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.

G. E. SAUER
L. B. BORST
Department of Physics and Astronomy, State University of New York at Buffalo, Buffalo 14214

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.

21 August 1967

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

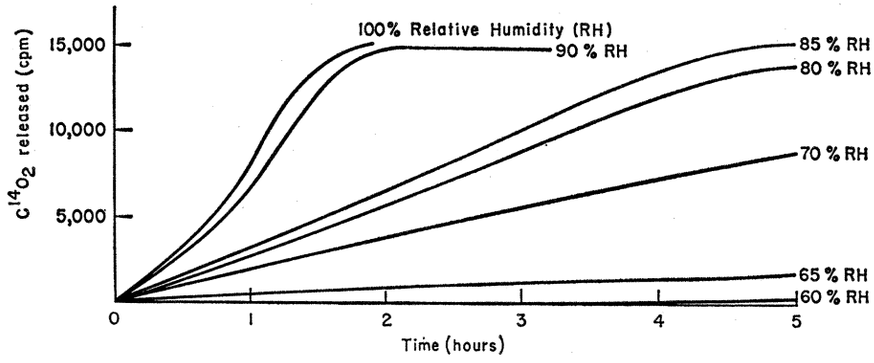

Fig. 1. Urease activity at selected relative atmospheric humidities. Lyophilized mixture of URC urease (*1*) and urea, 20°C.

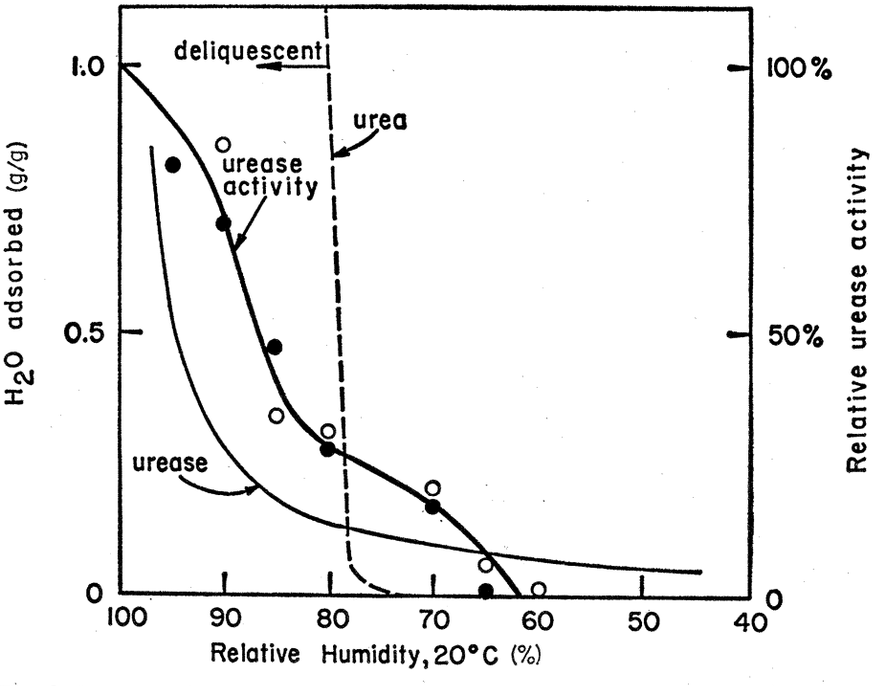

Fig. 2. Urease activity compared to water-vapor sorption isotherms of the substrate and of the enzyme. Open circles: lyophilized mixture of URC urease (*1*) and urea. Solid circles: mechanical mixture of NF-grade urease (*1*) and urea.

was maintained over phosphorus pentoxide in an evacuated desiccator.

A sample (1.1 mg) of the dry C^{14}-urea-urease preparation (containing 1 μc of C^{14}) was weighed on a glass planchet, and the radioactivity was measured in a chamber monitored with a Geiger-Mueller gas-flow tube connected to a scaler and to a rate meter. The integrated amount of $C^{14}O_2$ in the chamber was recorded on a strip chart. The atmosphere in the chamber was equilibrated at the desired humidity before the reactants were introduced, and the humidity was maintained in the chamber with appropriately diluted sulfuric acid (*2*) placed in an open dish.

Measurements of the reaction rate of the lyophilized urea-urease mixture with water vapor showed that, at 100-percent relative humidity (20°C), there was a maximum hydrolysis of urea in about 2 hours. With decreasing humidity, the rate decreased, and there was no measurable release of $C^{14}O_2$ below 60-percent relative humidity (Fig. 1). Similar results were also obtained with an NF-grade urease (*1*), and both sets of values of relative activities are shown in Fig. 2.

Isotherms of water-vapor adsorption were determined separately for urease and urea. Recrystallized urea and urease (*1*) were dried under vacuum and over phosphorus pentoxide. After the samples containing 50 to 100 mg of material reached a constant weight, they were placed in moisture chambers at 20°C and weighed again after constant weight was reached (*3*). The water-vapor adsorption isotherms of the substrate (urea) and the enzyme (urease) (Fig. 2) were compared with the relative reaction rates at the respective humidities.

The relative reaction rates follow the sorption isotherm of the enzyme rather than that of the substrate. In the range of relative humidities where there is no measurable water-vapor adsorption on the substrate (relative humidity 60 to 75 percent), a significant amount of urea becomes hydrolyzed. Evidently, the limiting factor in this reaction is the availability of water molecules to the active sites of the enzyme: at 60-percent relative humidity, the amount of water sorbed by urease is only 1.3 moles per mole of side-chain polar groups (*4*), corrected for side-chain amide. Thus, only loosely bound water can be utilized in hydrolysis, that is, water sorbed in excess of the stoichiometric minimum of one molecule of water per polar site (*3*).

Soils exhibit urease activity, and there is evidence that a certain amount of urease may be present in soils in a free state (*5*). Our results with soils containing added urea and equilibrated with water vapor show that considerable hydrolysis of urea may occur in "air-dry" soils at 80-percent relative humidity and above. Applications of enzymatic behavior at low humidities in food technology have been reviewed (*6*).

J. J. SKUJINS
A. D. MCLAREN
Department of Soils and Plant Nutrition, University of California, Berkeley

References and Notes

1. Worthington URC urease was used; NF-grade urease was obtained from Nutritional Biochemicals Corporation.
2. This was done in accordance with the International Critical Tables.
3. A. D. McLaren and J. W. Rowen, *J. Polymer Sci.* **7**, 289 (1951).
4. F. J. Reithel and J. E. Robbins, *Arch. Biochem. Biophys.* **120**, 158 (1967).
5. J. Skujins, in *Soil Biochemistry*, A. D. McLaren and G. H. Peterson, Eds. (Marcel Dekker, New York, 1967), p. 371.
6. L. Acker, *Advances Food Res.* **11**, 263 (1962).
7. Supported in part by NASA grant NsG 704/05.

22 September 1967

Solid-Phase Radioimmunoassay in Antibody-Coated Tubes

Abstract. *The adsorption of antibody to polymeric surfaces has been used to develop a new method of solid-phase radioimmunoassay. Incubation is performed in antibody-coated, disposable tubes that are finally washed-out with water and counted for quantitation of the bound tracer. The method is simple, rapid, inexpensive, and suitable for automation.*

The principle of solid-phase radioimmunoassay (*1*) is based on the ability of antibody-coated polymers specifically to bind radioactive tracer antigen. The use of antibody in this form allows rapid removal of the free radioactive tracer antigen by washing of the solid phase with water on completion of the immune reaction. The solid-phase material is then counted for quantitation of the bound tracer, which varies inversely with the total quantity of antigen present in the original incubation mixture. This simple and sensitive procedure can be used to measure nanogram quantities of protein hormones in plasma.

The materials originally used for solid-phase radioimmunoassay were prepared in powder form as either poly-(tetrafluoroethylene-*g*-isothiocyanatostyrene) (*2*) or Sephadex-isothiocyanate (*3*). More recently the former polymer has been prepared in the form of small discs (*4*), each of which, when coated with antiserum, represents a portion of specific antibody. Such discs have been used for radioimmunoassay of human growth hormone (*5*) and luteinizing hormone (*6*) in plasma; they are an improvement on the powder form of this solid phase. The discs have also been used in this laboratory for radioimmunoassay of human chorionic gonadotrophin, human placental lactogen, bovine luteinizing hormone, fibrinogen, and tetanus toxin; they appear to be suitable for radioimmunoassay of proteins in general.

During examination of various polymeric materials for applicability to solid-phase radioimmunoassay, it became apparent that certain unsubstituted polymers may adsorb antibody that can then bind an adequate quantity of radioactive tracer antigen for use in the assay. In contrast, adsorption of antibody to glass was negligible. Adsorption of antibody by polymer surfaces, from antiserums of moderately high titer, has been used to develop a simple and inexpensive form of radioimmunoassay.

The adsorption phenomenon has been applied to radioimmunoassay by coating of the interior of plastic tubes with uniform quantities of specific antibody. The two most commonly available disposable plastic tubes are manufactured from polypropylene or polystyrene, both of which give satisfactory results in the assay. Tubes (*7*) suitable for use in an automatic gamma counter were used without washing or other treatment; each was coated by addition of a uniform volume (1.0 or 2.0 ml) of diluted antiserum. Antiserum dilutions between 1:100 and 1:5000 proved to be satisfactory, the optimal *p*H of the buffer solution being 9.0 to 10.0. The duration of exposure to antiserum was not critical; results were identical with times ranging from 1 minute to 16 hours.

After removal of the coating solution, the tubes were washed-out three times with 0.15*M* NaCl and once with