tern of banding of the D-LDH-U system is modified but that of the D-LDH-L system remains normal. The variant pattern has six bands in the D-LDH-U system, three of which (numbers 1, 2, and 4 in Fig. 1) are identical in mobility with the three bands of the common phenotype, and three of which (numbers 3, 5, and 6) are unique. This pattern may be explained most easily by a dimer model of molecular configuration, and suggests that the three D-LDH-U isozymes are genetically controlled by two loci (y and z), one encoding a fast-migrating polypeptide Y, and the other a slow-migrating polypeptide Z. According to this model, the three D-LDH-U bands of the common phenotype correspond to the dimers Y_2, YZ, and Z_2, and individuals with the common phenotype are homozygous at both the y and z loci. The variant individuals are believed to be heterozygous at the z locus for a common allele (z) and an alternate allele (z') producing a slightly slower-migrating polypeptide Z'. In the presumed heterozygotes, associations of Y, Z, and Z' polypeptides yield a pattern of six bands in the D-LDH-U system corresponding to the dimers Y_2, YZ, YZ', Z_2, ZZ', and Z'_2 (bands numbered 1 through 6 in Fig. 1).

Other models accounting for the observed variation are less satisfactory. For example, if we assume that the D-LDH-U molecule is a tetramer rather than a dimer, we must further assume that all asymmetrical heterotetramers (Y_3Z, Y_3Z', and so forth) and the symmetrical heterotetramer Y_2ZZ' are enzymatically inactive or are not formed in appreciable amounts (*9*).

The fact that the electrophoretic banding pattern of the D-LDH-L triad is unmodified in the individuals exhibiting the variant D-LDH-U phenotype indicates that the postulated Z subunits of D-LDH-U do not participate in the formation of molecules of the D-LDH-L system. It is probable that the isozymes of the D-LDH-L triad are dimers and are under separate genetic control from those of the D-LDH-U system. Certainly, the evidence is not compatible with Massaro's model (*2*) of a tetrameric structure similar to that found in vertebrate L-LDH, with the entire D-LDH system being controlled by only two loci (*10*). We therefore conclude that the D-LDH isozymes of *Limulus* differ from L-LDH isozymes of vertebrates both in molecular configuration and in number of genetic loci encoding polypeptide subunits. If we were to assume that the polypeptide subunits of D-LDH in *Limulus* are of the same molecular weight as the subunits of vertebrate L-LDH, we would, according to the dimer model, expect the molecular weight of D-LDH to be roughly half that of tetrameric L-LDH. Following this line of reasoning, our findings are easier to reconcile with Long and Kaplan's (*1*) determination of molecular weight (65,000) for D-LDH of *Limulus* than with the value (140,000) reported by Massaro (*2*).

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5. Horizontal starch-gel electrophoresis was carried out as described by R. K. Selander, S. Y. Yang, W. G. Hunt [*Studies in Genetics V*, Univ. of Texas Publ. No. 6918 (1969), p. 271]. The 12.5 percent gels were made with Electrostarch (Electrostarch Co., Madsion, Wis.; Lot 682). Electrophoresis was conducted at a constant gradient of 9 volt/cm for 3 hours, with the following tris-citrate buffer system. Gel buffer: 0.008M tris (Sigma) and 0.003M monohydrate citric acid, pH 6.7 (adjusted with 1.0M sodium hydroxide). Electrode buffer: 0.223M tris and 0.086M monohydrate citric acid, pH 6.3 (adjusted with 1.0M sodium hydroxide). Gels were stained for LDH activity by the nitro-blue tetrazolium (NBT) method [C. L. Markert and H. Ursprung, *Develop. Biol.* **5**, 363 (1962); T. B. Shows and F. H. Ruddle, *Proc. Nat. Acad. Sci. U.S.* **61**, 574 (1968)], with the lithium salt of DL-lactic acid (Sigma). NAD, NBT, and phenozine methosulfate (PMS) used in the stain were obtained from the Sigma Chemical Co. (St. Louis, Mo.).
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7. Extracts of hepatopancreas were electrophoresed on tris-EDTA-borate gels (pH 8.0) and stained for alcohol dehydrogenase with a staining solution composed of 10 ml of 0.5M potassium phosphate buffer (pH 7.0), 66 mg NAD, 35 mg NBT, 2 mg PMS, and 80 ml water. For substrates we added 5 ml of ethanol, isopropanol, or methanol, and some gels were stained with the mixture lacking substrate. Enzyme X bands appeared on all gel slices, but were darkest on those for which no substrate was provided. Thus, the alcohols appeared to inhibit the staining reaction.
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The Hydrogen Atom and Its Reactions in Solution

Abstract. *Hydrogen atoms have been generated in solution by photolysis of thiols in solutions of organic compounds, and the relative rate constants,* k_H, *have been measured for the reaction* $H\cdot + QH \rightarrow H_2 + Q\cdot$, *where QH is any organic compound which contains hydrogen. This represents the first kinetic study of the hydrogen atom in which it is generated in solution by a technique not involving ionizing radiation. The relative values of* k_H *are in agreement with the values from radiolysis for most of the substances studied; however, for some compounds significantly different results have been obtained.*

When either aqueous solutions or organic solvents are subjected to ionizing radiation, a series of reactive, transient intermediates is produced which includes electrons, ions, excited molecules, and free radicals (*1*). Among these species, the hydrogen atom is particularly interesting since it is the simplest possible radical and its reactions are of great theoretical interest. Furthermore, this species is responsible for some of the damage caused to organisms by radiation (*2*). In order to clarify the reactions of the hydrogen atom in solution, it is highly desirable to generate it by a technique which does not produce other reactive fragments which can confuse the analysis (*3*). In the past this has been accomplished by allowing hydrogen gas to flow past a discharge and then into solution (*4, 5*); this method has some disadvantages (*4*). We here report the first kinetic study of the hydrogen atom in solution in which this species is generated in situ by a technique not involving ionizing radiation. We have obtained relative rate constants for reaction 1 in organic solvents, where QH is any organic molecule that contains hydrogen, and we

have compared our data with those obtained from radiolysis of aqueous solutions (*6*).

$$H\cdot + QH \xrightarrow{k_H} H_2 + Q\cdot \quad (1)$$

We have generated hydrogen atoms by the photolysis of thiols with 3000-Å lamps, and we have used two methods for obtaining values of k_H.

Method I. In the simplest method (*7*) a deuterated thiol, RSD, is photolyzed in the presence of a QH molecule. The key equations are shown below.

$$RSD \xrightarrow{h\nu} RS\cdot + D\cdot \quad (2)$$

$$D\cdot + RSD \xrightarrow{k_3} RS\cdot + D_2 \quad (3)$$

$$D\cdot + RSD \xrightarrow{k_4} \dot{R}SD + HD \quad (4)$$

$$D\cdot + QH \xrightarrow{k_D} Q\cdot + HD \quad (5)$$

where $\dot{R}SD$ is the thiol radical which results when the thiol side chain loses a hydrogen atom. Kinetic analysis of this system gives Eq. I. According to this equation

$$\frac{[HD]}{[D_2]} = \frac{k_4}{k_3} + \frac{k_D}{k_3}\frac{[QH]}{[RSD]} \quad (I)$$

plots of the ratio of the yields of HD to D_2 as a function of the initial ratio of the solvents QH/RSD should give straight lines with slopes that are proportional to k_D. We have tested this for both t-C_4H_9SD and C_6H_5SD, and straight lines are obtained for solvent mole ratios from about 5 to 25. If the isotope effect $I_1 \equiv k_D/k_H$ is defined as the ratio of rate constants for Eqs. 1 and 5, then the slopes of these plots yield values of k_HI_1. It is expected that I_1 is very nearly unity for any QH molecule (*8*), and therefore changes in I_1 in a series of QH molecules will be quite small. Thus, our relative values of k_HI_1 should parallel values of k_H obtained from aqueous radiolysis.

Method II. In a second method we have photolyzed tritiated propanethiol, PrSH(T), in the presence of a variety of QH molecules (*9*). This system produces Q· and ·C_3H_6SH radicals by reactions analogous to reactions 4 and 5, and these species then react with the tritiated thiol to become labeled as follows

$$PrSH^* + Q\cdot \rightarrow PrS\cdot + QH^* \quad (6)$$

$$PrSH^* + \cdot C_3H_6SH \rightarrow PrS\cdot + \overset{*}{P}rSH \quad (7)$$

Kinetic analysis of this system yields an equation which indicates that values of k_HI_6 can be obtained by measuring the rate of incorporation of tritium into QH as a function of the QH/thiol solvent ratio (*9*). The isotope effect I_6 equals k_T/k_H for reaction 6. (In each run we also measure the activity incorporated into the propyl side chain of the thiol as an internal control.) We have measured values of I_6 for several Q· radicals, and, as might be expected (*10*), I_6 does depend on the structure and reactivity of the organic radical (*11*). However, we believe that I_6 values within a series of similar QH compounds will be fairly constant.

Table 1. Relative rates of reaction of hydrogen atoms. Data in each column are relative to the value in parentheses.

QH	k_HI_1 Method I*	k_HI_6 Method II†	k_H Aqueous radiolysis‡
n-Hexane	(1)	(1)	
n-Dodecane	2.22 ± 0.12	2.30	
2,3-Dimethylbutane	2.13 ± 0.10	1.87	
2,5-Dimethylhexane	2.41 ± 0.19		
Cyclohexane	1.23 ± 0.14	1.49	
Cyclopentane	1.21 ± 0.12		
Methanol	0.41 ± 0.09	0.42	(0.42)
Ethanol	1.19 ± 0.08	5.13	4.0
Isopropanol	2.00 ± 0.20	16.7	13.
t-Butanol	0.04 ± 0.03		0.03
Dioxane	2.84 ± 0.24	1.44	1.30
Tetrahydrofuran	8.21		7.6
Diethyl ether	5.16 ± 0.09		
Diisopropyl ether	4.69 ± 0.16		
Glucose			10.5
Glycerol			4.7
Ethylene glycol			2.1

* Average values of k_HI_1 for *t*-butyl mercaptan and thiophenol systems at 35°C. † Values of k_HI_6 for propanethiol-*t* at 40°C (*9*). ‡ Average values quoted in reference (*6*).

In Table 1 some of our data are compared with results from aqueous radiolysis. The data given in column 2 are average values obtained from runs with *t*-butyl mercaptan and thiophenol; the two thiols give the same relative values of k_HI_1. One of the difficulties with our system is that the thiyl radical is produced (Eq. 2) along with the hydrogen atom, and it is conceivable that this radical could invalidate our kinetic analysis. However, the $C_6H_5S\cdot$ and t-$C_4H_9S\cdot$ radicals have very different reactivities (*12*), and the fact that runs with these two thiols yield the same values indicates that method I gives true relative k_H values (*13*). In method II it is less clear that this is true. In this method, the primary product of the reaction of the H atoms, H_2, is not measured, but rather a subsequent product QT is followed. If RS· abstracted hydrogen from QH, this Q· would react according to Eq. 6 and produce a spuriously high activity in the QH. Calculations based on known rate constants for models of these steps suggest that RS· could only produce kinetically significant yields of Q· in cases where the Q–H bond has the same or a lower strength than the PrS–H bond (*12*). This could occur for some of the oxygen compounds in Table 1, but it does not appear likely for the hydrocarbons. An indication that the H atom is the only radical which attacks QH at a kinetically significant rate for QH molecules which have high C–H bond strengths is the fact that in method II both propanethiol and thiophenol give very similar relative values of k_H for cyclohexane, hexane, dodecane, and 2,3-dimethylbutane (*14*).

Almost all the data in Table 1 are in very good agreement; only in the cases of ethanol and isopropanol do the different methods give results which differ significantly. We believe that the use of propanethiol in method II gives values that are spuriously large for both these alcohols, probably because of reactions in which the thiyl radical abstracts the α-H from the alcohol (*12*, *15*). This hypothesis is supported by the finding that the use of thiophenol in method II gives values for ethanol and isopropanol that are much lower than those obtained with propanethiol and are in good agreement with the data obtained by the use of method I (*14*). The aqueous radiolysis values for ethanol and isopropanol also are quite high, and we tentatively suggest that there is some as yet unevaluated feature of the aqueous radiolysis of alcohols that makes data from that system not exactly comparable with similar data for ethers and hydrocarbons. This would be a significant finding since these alcohols were among the first substrates for which the reactions of the hydrated electron and the H atom were separated and identified (*16*). Finally, our data indicate that the H atom has a selectivity which is quite similar to that of the methyl radical (*17*).

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17. The data from aqueous radiolysis of methanol, ethanol, and isopropanol give a reactivity per hydrogen for primary, secondary, and tertiary hydogens which is not at all what should be expected of the hydrogen atom. Our data obtained by method II yield relative rates for the attack of the H atom on these alcohols which closely parallel the data for the methyl radical. Since the methyl radical and H atom form bonds with hydrogen of the same strength (104 kcal/mole), it would be expected that they would show similar selectivity (*10*). These points are discussed in a review [W. A. Pryor and J. P. Stanley, *Intra-Sci. Chem. Rep.*, in press].

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Calcium Oxalate: Crystallographic Analysis in Solid Aggregates in Urinary Sediments

Abstract. *A relationship between crystallographic structure and morphological form of calcium oxalates in urinary sediments is established. The common tetragonal bipyramids have been confirmed as weddellite from their electron diffraction patterns. Other solid forms, such as needles, biconcave disks, and dumbbell forms, that can appear in hyperoxalurias, of both metabolic and alimentary origin, have been identified as whewellite. Micrographs reveal fibrous structure on those whewellite polycrystalline aggregates.*

Urinary calcium oxalate sediments of healthy persons and patients with renal stones have been studied by electron microscopy (Siemens Elmiskop IA), and the different crystalline types have been identified by diffraction analysis of selected areas. Specimens were prepared as follows. Immediately after the urine was collected, it was centrifuged in warm, conical glass tubes (1500 rev/min, 1 minute), and the sediment was washed with distilled water and dried. Crystals were observed directly and by replica with the Bradley method (*1*).

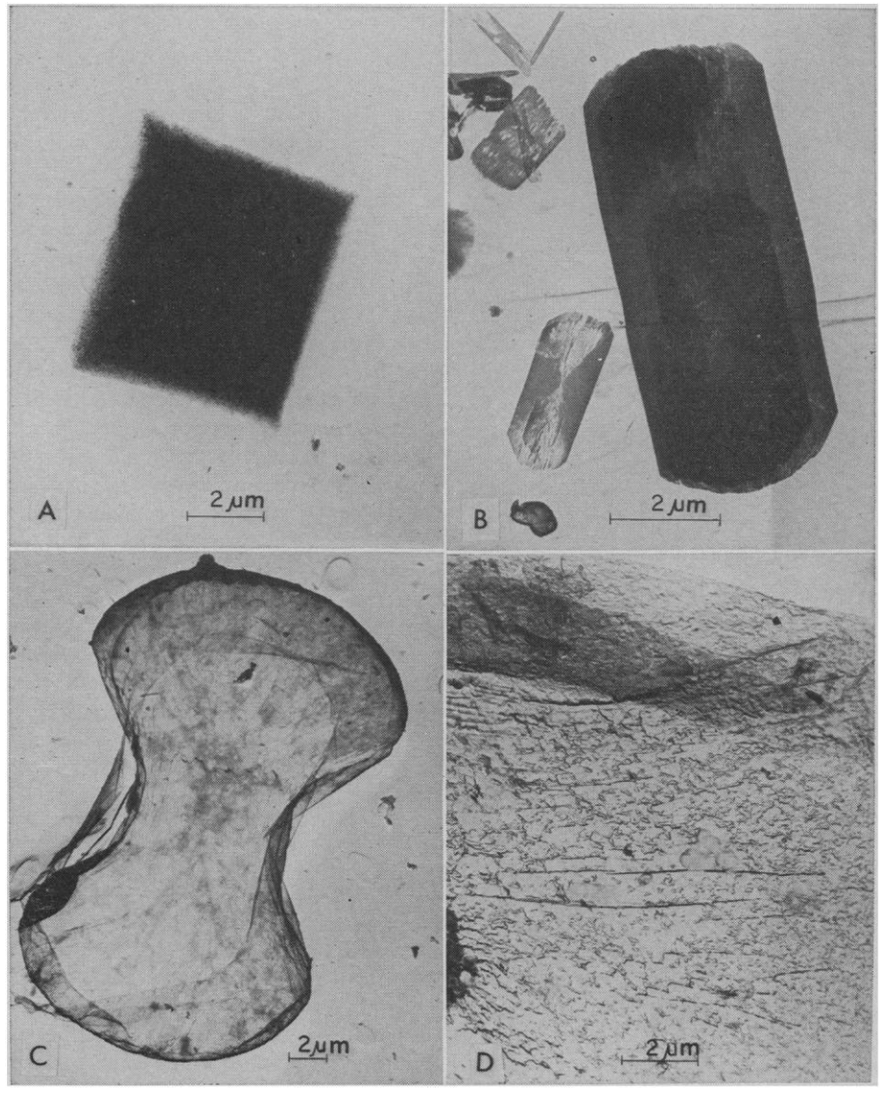

Fig. 1. (A) Bipyramid tetragonal crystal of weddellite. (B) Aggregated prismatic crystals. (C) Carbon replica of a typical dumbbell form of polycrystalline aggregates. (D) Replica of a dumbbell form with high magnification. On its surface the fibrillar structure is observed.