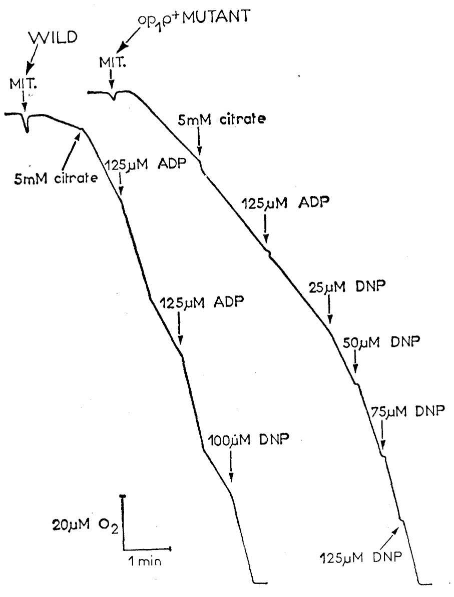

Fig. 3. Effect of adenosine diphosphate (ADP) and 2,4-dinitrophenol (DNP) on oxidation rates of mitochondria from a wild strain and $op_1\ \rho^+$ mutant. Respiration was measured polarographically at 30°C. The reaction mixture contained in 2.0 ml: 0.6*M* mannitol, 20 m*M* KCl, 1.5 m*M* EDTA, 10 m*M* tris maleate, and 10 m*M* potassium phosphate; final *p*H, 6.4. To start the experiments, 1.6 and 2.3 mg of wild-type and mutant mitochondrial protein respectively were added. The other additions are indicated in final concentrations.

fluoride-resistant like the wild-type adenosine triphosphatase.

Two important conclusions may be drawn from the study of the op_1 mutants. First, a single recessive gene mutation can lead to a deficiency that is common to all the phosphorylation steps in the respiratory chain. It can be inferred from it that either the same enzyme is involved in the energy transfer at all the phosphorylation sites or that the same structural component of mitochondria is required for the normal functioning of all sites. Second, this common component or enzyme is correlated with the high activity of the *p*H 9.5 oligomycin-sensitive adenosine triphosphatase.

It is conceivable that the diminution of adenosine triphosphatase activity is not a primary effect of the op_1 mutation but is secondary to other modifications in the organization of proteins or structures in mutant mitochondria. Another possibility would be a lesion in the last step of the mitochondrial phosphorylation system, that is, in the adenine nucleotide translocation through the membrane. All these possibilities will be considered in further studies of the mutant.

It is clear that an analysis of a single mutant is unable to solve the problem of the mechanism of oxidative phosphorylation. It seems to us, however, that by systematically applying the approach presented in this report one should be able to dissect in a stepwise manner the mechanisms of this highly integrated process. It may be hoped that the use of nonallelic complementing mutants could permit a reconstitution of functional machinery from its mutationally deficient parts. It may be of interest, in this respect, to mention that the mutants that have lost cytochromes $a + a_3$ and b (ρ^- cytoplasmic mutants) still maintain at least part of the oxidative phosphorylation reactions. This is indicated by the fact that their assimilation processes are still sensitive to dinitrophenol and azide (*8*). As shown in Table 3, ρ^- mutant has a normal activity of the *p*H 6.2 adenosine triphosphatase while the *p*H 9.5 component is very similar in its specific activity to that present in the op_1 mutant but is no longer sensitive to oligomycin.

Although yeast presents considerable advantages in the mutant approach to oxidative phosphorylation the same methodology could be tried with other eucaryotic microorganisms that possess mitochondria, or with bacteria.

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References and Notes

1. E. Racker, *Mechanisms in Bioenergetics* (Academic Press, New York, 1965); A. L. Lehninger, *The Mitochondrion* (Benjamin, New York, 1964).
2. B. Ephrussi, *Nucleo-Cytoplasmic Relations in Micro-organisms* (Clarendon Press, Oxford, 1952); P. P. Slonimski, *La Formation des Enzymes Respiratoires chez la Levure* (Masson, Paris, 1953).
3. F. Sherman and P. P. Slonimski, *Biochim. Biophys. Acta* **90**, 1 (1964).
4. J. C. Mounolou, H. Jakob, P. P. Slonimski, *Biochem. Biophys. Res. Commun.* **24**, 218 (1966).
5. P. Meuris, F. Lacroute, P. P. Slonimski, *Genetics* **56**, 149 (1967).
6. M. Ogur, R. St. John, S. Nagai, *Science* **125**, 928 (1957).
7. T. Bauchop and S. R. Elsden, *J. Gen. Microbiol.* **23**, 457 (1960).
8. L. Kováč and A. Istenesova, *Biochim. Biophys. Acta* **82**, 162 (1964); M. Greksak and L. Kováč, *Proceedings of the 2nd International Symposium on Yeast* (SAV Bratislava, 1967, in press).
9. E. A. Duell, S. Inoue, M. F. Utter, *J. Bacteriol.* **88**, 1762 (1964).
10. Valuable collaboration in different parts of this study of F. Charalampous, H. Bednarova, M. Greksak, E. Hrusovska, and K. Weissova is gratefully acknowledged. A detailed account of the work has been submitted for publication elsewhere. This work has been supported by a grant of D.G.R.S.T., Comité de Biologie Moléculaire.

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Lambda Transition in Liquid Sulfur

Abstract. *Density measurements on liquid sulfur near the transition at 160°C show a logarithmic singularity and discontinuous density. The transition shows the kinetic behavior of a zero-order reaction. The transition is expected to have a latent heat characteristic of a first-order phase transition but is of the cooperative nature associated with second-order transitions such as the lambda point in liquid helium.*

First-order phase transitions are characterized by a discontinuous first derivative of the Gibbs free energy, so that volumes and entropies are discontinuous. Second-order transitions have discontinuous second derivatives, so that heat capacities and expansion coefficients are discontinuous. Many second-order lambda-type transitions are known in magnetic substances, explained by the alignment of spins of electrons or nuclei. The classical example of a second-order transition occurs in liquid helium at 2.17°K. Spin ordering is not, however, an explanation in this case. The transition in liquid sulfur at 160°C is therefore of great interest, since it is the only recognized example (other than helium) among elementary liquids (*1*). The low-temperature μ form is structurally an eight-member ring, whereas the high-temperature λ form is a folded linear polymer of high molecular weight. Heat capacity measurements have shown the transition to have the form of a lambda point, unusual in the sense that the discontinuity falls on the low-temperature side of the transition (*2*). The transition is comprehensive. The viscosity changes by two orders of magnitude within a few degrees. The color changes from a yellow liquid to the dark-brown polymer. Surface-tension measurements show a

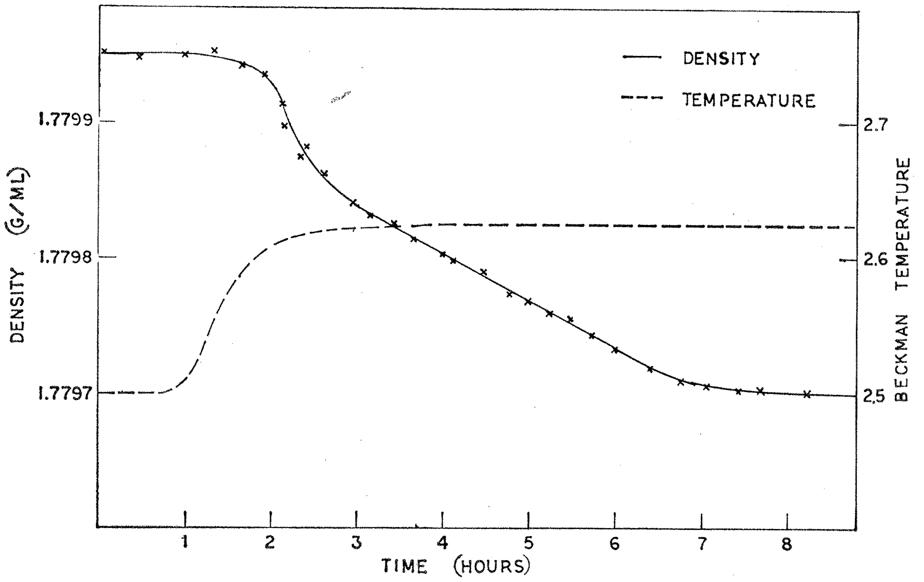

Fig. 1 (top). Density versus time after a sudden temperature change for the temperature at very nearly the transition temperature.

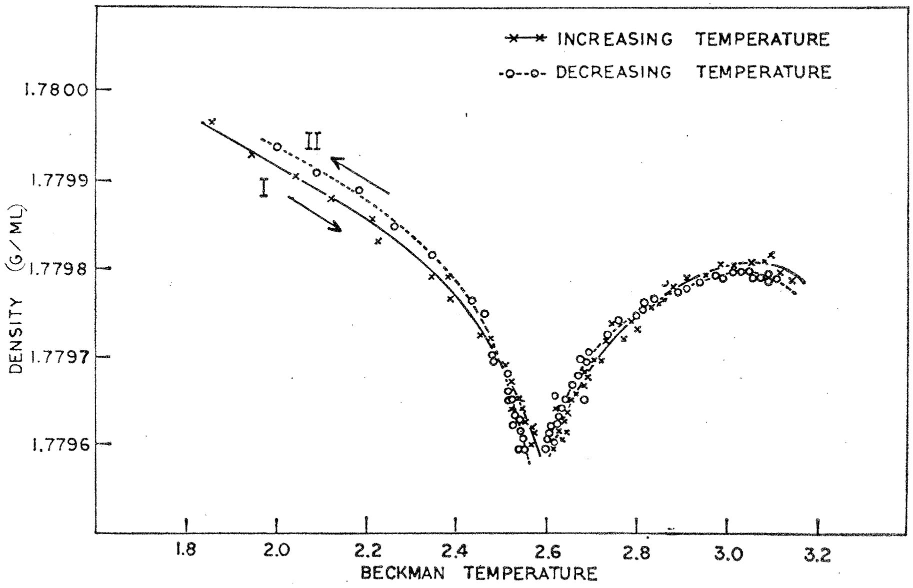

Fig. 2 (middle). Density as a function of relative temperature for a rate of change of temperature of 0.03°C per hour. Curve I, rising temperature; curve II, falling temperature.

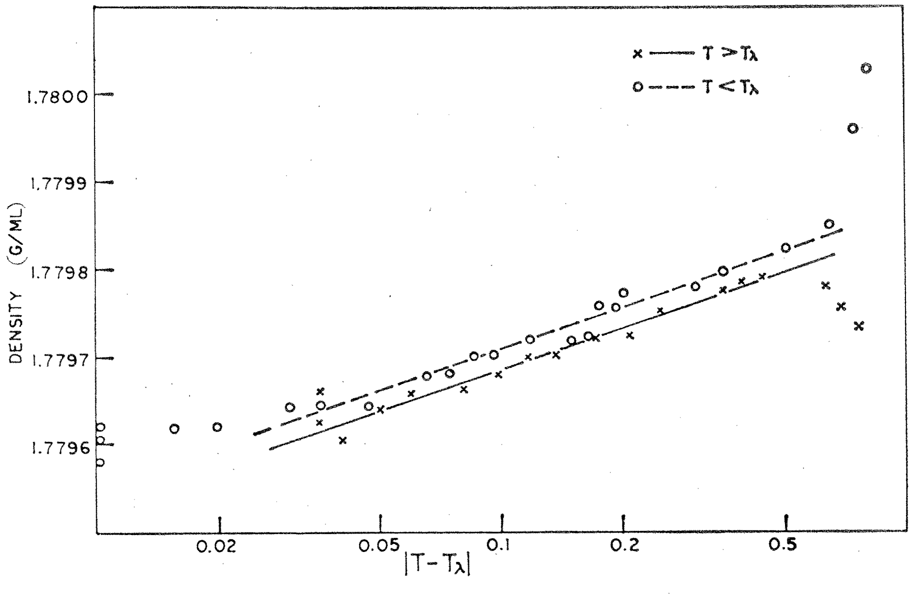

Fig. 3 (bottom). Density as a function of log $|T - T_\lambda|$ for rising temperature.

change of slope at the transition temperature, as do measurements of thermal conductivity. The diffusion coefficient changes discontinuously and the polarizability varies strongly (*3*).

Polymerization reactions are intrinsically slow, so that the measurement of dynamic variables such as heat capacity will necessarily be limited by calorimeter leakage. Measurements of stationary states will permit waiting for equilibrium to be achieved. The present investigation was therefore directed to measuring densities as a function of temperature and time.

Density measurements were made with a Westphal balance in liquid sulfur of commercial purity (>99.5 percent elementary sulfur). Careful control was maintained on bath temperature (±0.001°C) to permit the establishment of equilibrium. Instrument sensitivity was 5×10^{-6} g/ml in density and 5×10^{-3} °C in temperature. Figure 1 shows the response of density as a function of time after a rapid change of temperature of 0.13°C. More than 5 hours are required to achieve a steady-state density. The linear response of density to time at the exact transition ($T_\lambda \pm 0.02$°C) is evidence of a zero-order reaction mechanism. A linear polymer has only two active sites so that the rate of polymerization will be independent of concentration.

Density as a function of temperature is shown in Fig. 2 for a rate of change of 0.03°C per hour for rising and falling temperature. The data appear to indicate a singularity in density at the transition point. In second-order phase transitions, singularities are found in the heat capacity and expansion coefficient, but the entropy and density are continuous. The case of liquid helium has been carefully studied (*4*). Figure 3 shows that for rising temperature the data are well represented in the region $0.04 < |T - T_\lambda| < 0.5$ by the expression

$$\rho = 1.77985 + 0.00017 \log_{10} |T - T_\lambda| + 0.000025\,\Delta$$

where $\Delta = 0$ for $T > T_\lambda$, and $\Delta = 1$ for $T < T_\lambda$.

The data indicate that the lambda point in sulfur is of a stronger nature than those reported for other systems (for which the singularity occurs in the expansion coefficient and heat capacity). The logarithmic nature of the data is clear to within $dT/T = 10^{-4}$ of the transition. Only in the case of helium is the transition more nearly approached. The separation between the curves of Fig. 3 above and below the transition is unambiguous. A discontinuous density implies discontinuous entropy and a latent heat, characteristic of first-order phase transitions.

Indefinite extrapolation of the logarithmic density leads to a *reductio ad absurdum*: a negative infinite density at the transition. Likewise, an equilibrium gas phase would be predicted within an unattainably small region ($10^{-10,000}$ °C). The logarithmic law cannot be expected realistically to hold arbitrarily close to the transition temperature. Its range of validity is expected to exceed the range of observation, however.

The transition is clearly not second order and is distinguishable from those now observed in antiferromagnetism, in the gas-liquid critical point and in liquid helium. It is equally clearly not a first-order transition such as is found in melting and boiling. It is a cooperative transition associated with polymerization to a variable weight polymer, yet it shows a discontinuous density and shows evidence of a latent heat of transition.

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References and Notes

1. A. Smith and W. B. Holmes, *Z. Physik. Chem. (Leipzig)* **42**, 469 (1903).
2. H. Braune and O. Möller, *Z. Naturforsch.* **9a**, 210 (1954).
3. B. Meyer, *Elemental Sulfur* (Interscience (Wiley), New York, 1965), chap. 6.
4. W. M. Fairbanks, M. J. Buckingham, C. F. Kellers, *Proc. Intern. Conf. Low Temp. Phys. 5th, Madison, Wisc.* (1957), p. 50; E. Kerr and R. D. Taylor, *Ann. Phys. (N.Y.)* **26**, 292 (1964).
5. A report of this work was submitted by G. E. Sauer to the State University of New York at Buffalo in partial fulfillment of the requirements for the degree of Master of Arts.

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Enzyme Reaction Rates at Limited Water Activities

Abstract. *A well-mixed powder consisting of dry urea and urease exposed to air containing discrete amounts of water vapor showed a release of carbon-14 dioxide above 60-percent relative humidity. The relative activity of urease followed the water-vapor adsorption isotherm of urease. The minimum amount of water required for the reaction observed was 1.3 moles per mole of side-chain polar groups of the urease protein.*

Although the maximum rate of the urease activity on urea is achieved in dilute aqueous media, hydrolysis also takes place in concentrated urea solutions. We investigated the characteristics of this reaction with crystalline substrate and enzyme at controlled atmospheric water-vapor pressures.

A well-mixed preparation of dry enzyme and substrate was obtained by mixing 2 ml of urease suspension [cooled to 0°C and containing 10 mg of urease (*1*)] with 5 ml of a similarly cooled solution containing 100 μc of C^{14}-urea (100 mg) in a lyophilizing flask. The resulting solution was rotated for 10 seconds, frozen, and lyophilized. This lyophilized preparation

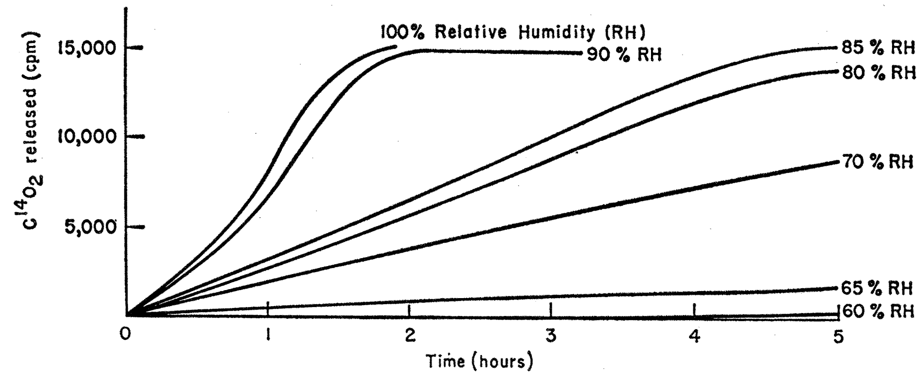

Fig. 1. Urease activity at selected relative atmospheric humidities. Lyophilized mixture of URC urease (*1*) and urea, 20°C.

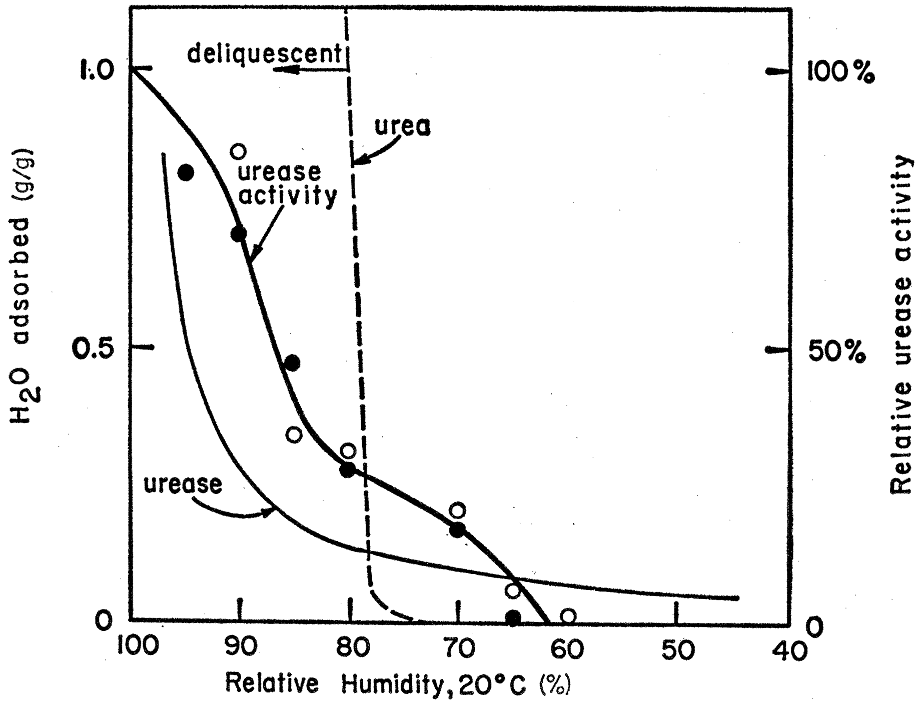

Fig. 2. Urease activity compared to water-vapor sorption isotherms of the substrate and of the enzyme. Open circles: lyophilized mixture of URC urease (*1*) and urea. Solid circles: mechanical mixture of NF-grade urease (*1*) and urea.