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12. Guinea pig complement (Baltimore Biological Laboratories) was screened for naturally occurring cytotoxicity, which, if present, was removed by absorption with either thymus or spleen cells at 4°C for 1 hour.
13. After lysis of red cells with tris-buffered ammonium chloride [W. Boyle, *Transplantation* **6**, 761 (1968)], 5×10^6 spleen cells were incubated with ^{51}Cr-labeled sodium chromate (250 μc/0.25 ml) at 37°C for 45 minutes, and then washed four times in medium.
14. ^{51}Cr-labeled lymphocytes (5×10^4 in 50 μl) were incubated in duplicate with an equal volume of a 1:10 dilution of antiserum for 15 minutes at 4°C. Complement (50 μl of a 1:4 dilution) was added, and the mixture was incubated for 30 minutes at 37°C. After centrifugation (1500 rev/min for 10 minutes), the radioactivity of 50 μl of supernatant was measured. Total releasable radioactivity was obtained by freeze-thawing the controls three times. Other controls contained cells and medium only or cells plus complement and medium or normal mouse serum (NMS). These controls gave 10 percent or less of chromium release. The percentage of lysis is equal to $(P - R)/(Q - R)$ where P is the radioactivity released by antiserums and complement; R is the radioactivity released by NMS and complement; and Q is the radioactivity released by freeze thawing.
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16 August 1971

Gonadotropin Secretion during Sleep in Normal Adult Men

Abstract. *Release of luteinizing hormone and follicle stimulating hormone during sleep in young adult men occurred in unrelated, random, arrhythmic peaks, with no consistency from night to night in the same subject. Release of luteinizing hormone was modestly but significantly larger (14 perecnt) during rapid-eye-movement sleep than it was in non-REM sleep, but release of follicle stimulating hormone was not clearly related to stages of sleep.*

Increased secretion of adrenocorticotropic hormone (ACTH) during sleep, as reflected by cortisol levels, occurs in the early morning hours at a time when rapid-eye-movement (REM) sleep is maximal (*1*). Increased secretion of growth hormone, on the other hand, occurs primarily in the first few hours after sleep onset and is closely related to slow-wave (stages 3–4) sleep (*2*). Whereas release of ACTH can be dissociated from REM sleep by acute sleep-wake reversal (*3*), release of growth hormone follows intimately the time of onset of slow-wave sleep (*4*) and is not alterable by manipulations which affect growth hormone during waking hours (*5*). The possibility that related neural mechanisms control both sleep patterns and the secretion of some anterior pituitary hormones is enhanced by evidence that biogenic amines have neurotransmitter roles both in sleep (*6*) and in the secretion by the hypothalamus of pituitary hormone releasing and inhibiting factors (*7*). Investigation of other pituitary hormones during sleep is thus warranted.

We now report a study of luteinizing hormone (LH; ICSH) and follicle stimulating hormone (FSH) release during sleep in normal young men. Patterns of release of these two hormones in normal men have not been extensively investigated. Concentrations of both hormones, measured every 2 hours for 1 day, in serums of ten subjects were quite stable (*8*); other studies, however, showed a circadian rhythm of serum FSH in men, with highest values occurring in the early morning hours (*9*). Some periodicity of plasma LH was observed during sleep in three men, although there was no obvious relation to sleep stages (*10*). Peaks of plasma testosterone, on the other hand, were found in conjunction with episodes of REM sleep in five male subjects (*11*).

We studied 16 healthy male volunteers, ages 21 to 30, on no drugs or medication, for four consective nights in the sleep laboratory. Men were chosen in order to obviate the cyclic influence of the female hypothalamus on gonadotropins. They were allowed normal meals and activity during the day. Continuous electrophysiologic (electroencephalographic, electrooculographic, and electromyographic) recordings were made each night during the hours of sleep (11 p.m. to 7 a.m.). On the first night, only electrophysiologic recordings were made; on the second night an antecubital venous catheter was placed. but no blood was taken; on the third and fourth nights blood was sampled from an adjoining room through a 10-foot tube connected to the catheter (*12*). The first eight subjects were sampled every 30 minutes from 11 p.m. to 7 a.m.; the second eight subjects were sampled every 10 minutes between 11 p.m. and 1 a.m. and between 5 and 6 a.m. Total blood withdrawal was kept below 200 ml each night. The plasma was immediately separated and frozen until radioimmunoassay for the hormones could be done (*13*). All samples were run in duplicate, and all samples from each subject were run in the same assay. Mean intra-assay variability was 5 percent; mean inter-assay variability was 20 percent.

Each thirty-second epoch of EEG recording was scored as awake; stage 1,2,3,4; or REM by established criteria

(*14*). In comparison with the subsequent three nights, the first adaptation night showed the usual greater sleep latency and lower REM time and REM percentage (*15*). The only significant difference between the first two adaptation nights and the two nights when blood was drawn was an increased wake time after sleep onset (*16*) during the second pair of nights; on several occasions subjects rolled over on the catheter while asleep and had to be momentarily awakened and untangled. There were no significant differences in sleep parameters between the two consecutive nights when blood was drawn.

Both LH and FSH showed patterns of nocturnal secretion that were different across subjects and inconstant between the two "blood-draw" nights, for the same subject. There were episodic but unrelated peaks of both hormones throughout most nights (Figs. 1 and 2).

The rhythmicity of these changes in hormone secretion was tested by fitting sine curves of 45, 60, 75, and 90 minutes and 2, 4, 8, and 24 hours for each subject (*17*). Although many of the correlations between the hormone data and the sine curves with optimum start points were high, the across-subjects correlation of optimal ultradian periods (*18*) for the two "blood-draw" nights was not significant for either hormone. Many of the 24-hour sine curve correlations were high, but the acrophases (times of maximum amplitude) (*18*) were quite different between the two "blood-draw" nights; across-subjects correlations of the acrophases for the two consecutive nights were not significant for either hormone. These analyses indicate that there was no consistent ultradian or circadian pattern across subjects for either hormone. The analysis of hormone levels by sleep stage therefore was performed without correcting the hormone data for any effects of rhythm.

Generally, intersubject differences in hormone concentrations were great, and differences between sleep stages averaged across subjects were small. Because there were no stage 1 data for four of the 16 subjects, this stage was omitted in the sleep analyses. Because stages 3 and 4 had very few data points, these stages were combined in the analyses. Kendall's rank-order coefficient of concordance (*19*), comparing gonadotropin levels during awake, stage 2, stage 3 + 4, and REM across the 16 subjects, was low but statistically significant for both LH ($W = .24$) and FSH ($W = .19$). By successive comparison of the individual sleep stages and combination of those that were not significantly different, the overall significance for LH was found to reside in a difference between REM sleep and all other sleep stages combined; LH was 14 percent higher during REM sleep than it was in the other stages. The significance for FSH, on the other hand, was not clearly discernible by successive comparison and combination of the individual sleep stages; FSH was not significantly increased during REM sleep. (Preliminary measurements of growth hormone in these subjects indicate the usual increase in growth hormone during slow-wave sleep (*2*) which is consistent for the two consecutive nights.)

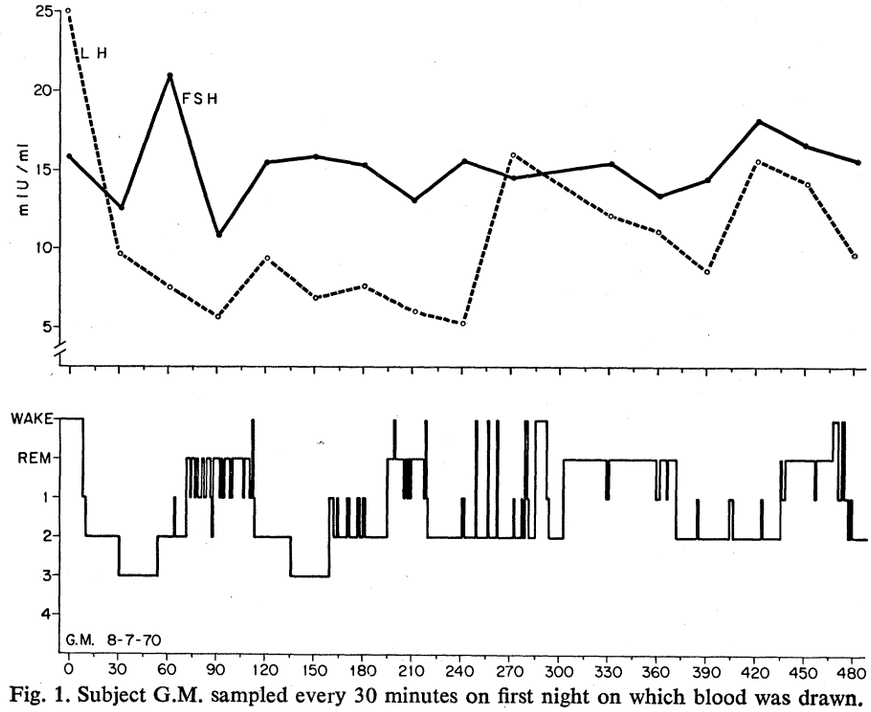

Fig. 1. Subject G.M. sampled every 30 minutes on first night on which blood was drawn.

Our results suggest that the secretion of gonadotropins during sleep in normal men is pulsatile rather than periodic (*10*) and varies considerably both from subject to subject and from night to

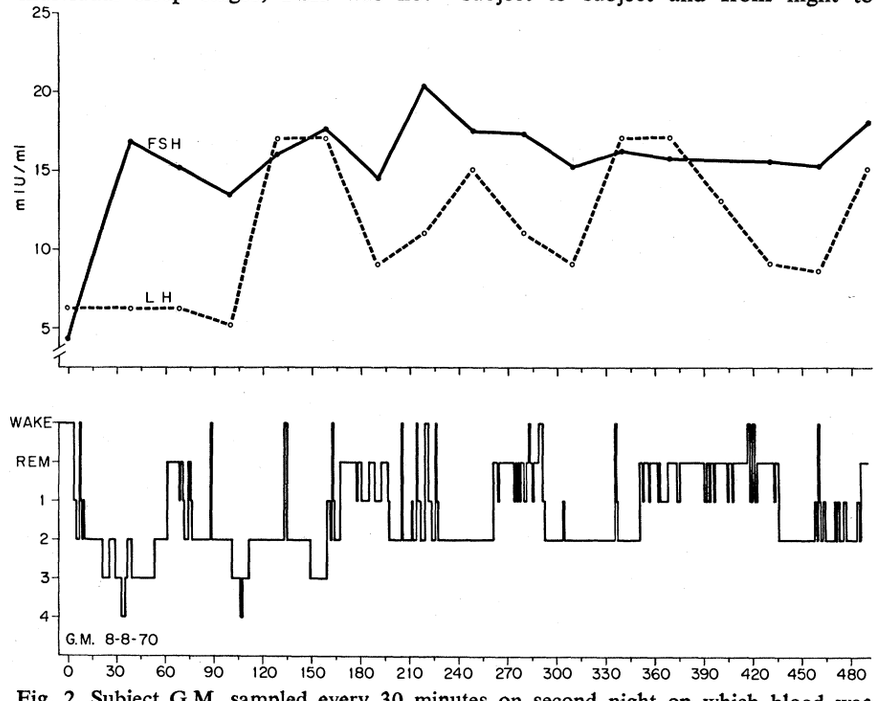

Fig. 2. Subject G.M. sampled every 30 minutes on second night on which blood was drawn.

night within the same subject. The discontinuous and arrhythmic nature of gonadotropin release is consistent with the pattern of release of other anterior pituitary hormones in man, particularly ACTH (*20*).

The modest but statistically significant increase in LH during REM sleep is of interest for several reasons. First, norepinephrine as a central neurotransmitter has been implicated both in REM sleep (*6*) and in the secretion of this hormone (*7*). Second, peak secretion occurs during paradoxical (REM) sleep in unanesthetized, unrestrained female rats at all times of the estrous cycle (*21*). Third, REM sleep-associated increases in testosterone, the release of which is stimulated by LH, were reported recently in male subjects (*11*). And fourth, penile erections occur during REM sleep (*22*).

Of major interest is the fact that the fluctuations in both LH and FSH are small in comparison with the changes in cortisol and growth hormone which occur during sleep. Recent studies of reproductive physiology by sensitive radioimmunoassay techniques indicate that major physiologic events may be associated with only small changes in LH and FSH concentrations (*23*). For example, the process of sexual maturation is accompanied by statistically significant increases in average concentrations of LH and FSH, but there is great overlap of values between sexually mature persons and prepubertal children. Also, the FSH increase which initiates follicle growth during the menstrual cycle is small. Thus the modest increase in LH found during REM sleep may have important, but as yet unknown, physiologic significance. However, it is also possible that these changes reflect only the modulation of control systems within the brain and are without importance to gonadal function.

Note added in proof: Similar repetitive, abrupt elevations in LH recently were reported in four normally active men sampled every 15 minutes between 6 a.m. and 6 p.m. (*24*).

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23 September 1971

Activation of Viruses in Human Tumors by 5-Iododeoxyuridine and Dimethyl Sulfoxide

Abstract. *Dimethyl sulfoxide added to cultures first treated with 5-iododeoxyuridine increased C-type virus production approximately tenfold in a human rhabdomyosarcoma cell line. 5-Iododeoxyuridine followed by dimethyl sulfoxide also activated a similar C-type virus in a metastatic tumor from a bronchial node taken from a 52-year-old male.*

Recently we described a method for activating a virus in a nonproducing, established cell line from a human rhabdomyosarcoma. This was accomplished by growing the cells in the presence of 5-iododeoxyuridine (IdU) for 3 to 4 days (*1*). The activated virus resembles the murine C-type oncornaviruses in size and morphology. It differs, however, from the animal oncornaviruses in that it does not bud from the outer cell membrane of the cell, but only from the endoplasmic reticulum. One to ten virus particles were observed in about 1 percent of the cells examined.

We now describe a modification of the former procedure, whereby we can now produce much greater quantities of virus from the same rhabdomyosarcoma cell line. The method has also been applied to an established cell line developed from an adenacarcinoma metastasis to a bronchial node and has again yielded virus from a nonproducer line.

Dimethyl sulfoxide (DMSO), which is known to readily penetrate cell membranes, has been used to attempt enhancing the penetration of virus into

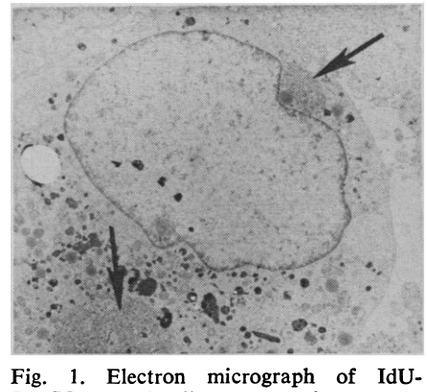

Fig. 1. Electron micrograph of IdU-DMSO treated cell. Arrows point to cytoplasmic inclusions (× 2400).