Table 2. Radial position and energy of deepest energy minimums (maximum interaction) for varying test charge; r is the distance from nitrogen in angstroms; energies are expressed in kilocalories per mole.

Reaction to test charge									
−0.1		−0.3		−0.5		−0.7		−1.0	
Energy	r_{min}	Energy	r_{min}	Energy	r_{min}	Energy	r_{min}	Energy	r_{min}
				Choline					
−4.8	3.30	−10.3	3.20	−15.7	3.10	−21.2	3.00	−29.9	3.00
				Ethanolamine					
−4.0	3.00	−10.8	2.80	−17.8	2.80	−25.1	2.70	−36.1	2.60
				Difference					
+0.8	.30	−0.5	.40	−1.9	.30	−3.9	.30	−6.2	.40

was given steric (bulk) factors equivalent to those for the van der Waals radius of oxygen, and the negative charge was varied from −0.1 to −1.0.

The axes along which potential minimums occur are shown in Fig. 1. For the ethanolamine system, a single minimum occurs directly along the axis of the C–N bond. However, there are two minimums for the choline system, both on axes which bisect both tetrahedral angles at the nitrogen atom. Although there are three such axes, the third has a higher minimum because of the presence of the negative methylene carbon atom (second neighbor), which is *cis* to the test charge when it is brought in along this axis.

Table 2 shows the energies and the radial values for the minimums in the two molecules with different test charges. The energy minimums represent a combination of coulombic and steric forces, and the results reflect both parameters. The test charge reaches its energy minimum at radii which are consistently smaller for ethanolamine than for choline. This reflects steric repulsion by the choline methyl groups. Primarily because of this factor, the energy minimums are more negative for ethanolamine than for choline. However, as the coulombic part of the energy becomes less significant (smaller test charge), the energy differences become less and steric factors are most predominant.

These data do not take into account any polarization effects by atoms beyond those shown in Fig. 1. Polarization through such effects as internal salt formation are not considered, because definite information on the stereochemical aspects of cell surface components such as phospholipids is scarce. In regard to this point, Sundaralingham (*16*) has shown that crystalline α-L-phosphorylcholine glycerol and similar compounds are in a *gauche* conformation about the choline carbon-carbon bond. If this conformation exists in these compounds at cell surfaces, then the positions and magnitude of the energy minimums calculated here will be significantly altered. In any case, it is clear that both choline and ethanolamine possess distinct potential energy fields for interaction with anions, either through internal or external salt formation.

It is of interest to consider the probable effect of these differences on cellular adhesion. If adhesion depends on matching a surface charge with another surface charge, or on matching a surface charge with a linking macromolecule, then one would predict that membranes containing large amounts of ethanolamine relative to choline would adhere more strongly to one another. Negative sites apposed to a cell surface would be more strongly bound to ethanolamine-positive sites than to choline-positive sites. This is especially so if the negative sites carry a full or large negative charge on one atom, or can be polarized so that the negative charge is localized. Although the differences between the two systems become smaller as the negative charge is reduced (Table 2), these differences may well be significant down to values as small as 0.1 kcal/mole. Since surface charge matching is a cooperative effect, one must consider the sum of the interactions rather than the isolated case. An example of this type of situation is found in the work of Scheraga (*7*), who predicted the relative stabilization of right- and left-handed helices based on energy differences of less than 0.1 kcal per mole residue. The net energy difference clearly is the significant value.

In fact, Tomasz showed that cellular adhesion in pneumococcal cells with a high surface content of ethanolamine was far stronger than that in cells with a high surface choline content (*3*), an observation consistent with our results. Further alterations in surface properties may also prove to be due to the different potential energy fields about nitrogen in choline and ethanolamine residues.

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Human Growth Hormone Release: Relation to Slow-Wave Sleep and Sleep-Waking Cycles

Abstract. *Release of human growth hormone during sleep is significantly related to slow, synchronized stages of sleep and therefore would seem to be controlled by related neural mechanisms. When sleep-waking cycles are reversed by 12 hours, the release of growth hormone with sleep is reversed; thus release does not follow an inherent circadian rhythm independent of sleep.*

Human growth hormone (HGH) is spontaneously released during sleep without prior change in concentrations of glucose or insulin in plasma (*1, 2*) and in amounts comparable to maxi-

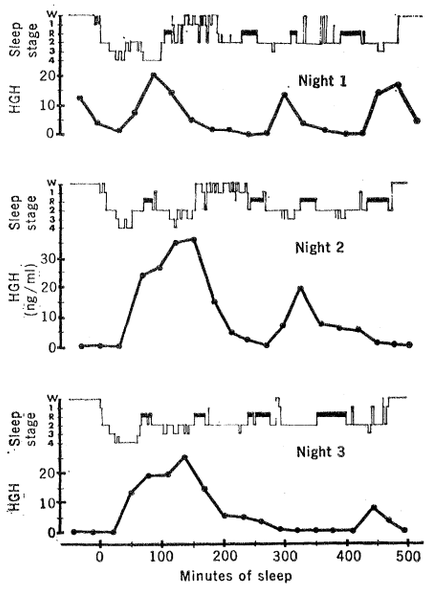

Fig. 1. Histograms of sleep cycles over three consecutive nights with simultaneous plot of HGH from one representative subject; *W*, wakefulness; *R*, stage REM. Subject illustrates night-to-night similarity in HGH secretion pattern and sleep cycles. Note also typical, large, early sleep rise and relation of later rises to slow-wave cycles.

mum concentrations induced by arginine infusion (*3*). That release of HGH may be related to sleep stages 3 and 4, as defined by electroencephalographic criteria (*4*), has been suggested in these reports. The effect of reversing sleep-waking cycles and the possibility of an endogenous rhythm of nocturnal release were not fully investigated.

We demonstrate a significant relation between release of HGH and the cyclic recurrence during sleep of stages 3 and 4, which we refer to as slow-wave sleep, and show that a 12-hour reversal of sleep-waking cycles results in prompt reversal of HGH release. There is no evidence for an endogenous rhythm independent of sleep.

Ten healthy, adult, male volunteers, aged 19 to 28, were allowed normal meals and activity when not under study, but were kept fasting and at rest during experimental sessions. Their last meal was 4 hours before each session. The electroencephalogram (EEG), submental electromyogram, horizontal eye movements, heart rate, respiration, and rectal temperature were recorded (*5*). Blood was sampled every 30 minutes through an indwelling, lightly heparinized, antecubital venous catheter. Plasma was immediately separated and frozen until radioimmunoassay for HGH was performed. Free HGH was separated from HGH-antibody complexes on charcoal-dextran (*6*) modified for HGH (*7*). All assays were run in duplicate. Maximum sensitivity ($P = .05$) of duplicates was 0.6 ng/ml. Mean sample difference over the entire range of HGH values in two consecutives assays was ± 0.4 ng/ml. Therefore, values for HGH that were greater than 1 ng/ml were accepted as readily detectable and reproducible concentrations.

Each 30-second epoch of EEG recording was scored as wakefulness (*W*); stage 1, 2, 3, 4; or REM (rapid eye movement) (*4*). Percentages of sleep in each stage were within range of normal for all subjects (*8*). Recordings were divided into recurrent sleep cycles, each cycle terminating with the end of a period of stage REM. Cycles were then divided into two types, slow-wave and non-slow-wave, on the basis of appearance of stages 3 and 4.

Rises in concentrations of plasma HGH occurred during each of 21 sessions of normal nocturnal sleep. On 18 of the 21 nights HGH was first released within 90 minutes of the onset of sleep. Nineteen additional rises occurred later in sleep. If a subject had more than one rise in HGH during sleep, the initial peak was usually the greatest. Frequency, magnitude, and pattern of peaks varied from subject to subject, but each subject had a characteristic pattern of both release of HGH and sleep cycles reproducible over as many as three consecutive nights (Fig. 1). One subject, kept awake in bed in the dark with eyes closed for 4 hours, did not secrete HGH.

We compared concentrations of HGH in plasma in both slow-wave and non-slow-wave sleep cycles. Thirty-two of the 37 rises of HGH were associated

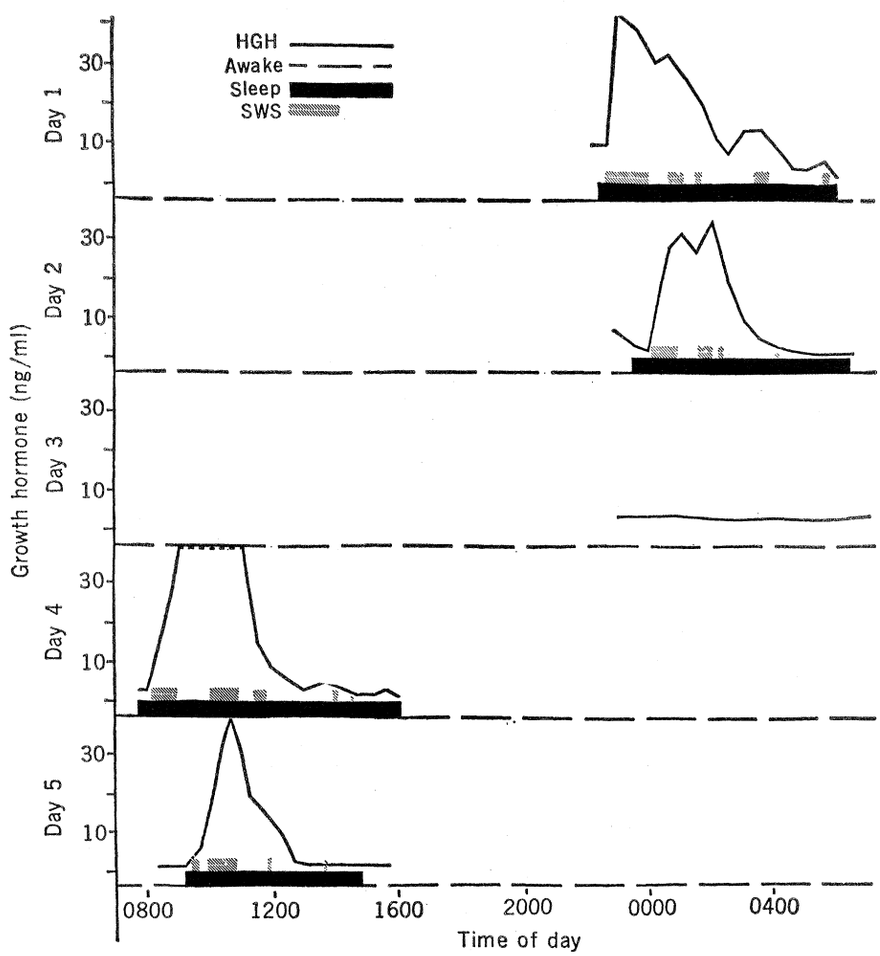

Fig. 2. Plots of five experimental sessions on consecutive days of a study of the reversal of the sleep-waking cycle from one representative subject. Peak concentration of HGH on the morning of 4th day reached 40 ng/ml. Time according to 24-hour clock. Graph illustrates absence of HGH secretion on 3rd night, when subject was kept awake, and resumption of secretion on 4th and 5th mornings when he was asleep. Base line and post-reversal release show relation to slow-wave sleep. *SWS*, Slow-wave sleep.

with slow-wave cycles. All 18 of those occurring within 90 minutes of the onset of sleep were associated with slow-wave cycles, but under normal conditions slow-wave sleep always occurs in the first third of sleep. More crucial to the relation of the release of HGH to slow-wave sleep are the 19 later peaks, recorded during that part of the night when slow-wave cycles are uncommon and stages 3 and 4 occupy only 5 percent of total sleep time. Fourteen of these peaks occurred in slow-wave cycles.

For seven subjects mean values of HGH per sample could be calculated for both slow-wave and non-slow-wave cycles in late sleep. All seven subjects had higher values during slow-wave cycles than during the non-slow-wave ones. Mean value per sample across subjects for these late-night, slow-wave cycles was 3.28 ng/ml, and for the non-slow-wave cycles, 0.65 ng/ml ($P < .02$).

Although the total amount of slow-wave sleep was similar for all subjects, its distribution over sleep cycles throughout the night was variable. Those subjects with more frequent slow-wave cycles had initial peaks of greater magnitude and more frequent secondary rises.

For five subjects sleep-waking cycles were reversed after one or two base line sessions by keeping them awake for 24 hours (Fig. 2). Four were reversed from night sleep to day sleep and one from day sleep, to which he had been accustomed for 2 weeks, to night sleep. None of the five secreted HGH during the wakefulness period corresponding in clock time to the previous 2 nights of base line sleep. Each then secreted HGH during sleep after reversal and during his next reversed sleep period. The basic pattern of release after reversal was similar to that subject's pattern in base line sleep and continued to show a relation to slow-wave sleep (Fig. 2). Three subjects who were reversed from night to day sleep had peaks of greater magnitude during their first sleep period after reversal.

Data on release of HGH during sleep in previous studies (*1–3*) and the data presented here favor the existence of a neural control mechanism of growth hormone releasing factor from the hypothalamus in addition to those mechanisms already studied (*9*).

We conclude from our data that this release is related not only to sleep but particularly to non-REM portions of the recurrent cycles within sleep, especially if these include EEG stages 3 or 4. The synchronization of release and sleep patterns implies that related or identical subcortical pathways from brainstem to cortex and hypothalamus are operative.

The reversal of sleep-waking cycles demonstrates further that the rhythmicity of HGH release in sleep over repeated base line records is not due to an inherent circadian rhythm as recently postulated (*9*) but is dependent on the rhythmic recurrence of sleep itself.

Release of HGH in sleep suggests an anabolic function of slow-wave sleep and provides impetus for further study of neuroendocrine rhythmicity in sleep.

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18 April 1969

Surveyor Alpha-Scattering Data: Consistency with Lunar Origin of Eucrites and Howardites

The alpha-scattering experiments carried out by Surveyor missions 5, 6, and 7 (*1*) supplied chemical analyses that are not inconsistent with the hypothesis that the eucrite and howardite achondrite meteorites originated on the moon (*2*). Briefly, this hypothesis maintains that the uplands consist of coarse-grained mafic to ultramafic igneous rocks which crystallized at depth, were then fragmented, and were repeatedly mixed to form polymict breccias, of which howardites are samples. The maria are considered younger basaltic rocks of distinctive iron-rich composition, from which eucrites have been derived. Rocks of the uplands are thought to have crystallized early in lunar history, perhaps 4.5×10^9 years ago, and have not undergone significant chemical alteration since then. Age determinations by potassium-argon dating suggest that eucrites are younger, with an average age of about 3.7×10^9 years (*3*). The hypothesis requires that a mechanism, such as meteorite or comet impact, is capable of removing material from the lunar surface, in order that samples can arrive at the earth.

Surveyor 5 and 6 supplied analyses of samples in Mare Tranquillitatis and Sinus Medii, two widely spaced locations covered by dark, flat, typical mare material. The similarity of these analyses to those of typical eucrites (Table 1) has been pointed out by O'Keefe *et al.* (*4*). Surveyor 7 made three similar analyses of a rock and soil in the vicinity of the young upland crater Tycho, apparently on ejecta from the

Table 1. Comparison of composition of eucrites with results of Surveyor 5 and 6 analyses.

Element	Surveyor 5 (atom %)	Surveyor 6 (atom %)	Juvinas eucrite (atom %)*
C	< 3	< 2	
O	58 ± 5	57 ± 5	60.6
Na	< 2	< 2	0.3
Mg	3 ± 3	3 ± 3	3.8
Al	6.5 ± 2	6.5 ± 2	5.6
Si	18.5 ± 3	22 ± 4	18.7
"Ca"	13 ± 3	6 ± 2	4.6
"Fe"		5 ± 2	6.2

* Composition of Juvinas eucrite (percent by weight) and range of eucrites (in parentheses): SiO_2, 49.32 (48.6–49.6); TiO_2, 0.68 (0.4–1.0); Al_2O_3, 12.64 (11.7–13.9); FeO, 18.49 (15.3–20.1); MgO, 6.83 (5.4–7.4); CaO, 10.32 (8.6–11.5); Na_2O, 0.42 (0.4–0.9); K_2O, 0.05 (0.04–0.22); Cr_2O_3, 0.30 (0.06–0.9); MnO, 0.53 (0.3–0.8); P_2O_5, 0.09 (0.09–0.16); FeS, 0.53 (0.03–0.57). See (*2*).