layer chromatography on silver-impregnated silica gel (*14*) with a developing system of chloroform and 0.75 percent ethanol, the enzymatic product chromatographed with the authentic compound. In addition, mass spectroscopy of the trimethylsilyl derivative of the methyl ester was used to identify the β-hydroxydodecanoic acid.

Our results, along with those of Toomey and Wakil concerning the shorter chain saturated acyl-ACP's (*15*), indicate that this condensing enzyme can account for both saturated and unsaturated products of fatty acid biosynthesis in *E. coli*. The specificities indicated by the kinetic data are consistent with the reports of Lennarz *et al.* (*16*) and of Knivett and Cullen (*17*), who found that predominating among saturated fatty acids in whole cells is palmitate and among unsaturated fatty acids are palmitoleate and *cis*-vaccenate, or their cyclopropane derivatives. Chain termination in the yeast fatty acid synthetase is also strongly dependent on the efficiency of the rate-limiting reaction catalyzed by the condensing enzyme of that system (*18*).

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References and Notes

1. M. D. Greenspan, A. W. Alberts, P. R. Vagelos, *J. Biol. Chem.* **244**, 6477 (1969).
2. M. D. Greenspan, C. H. Birge, P. R. Vagelos, *Fed. Proc.* **28**, 537 (1969).
3. K. Hofmann, W. M. O'Leary, C. W. Yoho, T. Lin, *J. Biol. Chem.* **234**, 1672 (1959).
4. C. A. Brown and H. C. Brown, *J. Amer. Chem. Soc.* **84**, 2829 (1962).
5. F. Davidoff and E. D. Korn, *J. Biol. Chem.* **238**, 3199 (1963).
6. G. Wittig and U. Schoellkopf, *Org. Syn.* **40**, 66 (1960).
7. I. D. Bergelson and M. M. Shemyakin, *Angew. Chem. Int. Ed. Engl.* **3**, 250 (1964).
8. A. Bouers, C. Djerassi, R. R. Engle, *J. Org. Chem.* **21**, 1547 (1956).
9. C. H. Birge, D. F. Silbert, P. R. Vagelos, *Biochem. Biophys. Res. Commun.* **29**, 808 (1967).
10. H. van den Bosch and P. R. Vagelos, *Biochim. Biophys. Acta*, in press.
11. P. R. Vagelos, A. W. Alberts, P. W. Majerus, *Meth. Enzymol.* **14**, 60 (1969).
12. L. R. Kass, D. J. H. Brock, K. Bloch, *J. Biol. Chem.* **242**, 4418 (1967).
13. G. Scheuerbrandt and K. Bloch, *ibid.* **237**, 2064 (1962).
14. C. B. Barrett, M. S. S. Dallas, R. B. Padley, *J. Amer. Oil Chem. Soc.* **40**, 580 (1963).
15. R. E. Toomey and S. J. Wakil, *J. Biol. Chem.* **241**, 1159 (1966).
16. W. J. Lennarz, R. J. Light, K. Bloch, *Proc. Nat. Acad. Sci. U.S.* **48**, 840 (1962).
17. V. A. Knivett and J. Cullen, *Biochem. J.* **103**, 299 (1967).
18. M. Sumper, D. Oesterhelt, C. Riepertinger, F. Lynen, *Eur. J. Biochem.* **10**, 377 (1969).
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Electron Probe X-ray Microanalysis of a Normal Centriole

Abstract. *A scanning spectrometer with lithium fluoride, ammonium dihydrogen phosphate, and gypsum crystals was used to detect the x-ray spectrum of a normal centriole, the transmitted electron image of which was used to focus the exciting electron beam to the size, shape, and position of the centriole in a 700-angstrom section of intact tissue.*

We have used electron microprobe image-forming and x-ray microanalysis in the identification of the elements present in the centriole. Although huge by molecular standards, centrioles are so small as to be barely distinguishable at the limit of visible light microscopy, and then in only the thinnest of sections.

Their study requires high-resolution magnification ranging from ×2000 to ×10,000 of sections not much in excess of 1000 Å. Essentially nothing is known of the molecular species which massively aggregate into the nine triplet microtubules of the centriolar wall. Conventional analytical methods have not been applied to centrioles for the following reasons: (i) these organelles measure only 0.2 by 0.5 μm, (ii) they occur generally in the number of only two per cell, and (iii) no one has managed to isolate them from other cell constituents. Even from electron microscopy of random sections of intact tissue one cannot consistently determine whether one is observing an inside or an outside member of the pair or whether one is approaching or retreating from the intercentriolar angle. However, a procedure has been perfected which consistently yields information from consecutive sections of intact tissues prepared for ultramicrotomy and electron microscopy by conventional methods (*1*).

This procedure was used in the location and identification of the centrioles of the distal convoluted tubule of a normal guinea pig kidney. The sections were then analyzed in an electron microscope-microanalyzer (EMMA-4, AEI) (*2*). In preliminary nondispersive analyses and spectrometer scans we found that the sections contained very small amounts of the various elements. To obtain sufficient x-ray output, we used a 100-kv accelerating potential and maximum available beam current. Discrimination at the resultant level of output precluded nondispersive detection. Thus we had to use crystal spectrometer scans to locate all of the heavier elements which were present. To reduce the chance of specimen damage due to the high beam current, we used relatively fast scanning rates (crystal movements of 0.25 to 0.5 inch/min).

Figure 1 is a survey electron photomicrograph of the section selected for detailed analysis. The 12 cells comprising the intact tubular wall are overlapped in such a way that the section passes through various amounts of cytoplasm and through only three nuclei. All of the cytoplasmic organelles ranging in size up to 1.0 μm are mitochondria, with one exception. The outside centriole, in nearly perfect cross section, whose analysis is here reported, is distinguishable from the other organelles by its intense electron opacity, its circular form, and the constant curl-div configuration (*3*) of its microtubules.

The EMMA-4 electron beam was focused to precisely the size and shape of this particular centriole, simultaneously with its exact registration onto that target. The spectrometer was then scanned throughout its range of wavelengths, lithium fluoride, ammonium dihydrogen phosphate, and gypsum crystals being used in turn. The three traces obtained showed the presence of those elements listed in Table 1.

The ammonium dihydrogen phosphate crystal scanned at a rate of 0.5 inch/min with a chart speed of 1.0 inch/min yielding a horizontal calibration of 0.443 Å/inch (Fig. 2). Full-scale vertical deflection was made equal to a spectrometer output of 50 count/sec. Each of the elemental peaks encountered in these scans was confirmed by subsequent detailed examination. Without a change in its size, the microbeam was then moved to positions immediately adjacent to but not including

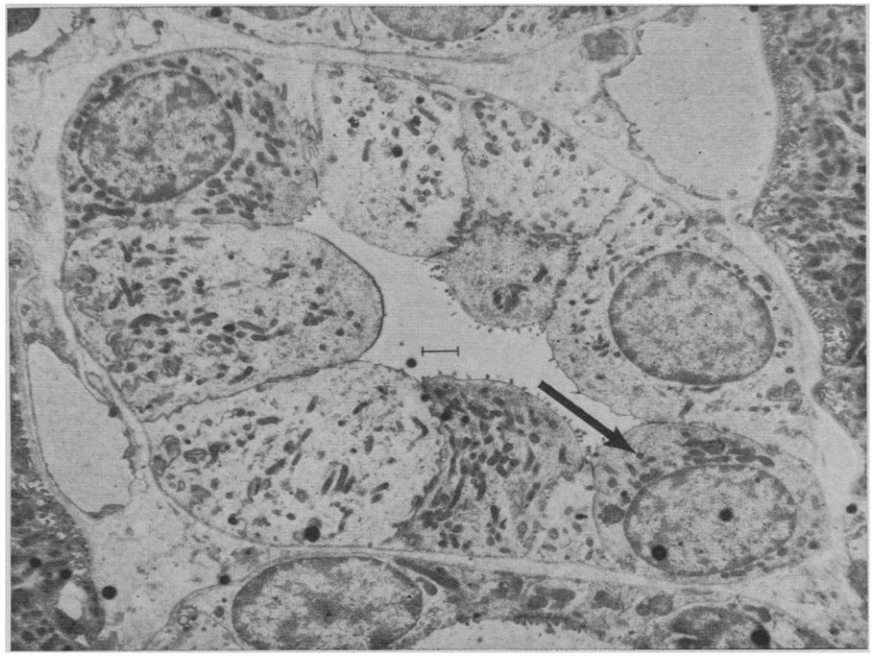

Fig. 1. Survey electron photomicrograph of distal renal tubule of a normal guinea pig (×2000). The arrow points to the only centriole visible in the section. It was this outside centriole which was subjected to detailed x-ray microanalysis. Scale is 1.0 μm.

Table 1. Elements detected by crystal spectrometer scans of a centriole. Trace I was made with a lithium fluoride crystal, trace II with an ammonium dihydrogen phosphate crystal, and trace III with a gypsum crystal.

Spectral line	Wavelength (Å)
Trace I	
Cu $K\beta$	1.392
Cu $K\alpha$	1.544
Cu $K\beta^2$	2.784
Cu $K\alpha^2$	3.088
Trace II	
K $K\alpha$	3.744
U $M\alpha$	3.910
Cl $K\alpha$	4.731
S $K\alpha$	5.374
P $K\alpha$, or Cu $K\alpha^4$	6.160
Os $M\beta$	6.267
Os $M\alpha$	6.490
Si $K\alpha$	7.127
Trace III	
Cu $K\alpha^4$	6.176
Si $K\alpha$	7.127
U $M\beta^2$	7.432
U $M\alpha^2$	7.811
Cu $L\beta$	13.053

any part of the centriole or its satellite appendage, and the scans were repeated. Only copper, phosphorus, osmium, and uranium were detected; the phosphorus count may really have been a fourth-order copper peak which could not be distinguished. The expected extraneous elements were copper, from the grid bars of the specimen support, and osmium and uranium, from the specimen preparation. Thus the elements native to the centriole are chlorine, potassium, silicon, and sulfur. The counting rates for sulfur and chlorine indicate the presence of approximately equal amounts of these two elements.

The detection of chlorine and potassium was not surprising. One might reasonably suspect that sulfhydryl groups or other organic forms of sulfur might be involved in the energy requirements of centriolar kinetics (*4*). Although silicon has recently been demonstrated in certain plant cells (*5*), its unexpected presence in an animal centriole is quite exciting. Further confirmation of this finding would be most desirable since it probably relates to the centriole-to-centriole interactions referred to in a previous publication (*1*). Perhaps even more significant is the observation that superconductivity was first demonstrated in compounds containing organic molecules, when these were sandwiched between the sulfides of niobium and tantalum (*6*).

The three crystals used in this experiment scanned the x-ray spectrum from 1 to 14 Å and thus did not include the soft x-rays diagnostic for elements with atomic numbers less than 12. However, with appropriate crystals, gratings, or ultrasensitive nondispersive detectors, such biologically significant elements as sodium, oxygen, nitrogen, and carbon can be detected. With full adaptation of electron microprobe theory and technology to the study of cellular ultrastructure, there exists the real promise of molecular as well as atomic characterization of cellular organelles.

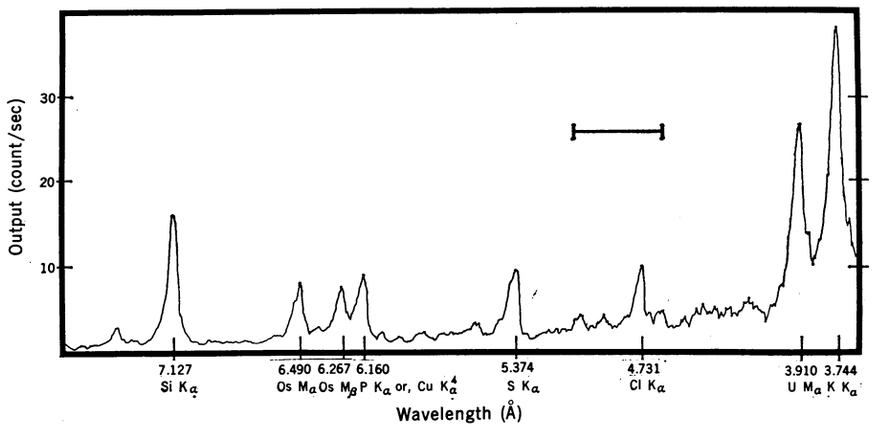

Fig. 2. Trace II of spectrometer scan with the ammonium dihydrogen phosphate crystal, the 100-kv beam of the probe having been focused to the exact size, shape, and position of the centriolar cross section in Fig. 1. Horizontal scale is 0.443 Å.

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References and Notes

1. P. W. Schafer, *Science* **164**, 1300 (1969).
2. C. J. Cooke and P. Duncumb, in *Fifth International Congress on X-ray Optics and Microanalysis*, G. Möllenstedt and K. H. Gaukler, Eds. (Springer-Verlag, Berlin, 1969), pp. 245–47.
3. J. Clerk Maxwell, *Proc. London Math. Soc.* **3**, 134 (1867).
4. T. W. James, *Ann. N.Y. Acad. Sci.* **90**, 550 (1960).
5. P. B. Kaufman, W. C. Bigelow, L. B. Petering, F. B. Drogosz, *Science* **166**, 1015 (1969).
6. F. R. Gamble, F. J. DiSalvo, R. A. Klemm, T. H. Geballe, *ibid.* **168**, 568 (1970).

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