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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References and Notes

1. E. F. Gale and J. P. Folkes, *Biochem. J.* 53, 493 (1953); C. L. Wisseman *et al., J. Bacteriol.* 67, 662 (1954).
2. F. Gibson *et al., J. Gen. Microbiol.* 15, 446 (1956).
3. D. J. Kushner, *Arch. Biochem. Biophys.* 58, 332 (1955).
4. S. S. Cohen in *Phosphorus Metabolism*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1951), vol. 1, pp. 148–58.
5. J. A. Loewus, *Anal. Chem.* 24, 219 (1952).
6. H. Niss, Ph.D. thesis, Purdue University (1958).
7. Nuclear Chicago, model 192.
8. G. K. Schweitzer and B. R. Stein, *Nucleonics* 7, 65 (1950).
9. M. Calvin *et al., Isotopic Carbon* (Wiley, New York, 1949).
10. H. Beevers and M. Gibbs, *Nature* 173, 640 (1954).
11. S. H. G. Allen, Jr., Ph.D. thesis, Purdue Univ. (1957).
12. W. R. Lockhart and D. M. Powelson, *J. Cellular Comp. Physiol.* 44, 177 (1954).

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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).

in cross section, and by their relatively low cells. Henle's loop and the collecting tubules were recognized in the medullary ray and in the medulla; in the papilla, collecting tubules only were found. When the various parts of the kidney had been identified in the stained section, they were dissected out of the adjacent unstained section with microscalpels under a dissection microscope (magnification ×40). Separate dissection of Henle's loops and collecting tubules was rarely possible because of their intimate anatomical relationship.

The dissected specimens were weighed on a quartz fiber fish-pole balance (sensitivity 0.4 mμg; useful range 10 to 100 mμg) (*2*). A manipulator made from a microscope mechanical stage was used to load and unload the balance pan. A horizontal microscope fitted with a vertical fine adjustment was used to read the displacement of the pan. Under the dissection microscope, each of the weighed specimens was transferred to the bottom of micro test tubes (inside diameter, 3 mm) by a fine glass needle. Both dissection and weighing were done in a room maintained at low humidity and relatively constant temperature.

The specimens were assayed to determine alkaline phosphatase activity by adding 3 μl of substrate reagent [0.5*M* 2-amino-2-methylpropanol-1 (*p*H 10.0), 8 mmole/lit of *p*-nitrophenylphosphate, 2 mmole/lit of 1*M* $MgCl_2$, and 0.05-percent bovine serum albumin (*3*)]. After 1 hour of incubation at 37°C, the reaction was stopped and color was developed by adding 50 μl of 0.1*N* NaOH. The optical density of the solution was read in Lowry-Bessey microcuvettes (*4*) at 410 mμ in the Beckman DU spectrophotometer, and the results were expressed in moles of substrate split per kilogram (dry weight) per hour.

Typical data for individual kidneys of dog, rat, and man are shown in Table 1. The results within each species were consistent, although somewhat different distributions were found in the tubules of each species. The experimental reproducibility for renal homogenates, expressed as standard deviation, was found to be ±0.8 moles split per kilogram (dry weight) per hour. The standard deviation for individually assayed proximal convoluted tubules in each kidney was much greater than this, indicating that the alkaline phosphatase activity of these tubules varied considerably between individual nephrons.

The results indicate accurate identification and dissection of the various parts of the nephron, particularly the proximal and distal convoluted tubules. For example, the values obtained for dog kidney are comparable to those of McCann (*5*), who used vital staining with trypan blue to differentiate proximal and distal convoluted tubules in the dog. Unfortunately this and other dyes which localize in the proximal tubules are too toxic for use in man, while fluorescence microscopy, phase microscopy, and polarization microscopy did not allow a distinction between the two types of convoluted tubules at the low magnifications which must be used with the dissection microscope. The technique presented here (*6*) has the advantages of being generally applicable, of allowing accurate identification and dissection of the desired structures, and of providing a permanent record in the form of the stained sections and maps.

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References and Notes

1. R. M. Kark and R. C. Muehrcke, *Lancet* 1, 1047 (1954).
2. O. H. Lowry, *J. Histochem. and Cytochem.* 1, 420 (1953).
3. The p-nitrophenylphosphate used in this study was obtained from The Sigma Chemical Co.; the bovine serum albumin was obtained from Armour & Co.
4. O. H. Lowry and O. A. Bessey, *J. Biol. Chem.* 163, 633 (1946).
5. W. P. McCann, *Am. J. Physiol.* 185, 372 (1956).
6. We are greatly indebted to Dr. Oliver H. Lowry for his help, especially for arranging for one of us (S.L.B.) to work in his department and learn some of the techniques employed in this investigation. The technical assistance of Miss Alta D. Tsoodle is gratefully acknowledged. This study was supported by contract No. DA-49-007-MD-637, Research and Development Division, Surgeon General's Office, U.S. Department of the Army.

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Preliminary Note on Kimzeyite, a New Zirconium Garnet

In 1953, during geological study of the Magnet Cove, Arkansas, carbonatite, R. L. Erickson and L. V. Blade, geologists of the U.S. Geological Survey, noted well-crystallized dark brown garnets about 5 mm in diameter in calcite rock in the Kimzey Calcite Quarry. With the garnets was a variety of other minerals, including monticellite, magnetite, perovskite, and apatite. The garnets themselves are shot through with those minerals, and in addition, with sharply euhedral, almost microscopic, crystals of anhydrite.

In 1953, A. T. Myers, spectrographer, U.S. Geological Survey, reported more than 10 percent zirconia, which led to further examination of the garnet. Myers' work was confirmed in 1954 by H. J. Rose (U.S. Geological Survey) who, on another sample, found zirconium and titanium in the X percent range, XO percent Fe and Ca, and O.X percent Mg and Al.

Table 1. Composition of kimzeyite. Looked for but not found: Ag, Au, Hg, Ru, Rh, Pd, Ce, Ir, Ge, Pb, As, Sb, Pt, Mo. W, Re, Bi, Zn, Cd, Te, In, Co, Ni, Ga, Cr, V, Y, La, Hf, Th, Ta, Be, Li, Na, K, B. Not tested for: P_2O_5, H_2O, F, S, and CO_2.

Element	Amt. %	Computed as oxide	
		Oxide	(%)
Si	10	SiO_2	21.4
Al	6	Al_2O_3	11.4
Ca	12	CaO	16.8
Fe	11.5	Fe_2O_3	16.45
Ti	3.5	TiO_2	5.8
Zr	15	ZrO_2	20.25
Nb	0.5	Nb_2O_5	0.72
Mg	0.3	MgO	0.5
Mn	0.1	MnO	0.13
Sn	0.07	SnO_2	0.09
Sc	0.06	Sc_2O_3	0.09
Cu, Ba, Sr	trace		
Total			94.

Finally, 35 mg of microscopically clean garnet was analyzed by Harry Bastron, spectrographer, U.S. Geological Survey. His analysis is shown in Table 1.

An x-ray diffraction pattern by F. A. Hildebrand (U.S. Geological Survey) showed "garnet group mineral; no perovskite detectable." The cell edge measured by J. M. Axelrod (U.S. Geological Survey) is $a_0 = 12.46$ A.

The garnet in thin section is isotropic, light brown, and has an index of refraction near 1.95. This is substantially higher than the index of refraction (1.895) of andradite, the pure calcium iron garnet, but is within the range of the indices of refraction of the calcium iron titanium garnets, schorlomite-ivaarite, which range up to 2.01.

Further work is in progress on this mineral, which is here named "kimzeyite." The Kimzey family has been actively associated with mineralogical developments in Magnet Cove for almost a century. Museums all over the world owe some of their best specimens of the remarkable Magnet Cove minerals to the intelligent zeal of the Kimzey family, notably William J. Kimzey, his son Joe Kimzey, former state geologist of Arkansas, and Lawton D. and John Kimzey (*1*).

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Note

1. Publication authorized by the director, U.S. Geological Survey.

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