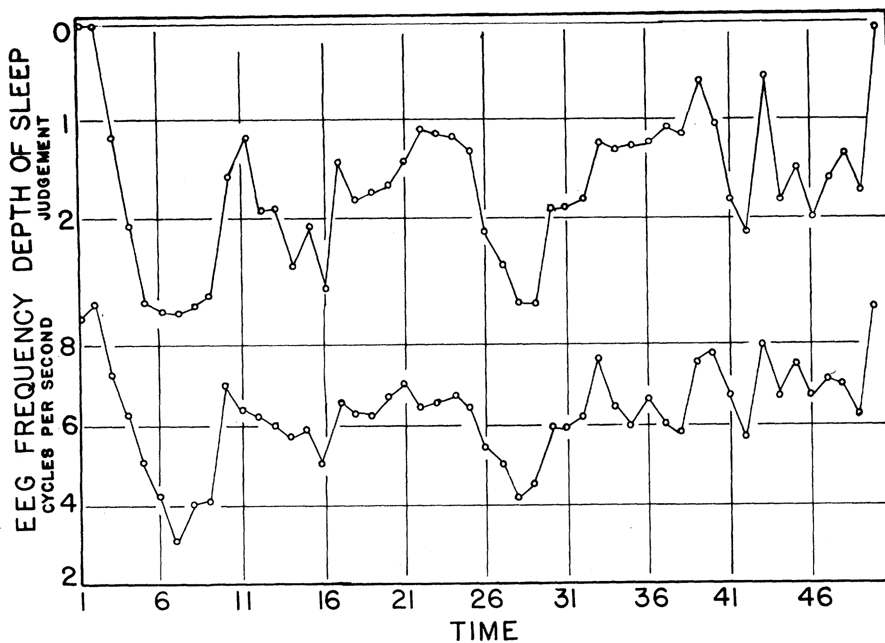

Fig. 1. (Lower curve) Electroencephalographic frequency during a night's sleep. (Upper curve) Judgments of depth of sleep made from the electroencephalogram, at corresponding times.

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.

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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

Table 1. Effect of chloramphenicol on the metabolism of growing *E. coli* during the first 30 minutes of treatment. Average deviation for glucose, O_2, or CO_2 = 4 percent.

Chloramphenicol (μg/ml)	Increase in total count (10^8 cells/ml)	NH_4-N uptake (μg/ml)	Glucose uptake (μmole)	Oxygen uptake (μmole)	Carbon-dioxide evolution (μmole)	C-1/C-6 ratio
0	4.7 to 6.0	13	2.3	5.4	6.0	12.2 ± 0.2
60	4.7 to 5.0	2	1.5	3.2	3.7	5.1 ± 0.6

of glucose-C^{14} substrates were combusted to $C^{14}O_2$ by the persulfate method (*9*). The activity of a C-1 preferential pathway was measured (*10*) by the ratio: (percentage of radiochemical yield of $C^{14}O_2$ from glucose-1-C^{14})/(percentage of radiochemical yield of $C^{14}O_2$ from glucose-6-C^{14}).

In preliminary experiments, data showed that chloramphenicol-treated cells took up less glucose than cells in control cultures and oxidized less of the glucose by a C-1 preferential pathway; the values of the ratio C-1/C-6 were 10 to 16 for the controls and about 5 for the treated cultures. In Table 1 are presented results of an experiment in which nitrogen assimilation was almost completely inhibited and glucose assimilation and oxidation continued during treatment of cultures with the antibiotic. The inhibited cultures used less glucose (65 percent as much as the controls) but assimilated the same proportion, about 60 percent. However, there appeared to be a shift in the proportion of glucose oxidized by way of a C-1 preferential pathway, as evidenced by the C-1/C-6 ratios. A large percentage of the glucose oxidized still was degraded by the C-1 selective mechanism. In the first 30 minutes of treatment, synthesis of deoxyribonucleic acid was slightly affected, while the synthesis of ribonucleic acid was 55 percent of that of the controls (77 percent on a "per cell" basis).

Further experiments are necessary to establish the sequence of events here. However, previous experiments (*11*) have shown that when limiting amounts of nitrogen halt the growth of cultures of *E. coli*, a similar shift of a C-1 preferential pathway in glucose metabolism occurs. In that case, too, assimilation of glucose continues, and so does synthesis of nucleic acids for a time (*12*). Possibly the interference with the assimilation of nitrogen by chloramphenicol indirectly affects the activity of the C-1 preferential pathway of glucose oxidation.

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Quantitative Histochemistry of the Nephron

The broad purposes of our investigations are to determine quantitatively the activity of enzymes and the concentrations of various substances in the anatomic units of the human kidney in health and disease. This report describes the technique used to identify and dissect out various microscopic portions of the nephron. The accuracy of the method was checked by determining the distribution and concentration of alkaline phosphatase in different parts of the nephron, because histologic staining techniques have demonstrated clear-cut differences in the concentration of this enzyme in proximal and distal convoluted tubules.

Renal tissue from animals was obtained as rapidly as possible after death. Human samples were obtained at autopsy or by percutaneous renal biopsy during life (*1*). The ultramicrotechniques developed by Lowry (*2*) for analysis of brain tissue were adapted for analysis of renal tissue. Immediately on removal from the body, the fragment of kidney, weighing a few milligrams, was placed on a layer of tragacanth jelly covering the surface of a microtome sample holder and was frozen rapidly in liquid nitrogen. Serial sections, 16 μ thick, were cut by a microtome in a cryostat at −20°C; these were placed seriatim in the numbered wells of a section holder, which was inserted into a lyophilizing tube. The sections in the lyophilizing tube were frozen-dried in the cryostat at −20°C, and the evacuated tube was stored, until needed, in a freezer at −35°C.

Alternate sections of frozen-dried tissue were stained by means of the periodic-acid-Schiff technique, counterstained with hematoxylin, and studied under a microscope equipped with a Galileo projection viewer. The whole section was mapped out, blood vessels and glomeruli serving as landmarks. The convoluted tubules were recognized by their relative proximity to the glomerulus. The proximal convoluted tubules were identified by the presence of a brush border, by the small number of nuclei (4 to 6) in cross section, and by their relatively tall cells. The distal convoluted tubules were distinguished from them by their intimate relationship to the macula densa, by the absence of a brush border, by the greater number of nuclei (8 to 10)

Table 1. Distribution of alkaline phosphatase in the kidney, expressed in moles of *p*-nitrophenylphosphate split per kilogram (dry weight) per hour, with standard deviation and, in parentheses, the number of fragments analyzed.

Structure	Tissue sample					
	Dog		Rat		Man	
	No. 1	No. 2	No. 1	No. 2	No. 1*	No. 2†
Glomerulus	1.9 ± 0.3 (8)‡	1.8 ± 0.6 (5)§	6.6 ± 2.9 (5)	4.3 ± 2.7 (6)	0.4 ± 0.2 (4)	2.1 ± 1.7 (5)
Proximal convoluted tubules	10.4 ± 4.3 (13)	10.3 ± 3.5 (6)	16.9 ± 9.5 (7)	17.4 ± 6.5 (11)	6.8 ± 1.8 (6)	6.7 ± 1.1 (9)
Distal convoluted tubules	1.6 ± 1.5 (5)	0.3 ± 0.2 (5)	0.8 ± 0.4 (6)		3.2 ± 1.6 (5)	3.2 ± 0.9 (8)
Medullary ray	0.4 ± 0.4 (8)	0.3 ± 0.4 (5)	0.6 ± 0.5 (4)	0.4 ± 0.8 (5)		
Medullary tubules			1.0 ± 1.0 (6)			
Papillary tubules:				0.5 ± 0.6 (6)		1.2 ± 1.0 (11)
Base		0.3 ± 0.0 (2)	0.4 ± 0.2 (4)			
Apex			1.5 ± 0.7 (3)	0.9 ± 0.9 (6)		2.2 ± 1.3 (3)
Vessels	0.6 ± 0.1 (4)‖	0.1 ± 0.1 (4)‖		15. 5 ± 4.4 (6)		

* Biopsy from an adult male. † Autopsy specimen obtained 6 hr after death, from an adult female. ‡ Bowman capsule: ± 0.2 (1). § Glomerular vessels: 0.3 ± 0.5 (2). ‖ Vessel wall plus fibrous area: 0.6 ± 0.1 (4); vessel wall only: 0.1 ± 0.1 (14).