, and particularly structural proteins if these can ever be measured, will accord with Figs. 1 and 2.

Activity and rest

The hormones of wakefulness, mentioned earlier, are hormones that enhance catabolism and reduce protein synthesis, whereas the hormones of sleep enhance protein synthesis. However, hormones may be regarded as but additions to the more important control of protein synthesis by the energy state of the cell. It was in 1948 that Bullough [81] concluded that "the rate of epidermal mitosis normally increases during sleep, and decreases during hours of wakefulness and exercise ... the diurnal mitosis cycle is determined by the habits of the animal". We also would suppose that it is rest vs activity that determines the amplitude of 24 h variations. One of us has elsewhere [1] summarized grounds for believing that the differences between the rest and activity phases of the 24 h can be understood from the concomitant variations of Atkinson's cellular energy charge (EC) [96], which is a measure of the amount of cellular ATP in relation to the other adenine nucleotides, ADP and AMP. In brief, cellular work, which is higher during wakefulness, consumes ATP and so the level or concentration of ATP falls and with it the EC within the cell. This fall is a signal that leads to enhanced degradation, so to raise ATP levels, while simultaneously inhibiting synthesis and cell division. When work diminishes, ATP and EC rise, degradation is diminished, while

oxygen consumption falls, and synthesis is stimulated.

It must be emphasized that protein synthesis requires a high level of EC, and is necessarily diminished if there is a high rate of oxidative metabolism within the cell. A misconception sometimes encountered is that protein synthesis requires a lot of cellular energy, and that it will itself substantially lower EC, but this is not so: as Racker [97] has put it: "the energy requirements for biosynthesis of macromolecules such as protein and nucleic acids are relatively minor, probably less than 10% of the energy budget". Similarly Hommes estimates that only 2-3% of ATP produced is used for growth in the human newborn [96].

A variety of studies confirm that tissue ATP levels and EC are higher during rest and sleep, and this is an inevitable corollary of the lowered rates of oxygen consumption during sleep, especially slow-wave sleep, for it is a fall in EC that is the stimulus to oxidative metabolism.

Brain and muscle

The human brain uses a fifth of the resting body's blood supply and is rivalled only by the liver in intensity of metabolic activity. It is the brain that is most obviously renewed in its capabilities by sleep, and the reports agree that protein synthesis in the brain is enhanced during the sleep period of rodents [65, 66, 68, 69, 70]. In primates the rate of glucose utilization in the brain falls by some 30% during sleep [98], confirming the low rate of cellular work at this time. Others have reported raised brain ATP and EC levels during sleep, so that it can be understood that the lowered cellular work during sleep will be associated with a higher EC and thus stimulation of protein synthesis.

Many nutritionists have been interested in nutritional constraints on the development of the brain, but others have concentrated attention on muscles. Increasing the rate of work of muscles will enhance the rate of production of ATP several-fold (and oxygen consumption several-fold), but even light work for 2 min lowers the concentration of ATP in human muscle by 25% [99]. Not surprisingly therefore exercise increases the rate of muscle protein degradation and decreases muscle protein synthesis [100]. The finding of greater protein synthesis in rodent muscle during the sleep phase than during the activity phase [71, 72] may again be understood. Muscles can to a degree rest during wakefulness, but generalized muscle tone falls to its lowest levels during sleep, especially REM sleep. It must, of course, be understood that neither sleep nor feeding will alone make muscles stronger, for in the long run it is extra exercise that brings about hypertrophy of muscles; but first a deficit must be produced, to act as a stimulus to the processes of hypertrophy that occur many hours later [101]. We would suppose those processes to be facilitated by sleep.

Conclusions

In order to understand human tissue development and renewal it will be necessary to recognize the contribution of 24 h rhythms. The literature demonstrates that, in the majority of tissues, peak rates of protein synthesis and peak rates of cell division coincide with the time of sleep. In contrast, degradative metabolism is greater during wakefulness.

The evidence can be understood in terms of the 24 h variations in cellular work, and the simultaneous control of synthesis and degradation by the relative intracellular concentrations of adenine nucleotides, as may be represented by Atkinson's cellular energy charge (EC). High values of EC stimulate synthesis and cell division, but decrease degradative metabolism and oxygen consumption. Energy charge values are higher during sleep, in association with the low rates of cellular work. Sleep-dependent and circadian hormone rhythms can be seen to complement the more fundamental control mechanisms and to involve tissues not directly influenced by variations in cellular work, for example, the skin.

Note: Some statements made above are unsupported by references, in order to meet the Editor's space requirements. We can supply references upon request.

Acknowledgments

K.A. is grateful to the Scottish Hospital Endowments Research Trust for a Fellowship. We thank also Mrs Dorothy Duncan.

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