numeraries to associate with any particular chromosome. In this case, then, the apparent connection is probably an artifact. These meiotic chromosomes were found to match well with those of Japanese of the 48-chromosome type. Evidently the same supernumerary and 23 regular pairs are present in these Whites and Japanese. The finding of 46- and 48-chromosome individuals among Whites leaves little doubt that men with 47 chromosomes exist also in this human group. Thus, presumably the same three chromosomal constitutions exist in the two ethnic groups.

The present study has shown that the human supernumerary chromosome has the following characteristics: (i) It is a metacentric chromosome with the centromere located near its middle; (ii) its size is approximately that of the Y-chromosome; (iii) it never pairs or associates with any other chromosome except its own homolog; (iv) at metaphase I, two supernumerary chromosomes are conjoined more frequently at one arm than at two arms. In either case the attachment of the arms is always completely terminal, and the attachment region is sometimes strikingly attenuated. A similar manner of pairing is observed in the X-Y pair but not in other autosomes.

Since 1956, 15 individuals have been reported to have 46 chromosomes, including the recent one studied by Bender (*9*) and the seven described here. The White individual with 48 chromosomes in our sample is the only one with this number established since 1956. The ratio of the frequency of the 46- and 48-chromosome types in the present Japanese sample is 9:5 (a previously reported ratio, 4:16, was not based on a random sample). The numbers of Whites and Japanese studied so far are too small to provide the basis of reliable estimates of the frequencies of the three karyotypes in the two ethnic groups.

MASUO KODANI

Department of Urology and Radiation Research Laboratory, State University of Iowa, Iowa City

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Continuous Measurement of the Depth of Sleep

Electroencephalographic records characterizing levels of natural sleep in human beings (*1, 2*) appear to be marked by a diminution of the number of brain-wave peaks during the passage from wakefulness to deepest sleep. The present report deals with the validation of this observation.

Bickford (*3*) has used the integrated energy of the electroencephalographic output to control the level of anesthesia, the energy decreasing with the depth of narcosis. Forbes *et al.* (*4*) have measured the depth of barbiturate narcosis, in animals already lightly anesthetized, by counting manually the number of brain waves that exceed one-third the maximum amplitude in successive 40-second intervals. This brain-wave count appeared to measure the absolute depth of narcosis more reliably than did the Bickford energy record.

The measurement described in the present report differs from that of Forbes *et al.* in employing nonnarcotized human beings; in producing a continuous 12 in./hr record of the electroencephalographic frequency during sleep; and in obviating the need for knowledge of the eventual maximum amplitude of the brain-wave potentials. The use of the energy-output method would probably fail during natural sleep because such electroencephalographic records do not reveal a proportionality between energy output and depth of sleep.

The brain-wave potentials during sleep are obtained from two needle electrodes, placed ipsilaterally over the occipital and frontal areas of the scalp, with a third silver disc electrode attached to the ear lobe on the same side and grounded. This electrode placement gives maximum alpha and delta waves.

The potentials were amplified by a Type 122 Tektronix low-level preamplifier, the low impedance output being fed into an electrocardiograph, whose "voltage gain" was set so that the maximum amplitude of the alpha rhythm in the waking state was 7 volts. This amplified signal then passed into a Schmidt trigger circuit set to pass only positive-going pulses greater than 2 volts. The square-wave output of the Schmidt circuit actuated an electronic counter and rate transducer (*5*) to produce, finally, a record of frequency versus time on a Leeds and Northrup Speedomax recorder. The frequency scale was set to cover the range from 3 to 16 cycles per second on the 10 in. recorder paper; the relationship between millivolts and the reciprocal of frequency is linear. The electronic counter keyed a relay every 32 counts; the transducer thus produced a rate based on the mean of successive trains of 32 positive-going waves of greater than 2 volts amplitude.

If the amplified output of the alpha rhythm is set lower than 7 volts or if the trigger threshold is set higher than 2 volts, the so-called transition or "B" stage of sleep (*2*), a relatively fast rhythm of low amplitude, will appear to be of low frequency and will consequently be interpreted as a deeper stage of sleep. There is no reason to believe that marked differences will occur if this ratio is varied between 3:1 and 4:1.

The use of the electroencephalographic frequency as a continuous measure of the depth of sleep was validated as follows: At intervals of 8 to 10 minutes throughout the night a 30-second written record of the electroencephalographic potentials was obtained on the strip chart of the electrocardiograph; the time at which this record was taken was signal-marked on the continuous electroencephalographic frequency recording. This record was then cut in half, and each 15-second strip was randomly numbered; at the end of the night, 100 such strips had been accumulated. These strips were then shuffled so that their subsequent order would differ from the order in which they had been obtained. Three judges then independently evaluated each record on a scale of 4, zero being the waking state and 1, 2, and 3 being light, moderate, and deep sleep, respectively. Prior to classifying the records, each judge was shown sample sleep records from the electroencephalographic literature. After 24 hours or more had elapsed the judges again evaluated the same records after they had been shuffled into a different order. Thus, each 30-second record received six scores on each of two occasions. The phi correlation coefficient was computed for the means of the first and second judgments and in three different subjects was found to be 0.88, 0.88 and 1.00, respectively; in the first two cases this particular correlation coefficient probably indicated too low a degree of reliability of the judgments. The phi correlation coefficient was then computed as a validity coefficient for the correspondence between the mean of the 12 judgments of each record and the corresponding electroencephalographic frequency. For the two males and one female of this study, this coefficient was 0.76, 0.75, and 0.91, respectively. Although highly significant, this coefficient probably indicates too low a degree of reliability.

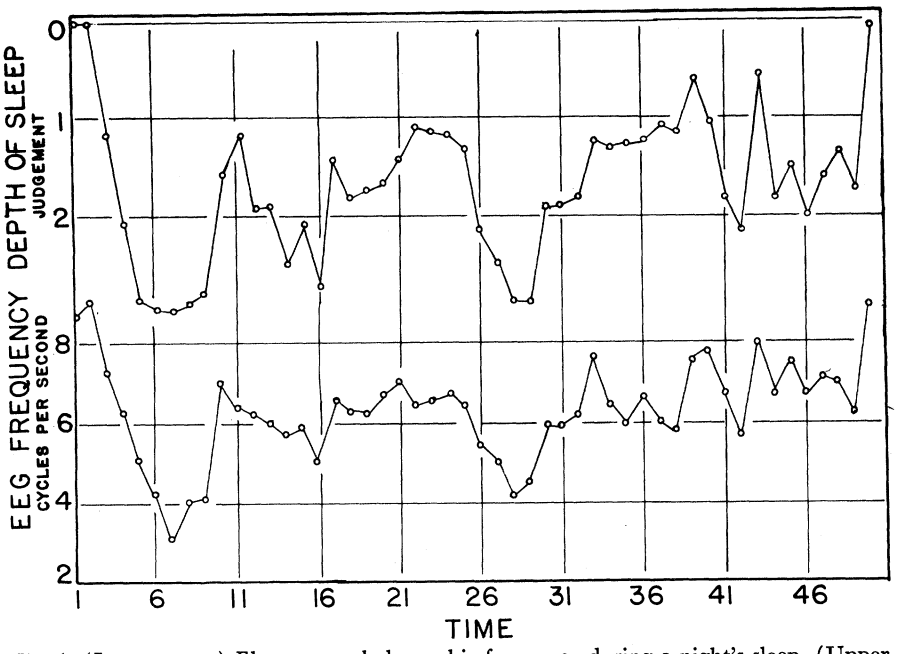

Fig. 1. (Lower curve) Electroencephalographic frequency during a night's sleep. (Upper curve) Judgments of depth of sleep made from the electroencephalogram, at corresponding times.

Figure 1 represents the depth of sleep of one subject during the night, as determined both by measurement of the electroencephalographic frequency and simultaneous scoring of the electroencephalogram. For this subject the phi correlation coefficients were 0.88 and 0.76.

If one assumes that there is basis for the belief that the electroencephalographic frequency represents the continuum of sleep depth, the use of a limited number of categories (observers' judgments) for correlation does not offer the best evidence for this assumption. It also appears somewhat circular to validate the electroencephalographic frequency with a measure from the same class. In the past many observers have noted, though not with the greatest rigor, that the electroencephalogram is correlated with the strength of stimulus necessary for arousal. The electroencephalographic frequency as measured here offers the possibility of making such a test by correlating frequency with strength of arousal stimulus, because such stimuli may be divided objectively into more categories than can observers' judgments of the electroencephalogram. Since the meaning of various levels of sleep depth is commonly believed to be associated with the reactivity of the nervous system, a correlation between the two continua would infuse both measures with additional meaning. The meaning would depend on the nature of the relationship and the definition of "depth of sleep."

In three other subjects preliminary observations were made as to the correspondence between minimum auditory awakening stimulus and electroencephalographic frequency. Although these observations are too few in number to have any substantive value, not one of them is at variance with the basic postulate: the lower the electroencephalographic frequency the louder the sound stimulus necessary for arousal.

Lindsley's (*6*) ingenious use of operant behavior may be related to the common concept of sleep depth; it suffers from the disadvantage of imposing continuously upon the sleeper either a sound stimulus or the necessity of his pressing a switch. The importance of using the electroencephalographic record to evaluate the operant response has been recognized by Lindsley.

The present method of continuous registration of the electroencephalographic frequency has been shown to represent with high reliability the time course of the depth of sleep through the night; additional evidence for this correlation should be sought by the use of another independent measure. Since the measurement may be carried out in the nonnarcotized individual, without the application of any external stimuli and with no discomfort to the sleeper, it offers many advantages to investigators in this field.

DAVID LESTER
Laboratory of Applied Biodynamics, Yale University, New Haven, Connecticut

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Effect of Chloramphenicol on Glucose Oxidation in Escherichia coli

When bacteriostatic concentrations of chloramphenicol are added to growing cultures of susceptible bacteria, protein synthesis and therefore reproduction are inhibited, while other cell processes continue at the same rate or at somewhat slower rates (*1*). Some specific reactions, however, such as indole synthesis in *Escherichia coli* (*2*) and oxidation of organic acids in *Pseudomonas fluorescens* (*3*), are also inhibited. One might expect inhibition by chloramphenicol to effect a shift in any metabolic reactions associated with protein synthesis and growth. Cohen (*4*) suggested that *E. coli* oxidizes glucose chiefly by the hexosemonophosphate shunt during growth and by the Embden-Meyerhof pathway during the "resting" state. The findings presented in this report show that inhibition by chloramphenicol does indeed alter the activity of a C-1 preferential pathway of glucose oxidation by growing cells of *E. coli*.

In all experiments *E. coli* was grown at 37°C in synthetic medium consisting of 5.4 g of KH_2PO_4, 1.2 g of $(NH_4)_2HPO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1.5 g of glucose, and glass-distilled water to make up 1 lit, at *p*H 7.1. The inoculum was grown in synthetic medium for two transfers before use and was added to growth flasks to give 3×10^8 cells per milliliter. Manometric and $C^{14}O_2$ determinations were made in double-sidearm Dixon-Keilin Warburg flasks. Total cell counts and substrate uptake during growth were measured in conventional double-sidearm Warburg flasks. Determinations were made for the 30-minute period before as well as after addition of chloramphenicol (final concentration, 60 μg/ml). Flasks for each manometric and isotopic determination were run in duplicate. Total cell counts were made with a Petroff-Hausser chamber and a bright high-contrast oil-immersion objective of a phase contrast microscope. Residual glucose was determined by the anthrone method (*5, 6*), ammonia nitrogen by the phenol hypochlorite test (*6*). The $C^{14}O_2$ collected was precipitated with a saturated $Ba(OH)_2$–10-percent $BaCl_2$ solution, plated on a porcelain disk, and counted with a windowless gas flow counter (*7*). The counts were corrected for self-absorption (*8*) and background. Aliquots