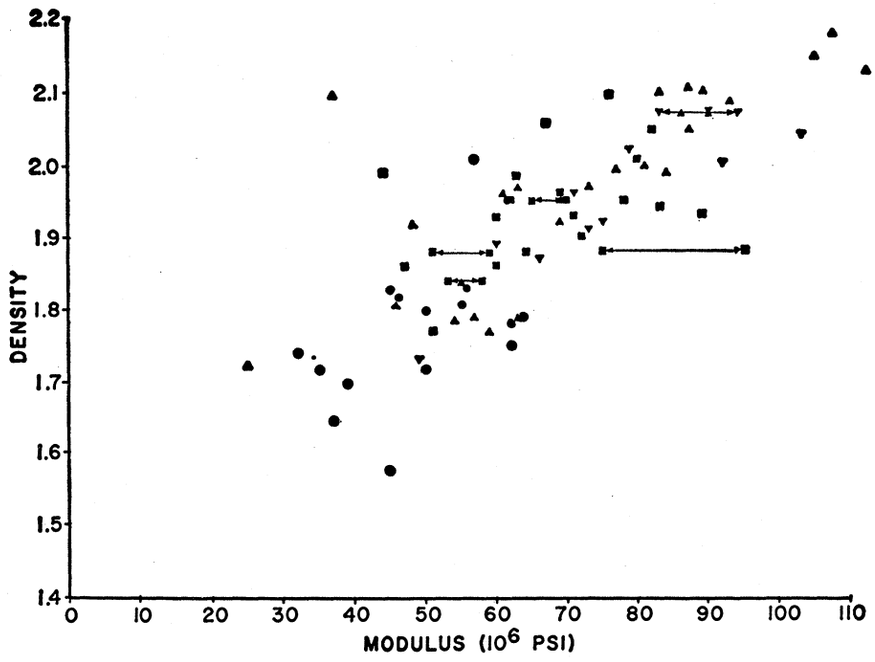

Fig. 2. Density (g/cm³) and modulus values for graphite fiber samples from all of the acrylic precursors: (●) Courtelle, (■) PAN, (▲ and ▼) Dralon T.

tionable; in these instances, both averages were plotted, and the values were connected by arrows.)

Two different sets of processing conditions were used in the first treatment stage for Dralon T; the graphite fibers from Dralon T were divided into two corresponding groups. The data for these fibers are shown in Fig. 1, C and D. The values in Fig. 1C can be represented by either a linear relation or by a curve for which the slope decreases slightly with increases in temperature. The same sort of slope could be drawn for the data in Fig. 1D, with the inflection more clearly defined above 90 million psi.

Data for all the samples are plotted in Fig. 2 and show that all of the series fit the same general linear relation between density and modulus. Several other variables were under investigation in these experiments and may account for the considerable scatter in much of the data.

Certain parameters can influence density or modulus, or both, at a specific graphitization temperature (*3*). However, the data in Fig. 2 show a relation between the two properties which holds in spite of these precursor and processing variations.

Data for the tensile strengths of these fibers is much more scattered. Least-squares lines were plotted by computer for the changes in fiber density as tensile strength was increased. These showed a slight decrease for Courtelle-precursor samples, an even slighter increase for PAN-derived fiber, and no density change for the fibers from Dralon T.

The data on the density and modulus properties for the acrylic-derived fibers should be compared with those for graphite fibers prepared from other precursors. Although other polymers have been used in precursor yarns, rayon and polyacrylonitrile give the graphite fibers with the highest moduli (*4*). Some investigators have reported on density-modulus relations in rayon-derived fibers. Gibson and Langlois (*5*) showed that the apparent density increased directly with the Young's modulus between 20 and 50 million psi. Bacon and Schalamon (*6*) found a similar increase; they stated that the behavior had not yet been well characterized but gave values of 1.35 and 1.95 g/cm³ for fibers with 10 and 100 million psi moduli, respectively. A linear interpolation between the two values was considered an adequate representation of the available data. The density-modulus line for these graphite fibers would be substantially lower than the trend shown in Fig. 2 for fibers from acrylic polymers.

A higher modulus at equivalent density cannot be assumed to be due to a higher degree of graphitization in the rayon-derived fibers. Rayon precursors form graphite fibers with long, needle-like micropores parallel to the fiber axis. These pores, described by Perret and Ruland (*7*), represent as much as 30 percent of the fiber volume; they are inaccessible to helium and account for the low density. On the basis of comparative x-ray diffraction patterns and interlayer spacings, the acrylic-derived fibers are actually more graphitic (*8*).

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Horseshoe Crab Lactate Dehydrogenases: Evidence for Dimeric Structure

Abstract. D-*Lactate dehydrogenase of* Limulus polyphemus *occurs in six molecular forms, not in five as does the* L-*lactate dehydrogenase of vertebrates. The electrophoretic pattern of bands in an apparent genetic variant is incompatible with a model of tetrameric molecular structure, and suggests, rather, that the three more anodally migrating isozymes are dimer molecules, the polypeptide subunits of which are encoded by two genetic loci and are not involved in the formation of the lower triad of isozymes.*

Long and Kaplan (*1*) reported that the lactate dehydrogenase (LDH) of the horseshoe crab *Limulus polyphemus* differs from the LDH of vertebrates in being specific for D-lactate rather than for L-lactate, in having a molecular weight of 65,000 as opposed to 140,000 to 150,000, and in occurring in six

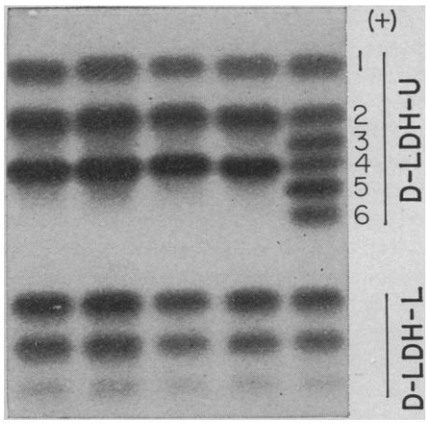

Fig. 1. Electrophoretic pattern of D-LDH in *Limulus polyphemus* in heart extracts (tris-citrate gel, *p*H 6.7). Samples 1–4 represent normal phenotype, and sample 5 is presumed heterozygote at *z* locus of D-LDH-U system.

rather than in five molecular forms that differ in electrophoretic mobility on starch gels. However, Massaro (*2*) has recently claimed that the D-LDH of *Limulus* has a molecular weight similar to that of vertebrates (140,000) and occurs in five isozymic forms. Additionally, he suggested that the *Limulus* D-LDH molecule is, like the L-LDH molecule of vertebrates, a tetramer of two physicochemically distinct subunits, which are presumably encoded by two genetic loci.

Results of an analysis in our laboratory (*3*) of electrophoretic variation in D-LDH in 64 individuals of *Limulus* from four localities (*4*) are not compatible with Massaro's interpretation. We now confirm the report (*1*) that D-LDH in *Limulus* occurs in six isozymic forms patterned on starch gels in two triads. We also present evidence suggesting that the D-LDH molecule is a dimer and that the polypeptide subunits of the two triads of isozymes are under separate genetic control.

When aqueous extracts of heart tissue from most individuals in our samples are electrophoresed on tris(hydroxymethyl)aminomethane-citrate gels (*p*H 6.7) and stained for lactate dehydrogenase (*5*), six isozymes are demonstrated (Fig. 1). (Two individuals show a variant pattern of nine isozymes; see below.) The pattern of distribution, which is identical to that reported by Long and Kaplan (*1*), consists of three upper (more anodally migrating) bands and three lower bands separated by a relatively large gap. For reference purposes, we have designated the upper triad of bands D-LDH-U and the lower triad D-LDH-L. The D-LDH-U triad is prominent in muscle, as noted by Long and Kaplan (*1*), but the two upper bands of the D-LDH-L triad stain darker than the D-LDH-U bands in extracts of hepatopancreas and stomach-intestine, and all six bands appear in extracts of heart (Figs. 1 and 2).

When extracts of hepatopancreas, gizzard, or stomach-intestine are electrophoresed on tris–ethylenediaminetetraacetic acid–borate gels (*p*H 8.0 to 8.6) and stained for LDH, a sixth band ("X" in Fig. 2) appears in a position midway between the D-LDH-U and D-LDH-L triads. But the enzyme producing this band is not lactate specific, being, rather, a "nothing dehydrogenase" (*6*), the activity of which is expressed on all gels stained by the nitro-blue tetrazolium method, even in the absence of added substrate (Fig. 2). We have designated it "enzyme X." We initially suspected that enzyme X was an alcohol dehydrogenase, as shown for a "nothing dehydrogenase" in the deer mouse *Peromyscus maniculatus* (*6*), but tests with several alcohol substrates did not support this interpretation (*7*). Possibly enzyme X is a diaphorase (*8*).

Additional evidence that enzyme X is not an isozyme of LDH is provided by the discovery of a variant phenotype of this enzyme in two individuals in our samples from Panacea and Panama City, on the Gulf of Mexico coast of Florida (Fig. 2). These variant individuals have three bands, a slow-migrating band common to all other individuals, a fast-migrating band, and a relatively dark band of intermediate mobility. We interpret these variants as heterozygotes for alleles at a single locus encoding polypeptides which combine in pairs to form dimer molecules of the active enzyme X; the intermediate band presumably represents the heterodimer. Significantly, the observed variation in enzyme X is independent of that described below in the D-LDH system. That is, the two individuals showing the three-banded phenotype of enzyme X have normal D-LDH-U and D-LDH-L patterns.

Enzyme X further differs from D-LDH in the rate at which its activity decreases during storage. Whereas we detected no loss of activity of D-LDH after storage at −68° to −76°C for 6 months, the activity of enzyme X under the same conditions of storage decreased markedly after 1 week and was barely detectable after 6 months.

By electrophoresing tissue extracts on tris-EDTA-borate gels of the type used by Massaro (*2*), we have determined that the band labeled "LDH-4" in his figure 1 is actually enzyme X. We also find that the dark, elongated zone labeled "LDH-5" in Massaro's figure 1 represents the D-LDH-L triad, with the individual bands poorly resolved and overstained. In our experience, isozymes of the D-LDH-L triad do not band sharply on tris-EDTA-borate gels, although better resolution than that obtained by Massaro is possible (Fig. 2).

Two individuals from the gulf coast of Florida show a variant D-LDH phenotype (Fig. 1), in which the pat-

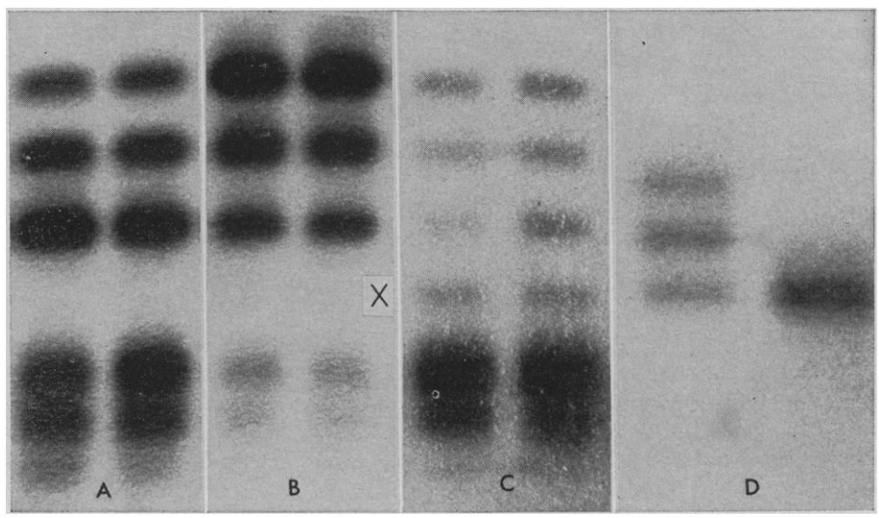

Fig. 2. Electrophoretic patterns of D-LDH and enzyme X in *Limulus polyphemus* (tris-EDTA-borate gels, *p*H 8.0). (A) D-LDH in heart; (B) D-LDH in muscle; (C) hepatopancreas extract stained for LDH, showing presence of band produced by enzyme X between D-LDH-U and D-LDH-L triads; (D) enzyme X on gel stained by nitro-blue tetrazolium method with no added substrate, showing phenotypes of presumed heterozygote (left) and homozygote (right).

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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4. Tissue extracts were obtained from specimens collected in July and August, 1969, as follows: 17 specimens from Woods Hole, Mass.; 14 from Chincoteague Island, Va.; 19 from Panacea, Fla.; and 14 from Panama City, Fla. Carapace diameters ranged from 13 to 30 cm. Blood (hemolymph) was taken by pipette, centrifuged for 30 minutes at 49,500g at 0°C, and the clear supernatant was removed. Samples of tissue from the gizzard, intestine (stomach-intestine), heart, leg and telson muscles, and hepatopancreas were homogenized in equivalent volumes of buffer (0.01M tris, 0.001M EDTA, $5 \times 10^{-5}M$ nicotinamide adenine dinucleotide phosphate; pH adjusted to 7.0 with HCl), and centrifuged to obtain a clear supernatant. Tissue extracts were stored at −68° to −76°C for from 12 hours to 3 weeks before electrophoresis.
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