of which had been rotated around its own horizontal axis (see Fig. 1). The text all came from popular psychology sources [described in (*3*)]. On each of 7 days each of the six male readers read 14 pages of this inverted text, 7 pages from the set of pages read previously, and 7 from the same sources, but of material not read previously. The order of pages was scrambled from reader to reader, with the proviso that the reread pages on each day sampled the range of page orders previously used. This proviso eliminated any differential bias in the results attributable to special practice. A single page in normal typographic orientation was read at the beginning of each test session. The material was read aloud into a tape recorder and was timed with a stopwatch.

Performance on the task of reading transformed typography aloud improves in a regular fashion, following the form of the standard learning curve (*4*). When the logarithm of the time taken to read a page of text is plotted against the logarithm of the number of pages read, the data appear as straight lines (Fig. 2). From an initial 4 minutes per page (the antilogarithm of 0.6) the readers improved to a terminal 1.7 minutes per page, on the average. The rate of learning was less than that for the same subjects when they had been without practice (*3*), but their initial reading speed in the present test, about 4 minutes per page, was considerably less than the 15 minutes first measured; that is, they revealed a marked savings of the skill acquired earlier.

The difference between the times to read new pages and pages read earlier is shown by the different symbols associated with the learning curve for inverted typography. Many more strokes (new pages) appear above the least-squares line, and many more closed circles (repeated pages) appear below it. (As the order of pages was scrambled from reader to reader, a particular position in the order could be filled by an old page for one reader and a new page for another. Hence usually two observations appear for each value of the abscissa, one for pages read for the first time and the other for reread pages.)

Reading time was subjected to an analysis of variance that partitioned performance into the learning component, a component that evaluated the difference between pages read previously and pages read for the first time, and an error term. All of the readers revealed very large learning effects, as reported previously (*3*). In this experiment the difference in speed for pages read for the first time and pages reread after 13 to 15 months was significant ($P < .0001$).

One view of my results could be that the readers recognized the semantic content of the pages and that this recognition facilitated speed of reading the text. Such a view was tested and found wanting in a study that compared the relative worth of semantic and pattern-analytic practice at reading (*5*). An alternative implicated here is that the skills involved in literacy are associated with pattern-analyzing operations that are directed at the surface lexical features of text; the encoding operations that acquire these features for the reader are themselves modifiable; moreover, they are "remembered." The memory is seen in a general way in the learning curve, and specifically in the differential performance on old and new pages (Fig. 2). This operational memory is to be distinguished from memory for the results of encodings, the semantic or other grammatical content of text that is the subject of most contemporary studies (*1*). Models directed only to an account of the semantic and syntactic relations of text miss this crucial feature of performance and, hence, may overestimate the more conscious component of reading skill.

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REM Sleep Induction by Physostigmine Infusion During Sleep

Abstract. *Physostigmine (an anticholinesterase agent that increases acetylcholine at the synapse), in a dose of 0.5 milligram, was given intravenously to seven normal human volunteers. When injected during rapid eye movement (REM) sleep, physostigmine woke the subjects, and when injected during non-REM sleep, it induced REM sleep. This result suggests that cholinergic mechanisms play a role in the induction of REM sleep and in modulating cortical arousal mechanisms.*

Rapid eye movement (REM) sleep is distinguished from non-REM sleep and wakefulness on psychological and physiological grounds. It is closely, although not exclusively, associated with dreaming (*1*). Unlike the other two states, REM sleep is characterized by the combination of tonic components [such as loss of muscle tone and low voltage, fast, desynchronized brain waves as seen on the electroencephalogram (EEG)] and phasic events [such as rapid eye movements, variability of autonomic functions, penile erections, and, in animals, monophasic sharp waves occurring in the pons, lateral geniculate body, and occipital cortex (ponto-geniculo-occipital, or PGO, spikes)].

Differing biochemical control mechanisms of the three states of consciousness have been proposed (*2*). Although most research has focused on the role of biogenic amines (particularly serotonin and norepinephrine), laboratory studies of animals also suggest cholinergic modulation of REM sleep. The evidence is that (i) during REM sleep, increased amounts of acetylcholine are released from the neocortex (*3*) and caudate nucleus (*4*); (ii) REM sleep is induced by application of carbachol (a cholinomimetic drug) and physostigmine to the brainstem area in normal cats (*5*) or cats with pontine sections (*2*); (iii) REM sleep is inhibited, and EEG synchronization is induced by administration of hemicholinium-3, an inhibitor of acetylcholine synthesis (*6*), and by anticholinergic agents (*7*); and (iv) both tonic and phasic components of REM sleep are modified by cholinergic influences. Behavior resembling catalepsy has been induced by focal injection of cholinergic agonists in the brain (*8*). Bursts of REM and PGO spikes, as well as phasic suppression of spinal reflexes, have also been reported with administration of physostigmine to decerebrate cats (*9*).

In spite of these animal studies, few attempts have been made to demonstrate cholinergic mechanisms in human sleep. The anticholinergic agents scopolamine (*10*) and atropine (*11*) delay the onset and reduce the amount of REM sleep. Anticholinesterases, which enhance cholinergic activity, produce subjective reports of excessive dreaming and nightmares (*12*). Moreover, EEG sleep records from per-

sons previously exposed accidentally to organophosphates (irreversible anticholinesterases) indicate that REM periods are longer or occur sooner than is normal (*13*).

We evaluated the effects of physostigmine salicylate given intravenously during discrete stages of sleep in man. Physostigmine is a reversible, short-lived anticholinesterase agent that crosses the blood brain barrier, thereby increasing central as well as peripheral cholinergic actions (*14*). Subjects were paid, normal volunteers (five male, two female) between 20 and 29 years old (mean = 23.8). After one night of adaptation, subjects received one infusion per night, which was given either during the first non-REM sleep period (35 to 40 minutes from the onset of stage 2 sleep) or at the onset of the first REM period. The infusions were physostigmine (0.5 mg in 10 ml of 0.9*N* saline) or placebo (10 ml of 0.9*N* saline); the order of physostigmine and placebo nights was random. An intravenous catheter was inserted into a forearm vein and connected to a thin 10-foot (1 foot = 0.3 meter) polyethylene tube that extended out of the subjects' rooms. A very slow 0.9*N* saline drip was maintained to ensure patency, and, at appropriate times, infusions were given over a 4-minute period from outside the room. In order to block peripheral cholinergic effects induced by physostigmine (such as increased secretions, vomiting, and bronchoconstriction) subjects were given intramuscular injections of methscopolamine bromide (0.5 mg) each night when they retired (about 11 p.m.). Methscopolamine is a peripheral anticholinergic agent that does not cross the blood brain barrier (*14*) and has no effect on human sleep (*10*). The EEG, electrooculogram, and electromyogram were monitored. Other details of intravenous infusion and human sleep study techniques have been described (*15*). All records were scored according to standard criteria (*16*) by a single investigator who did not know the drug status of the subjects. Sleep stages during drug nights were compared to those during placebo nights by a paired *t*-test.

After infusions during the first non-REM sleep period, REM sleep appeared significantly sooner with physostigmine (9.8 ± 2.6 minutes) than with placebo (59.0 ± 16.7; $P < .02$, two-tailed *t*-test) (Fig. 1). The REM latency (elapsed time from onset of stage 2 to first REM period) was significantly shorter with physostigmine (48.1 ± 3.0) than with placebo (94.5 ± 16.9; $P < .05$, two-tailed *t*-test).

There was no significant difference in either the length of the first REM period or the amount of eye movement activity during REM sleep (REM density, scored on a scale of 0 to 8 per minute of REM) when REM sleep induced by physostigmine was compared with that occurring with placebo (*17*).

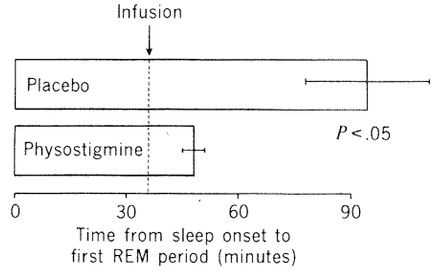

Fig. 1. Effect of physostigmine infusion during non-REM sleep on REM latency. Physostigmine (0.5 mg) or placebo was administered about 35 to 40 minutes after the onset of stage 2 sleep; mean ± standard error of the mean.

When infusions were given at the onset of REM, subjects awoke significantly more often (*18*) with physostigmine (five of seven infusions) than with placebo (none of seven) ($\chi^2 = 4.98$, d.f. = 1, $P < .05$). Moreover, physostigmine woke subjects more frequently when infused during REM (five of seven) than during non-REM sleep (none of seven) ($\chi^2 = 4.98$, d.f. = 1, $P < .05$). These waking periods lasted from 10 to 50 minutes (mean = 26.5).

Compared with the placebo, physostigmine did not alter total REM sleep, non-REM sleep, REM density, or delta sleep (stages 3 and 4) (*19*). When a higher dose of physostigmine (1.0 mg) was infused during non-REM sleep, the subjects woke for long periods in two out of four infusions; for the two subjects who continued sleeping, REM sleep appeared about 13 minutes after the infusion. When the 1.0-mg dose was infused at the onset of REM sleep, subjects awoke on three out of three occasions.

Among subjects who woke after physostigmine infusions, two reported frightening nightmares; each had received a dose of 1 mg, in one subject during REM and in the other during non-REM sleep. A third subject who received a dose of 0.5 mg during REM sleep reported "a golden thickness and fluidity throughout my body." Placebo infusions in the same subjects did not produce any of these experiences.

In order to determine whether REM sleep induced by physostigmine was accompanied by dreaming, we gave physostigmine (0.5 mg) infusions to two additional normal subjects during non-REM sleep. About 7 minutes after REM was induced, we woke the subjects and asked each to describe everything that had gone through his mind. Both subjects reported having had dreams; one subject, for example, dreamed of playing his guitar in the company of his grandfather and a girl.

These data suggest that the onset of REM sleep is induced by cholinergic mechanisms in man; they confirm similar animal studies. Moreover, our data are consistent with reports that cholinergic mechanisms are involved in cortical activation and arousal (*20*). High doses of physostigmine (1.0 mg) tended to wake subjects. A lower dose (0.5 mg) induced REM when infused during non-REM sleep and arousal when infused at the onset of REM. This is consistent with the hypothesis that non-REM, REM, and "awake" states form a continuum of increasing levels of arousal (*21*). Cholinergic mechanisms may modulate cortical arousal and shift the level of consciousness from non-REM to REM sleep and from REM sleep to waking states. (We cannot, however, determine if this arousing effect of physostigmine represents a specific cholinergic activation mechanism or a nonspecific psychological or physiological change.) Also, cholinergic neurons may modulate and be modulated by neuronal systems through other neurotransmitters, such as norepinephrine (*22*).

It is of clinical interest that short REM latencies have been described in depression (*23*) and in narcolepsy (*24*). The REM-inducing effect of increasing functional acetylcholine in the human brain may be relevant to the pathophysiology of these disorders (*25*).

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18. A waking is scored if subject wakes within 15 minutes of infusion and remains awake for more than 3 minutes.
19. Total REM time: placebo, 93.8 ± 7.9 minutes; physostigmine, 93.4 ± 7.8 minutes. REM density: placebo, 1.57 ± 0.18; physostigmine, 1.65 ± 0.38. Total non-REM time: placebo, 280.1 ± 31.2; physostigmine, 285.2 ± 24.0. Total delta sleep: placebo, 26.5 ± 9.6; physostigmine, 27.5 ± 3.5.
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β-D-Galactosidase Activity in Single Yeast Cells During Cell Cycle of *Saccharomyces lactis*

Abstract. *Single* Saccharomyces lactis *cells taken from a random population were assayed for β-D-galactosidase activity under a microscope equipped for fluorogenic measurements. The cells were also photographed, and enzymatic activity was correlated to the size of cell buds. A periodic pattern of enzyme synthesis was found during the cell cycle.*

Although most enzymes are synthesized discontinuously at a particular stage of the yeast cell cycle, examples of continuous synthesis have been reported (*1, 2*). Previous methods that made it possible to observe and investigate these patterns of changes in enzymes levels are based either on the use of synchronously growing cultures (*2*), or on gradient centrifugation of a random population of yeast (*3*). These methods provide only a close approximation of events which occur at the single cell level. The present work is concerned with the measurement of the activity of β-D-galactosidase (E.C. 3.2.1.23) in single yeast cells of *Saccharomyces (kluyveromyces) lactis* taken from a random population. The enzymatic activity of individual cells was determined under a microscope equipped for fluorogenic assays (*4*). Photographs of the cells after measurements of enzymatic activity were used to obtain an index of the size of the cell buds and, from this information, to establish the stage of the growth cycle of individual yeast cells. Thus, it was possible to examine directly the correlation between levels of β-D-galactosidase in a given cell and stage of the growth cycle.

Cells of *S. lactis*, strain Y-14 (*5*), were grown aerobically at 25°C in 0.2 percent succinate synthetic medium (*3*) supplemented with 2 percent lactose and harvested during exponential growth. Cells were washed with 0.025*M* phosphate buffer (*p*H 7.2) containing $10^{-3}M$ $MnCl_2$ and then resuspended in the same buffer to a density of about 10^9 cells per milliliter.

The assay of β-D-galactosidase in single cells was performed as follows. The cell suspension was treated with 0.2 volume of isoamyl alcohol at 0°C for 10 minutes with occasional shaking. The suspension was then diluted 1 : 10 with 0.02*M* phosphate buffer, *p*H 7.2, and 0.05 ml was mixed with 0.05 ml of $4 \times 10^{-4}M$ fluorescein-di-(β-D-galactopyranoside) (purchased from Nortok Associates, Lexington, Massachusetts). The mixture then was dispersed into a microchamber filled with silicone oil [SF-96 (200) from General Electric] as previously described (*4*). The time of mixing with the substrate was taken as the zero time for the enzymatic reaction. Immediately after dispersion, the microchamber was centrifuged to increase the rate of settling of the microdroplets to the bottom of the slide. The microchamber was then placed on the stage of microscope equipped for quantitative measurements of single molecules of β-D-galactosidase by fluorogenic methods (*4*). Microdroplets smaller than 100 μm containing a single yeast cell were selected for assay. The course of the enzymatic reaction in a given microdroplet was followed by recording the fluorescence every 60 seconds for at least 5 minutes. The fluorescence of droplets without cells was used as blank correction.

At the end of the assay, two photographs were taken (Kodak Tri-X 35-mm film, 15-second exposure). One of the photographs was focused on the microdroplet and served to determine the diameter of the droplet. The other photograph, focused on the yeast cells, was used to measure the size of the bud. For this measurement, the photographs were enlarged tenfold and the cell images from the enlargements were

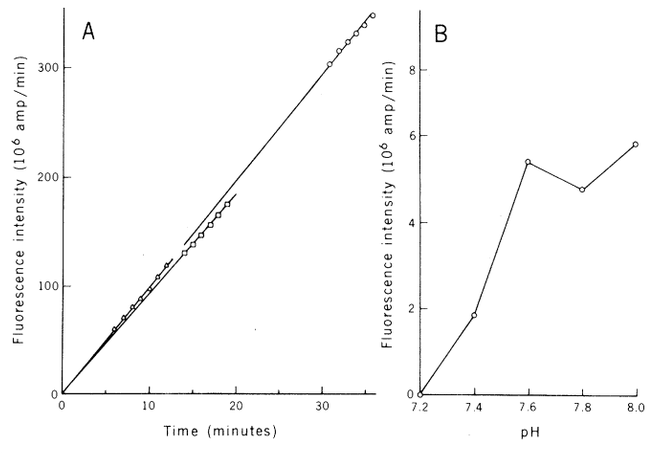

Fig. 1. (A) β-D-Galactosidase activity of single *S. lactis* cells in three different drops on the same slide. the activity of the budded cell in each drop was measured consecutively. A Zeiss Universal microscope equipped with a III-Z condenser was used. For photometry, the image from the objective was diverted to the monocular tube of the microscope where an Aminco photomultiplier microphotometer equipped with shutter and Wratten No. 12 filter, to exclude exciting light, was installed. The area measured by the photomultiplier (RCA 1P21) was reduced to about 100-μm diameter by placing a fixed pupil of 2-mm diameter near the point where the image of the object was focused. The amount of fluorescence was measured as the amperage recorded by the photomultiplier. (B) Effect of *p*H on β-D-galactosidase activity in single unbudded cells. The fluorescence of fluorescein increased 10 percent from *p*H 7 to 8.