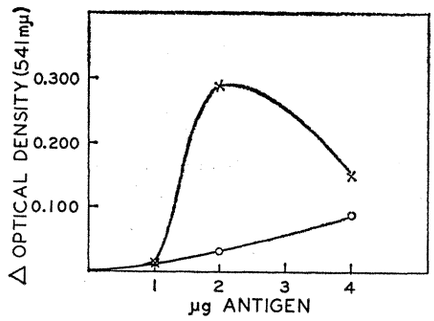

Fig. 2. Analysis of a rabbit antiserum, produced with calf-thymus DNA digested with deoxyribonuclease, for C'-fixing antibodies against a synthetic d-AT polymer: O———O, unheated polymer; ×———×, heated polymer. The volume of antiserum used was 0.1 ml diluted 1:100.

also reacted with heterologous DNA from monkey-heart cells and from several bacterial sources but, as expected, to a lesser extent than with homologous calf-thymus DNA. In addition, these antiserums did not react with calf-thymus DNA digested with deoxyribonuclease and they inhibited the transforming capacities of DNA from *Bacillus subtilis* and from *Diplococcus pneumoniae* (*7*). These findings support the conclusion that DNA-specific antibodies can be elicited by complexes of oligodeoxyribonucleotides with MBSA.

In view of such extensive cross-reactivity and the availability of a synthetic d-AT (polydeoxyadenylate-thymidylate) polymer (consisting of repeating units of adenine- and thymine-containing nucleotides) (*8*), it was of interest to determine whether or not a synthetic polymer also would cross-react.

A solution of the d-AT polymer (40 μg/ml) was boiled at 100°C for 10 minutes to disrupt any hydrogen bonds between adenine and thymine, chilled rapidly in an ice-water bath, and used immediately because of the ease with which hydrogen bonds can form between adenine and thymine. Significant cross-reaction was observed with each antiserum, the extent of the cross-reaction generally paralleling the reaction with homologous calf-thymus DNA. The cross-reaction of an antiserum with both heated and unheated d-AT polymer is shown in Fig. 2. As in the case of natural DNA, the heated preparation was more reactive, and as in the case of natural DNA, this may be attributed to a greater accessibility of the nucleotides for reaction with antibody.

The results show that oligodeoxyribonucleotides can serve as haptens for the production of DNA antibodies. If we take into consideration that the largest fractions occurring in appreciable amounts in a deoxyribonuclease digest of calf-thymus DNA are of the order of hepta-octanucleotides (*4*), and that the largest fraction formed on de-purination with formic acid is a hexanucleotide (*3*), then it would appear that oligodeoxyribonucleotides containing no more than six to eight nucleotides may suffice to serve as hapten when complexed to MBSA. It is unlikely that oligonucleotides containing less than four nucleotides would form stable complexes with MBSA in a physiological medium because the interaction between MBSA and the oligonucleotides is electrostatic in nature. Therefore, the minimum size of oligodeoxyribonucleotide that can serve as a hapten complexed to MBSA can be expected to be of the order of tetra- to hexanucleotide. Stollar *et al.* (*9*) have reported that the immunological specificity of a *Lupus erythematosus* serum is attributable to a pentanucleotide. Therefore, antibodies specific for unique sequences of some five nucleotides may eventually be produced through the use of penta- or hexadeoxyribonucleotides that are homogeneous with respect to size and sequence.

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1 March 1964

Polymorphism of Human Lactate Dehydrogenase Isozymes

Abstract. *Of 1200 individuals screened, four had variant erythrocyte lactate dehydrogenases. Three of these, identical electrophoretically to the Mem-1 described by Kraus and Neely, were heterozygous variants involving the* a*-locus. The fourth, involving the* b*-locus, appears to be the first example in man of a homozygous variant.*

Each of the five commonly encountered lactate dehydrogenase (LDH) isozymes is a tetramer containing varying combinations of two different monomers A and B (*1*). Genetic evidence that the synthesis of these two subunits is directed by two different structural genes *a* and *b* was obtained first in mice (*2*), and shortly thereafter in man (*3, 4*). Variants at either the *a*- or *b*-genetic locus were described and pedigrees were presented suggesting autosomal codominant transmission (*3, 4*).

The last and most extensive of these papers, in which eight variant LHD's were discovered among 980 individuals (*4*), raised questions concerning the relative frequency of variants at the two loci and among different populations.

To investigate these questions, erythrocytes from 600 white and 600 Negro patients at various hospitals in the Washington, D.C., area were examined for variant LDH isozymes. Hemolyzates were prepared (*5*) and were subjected to vertical starch-gel electrophoresis in a discontinuous tris-borate buffer system, with a voltage gradient of 12 v/cm^2 for 3 hours at 4°C. The gels were sliced horizontally, and identification of the LDH isozymes was accomplished by incubation for 1 hour at room temperature in a solution modified from that previously described (*5*) by addition of 4 ml of 0.06*M* sodium cyanide.

Three variants were observed among the 600 Negro patients, a frequency of 0.0050, and one among the 600 white patients, a frequency of 0.0017. Of these three unrelated Negro patients, one, a 65-year-old female, was hospitalized with a cerebrovascular accident probably secondary to atherosclerosis; the second, a 70-year-old female, had hypertensive heart disease, and the third, a 76-year-old male, had chronic lymphocytic leukemia. The pedigree of the second patient revealed six affected

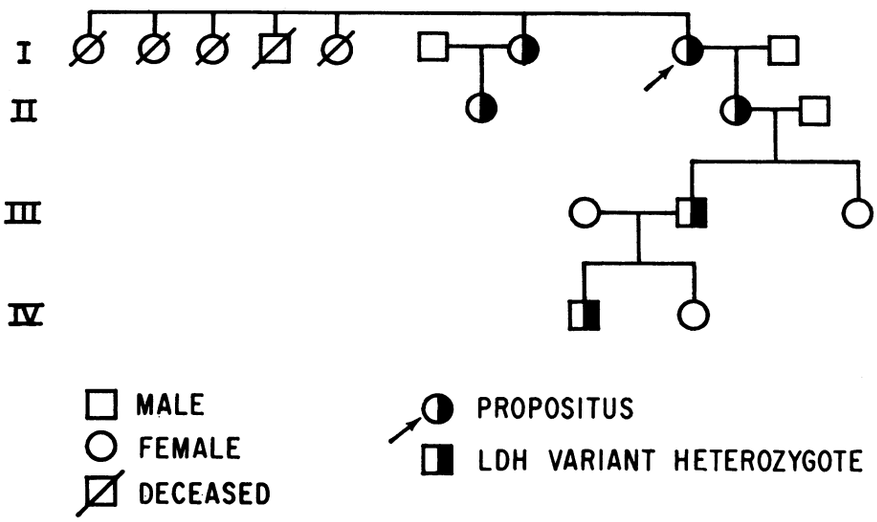

Fig. 1. Pedigree of individual with variant A subunit.

individuals in four generations (Fig. 1). All except the propositus were healthy. The pedigree suggests autosomal codominant inheritance of the variant phenotype (Fig. 2); male to male transmission of the variant from generation III to IV eliminates the possibility of X-linkage. All three Negro variants involved the A subunit and were electrophoretically indistinguishable from one another and from the Mem-1 variant of Kraus and Neely (*6*). All had normal, total erythrocyte LDH activity.

The multiple bands shown in Fig. 2 in regions where normally a single isozyme occurs probably result from hybridization in the heterozygote of the normal A subunit with the variant A′ subunit. Such hybridization would be expected to produce four bands in the LDH-4 position (AAAB, AAA′B, AA′A′B, and A′A′A′B), three in the LDH-3 position (AABB, AA′BB, and A′A′BB), and two in the LDH-2 position (ABBB and A′BBB). Since the B subunit is normal, LDH-1 is homogeneous and unaffected; LDH-5 is not observed in human hemolyzates on the starch gel (*5*), but the five bands expected at the LDH-5 position in A-subunit variants are visualized in leukocyte extracts. The four bands in the LDH-4 region of the A-subunit variants are observed in the distribution expected if, in the heterozygote, recombination of A and A′ subunits occurred in an entirely random fashion. That is, more activity resides in the two middle bands (AAA′B and AA′A′B) than in the two extreme bands AAAB and A′A′A′B.

The fourth variant occurred in a 74-year-old unmarried white female with lymphoblastic sarcoma. She was in remission without medication or recent transfusion at the time that four separate blood specimens were obtained at monthly intervals. All revealed identical results: LDH-1 and LDH-2 migrated more rapidly toward the anode than normal (Fig. 3). Each of these isozymes was homogeneous electrophoretically. However, an additional band not observed in normal hemolyzates migrated slightly anodal to the main band in the LDH-3 position (Fig. 3). The electrophoretic mobility of the main LDH-3 band appeared to be normal (Fig. 3). Leukocyte and platelet extracts revealed isozymes identical in electrophoretic mobility to those of the patient's erythrocytes. Total LDH activity of these three cell types was normal.

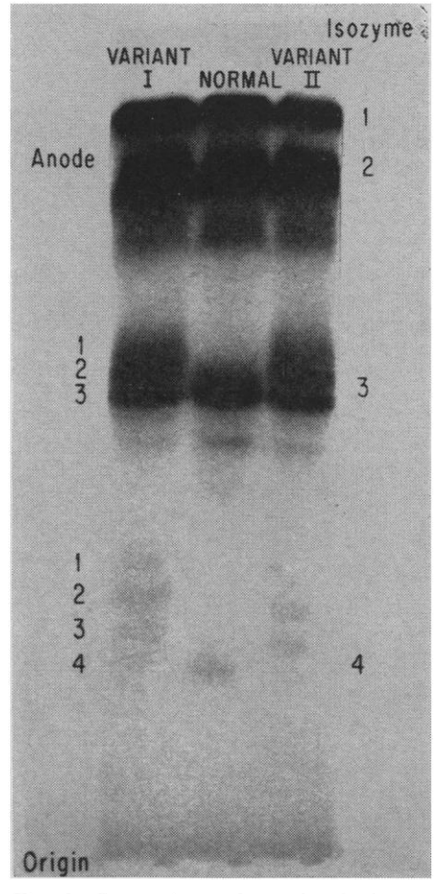

Fig. 2. Comparison of starch-gel electrophoretic pattern of three individuals, one normal and two with A-subunit variants. These two variants appear in the pedigree shown in Fig. 1.

Alteration in the electrophoretic mobility of LDH isozymes has never been described in disease states; the altered isozymic mobility of this individual's B subunit is probably best explained on a genetic basis, an interpretation supported by the identical electrophoretic mobilities of corresponding isozymes from leukocytes, platelets, and erythrocytes. Unfortunately, the hypothesis of homozygous involvement at the *b*-locus cannot be satisfactorily established since the propositus has no immediate family. Furthermore, involvement of both *b*-alleles accounts for the electrophoretic homogeneity and increased mobility of LDH-1 and LDH-2, but fails to explain the apparently normal mobility of the main LDH-3 band, the presence of a rapidly migrating sub-band in the LDH-3 region, and the greater distance between LDH-3 and its more rapidly migrating sub-band than between the normal and variant LHD-1 and the normal and variant LDH-2. The nature of sub-bands, which are consistently observed on starch-gel separation of LDH isozymes (*5*; Figs. 2 and 3), remains obscure; conflicting theories of their origin have been proposed (*7*). Perhaps in the case of certain variant isozymes the folding characteristics of alternative forms might affect the total charge, so that in the case of LDH-3 the form with AABB sequence might exhibit different electrophoretic mobility from the form with sequence ABAB or ABBA. A possible explanation of the apparently normal mobility of the main LDH-3 band is that the variant B subunit is so close in charge to that of the normal B subunit that only LDH-1 and LDH-2, containing predominantly B subunits, reveal differences in mobility between the variant and normal isozymes; these differences may not be ob-

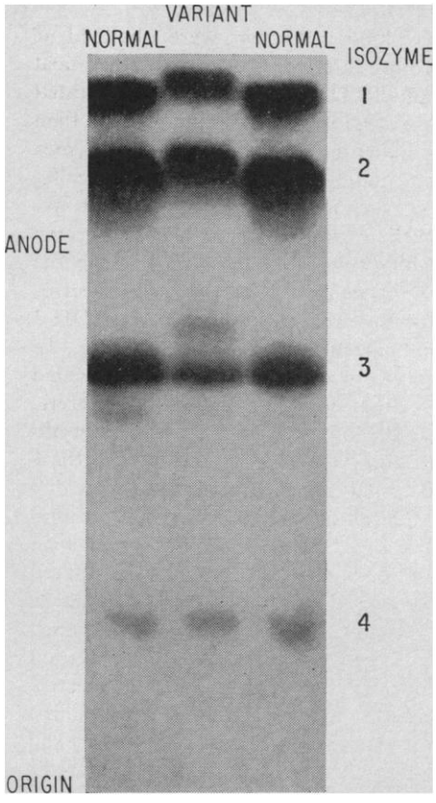

Fig. 3. Comparison of starch-gel electrophoretic patterns of the variant LDH erythrocyte isozymes presumably resulting from double affection at the *b* allele with two normal patterns.

served on the gel in LDH-3 and LDH-4 which contain progressively fewer B subunits and progressively more of the normal A subunits. Isolation and characterization of the variant B subunit are required for the elucidation of these problems. Unfortunately the normal and variant LDH-1 are so close in electrophoretic mobility that recombination experiments in which normal and variant isozymes were mixed and frozen according to the method described by Markert (*1*) failed to shed any light on the nature of the variant B subunit.

The frequency of 0.003 of LDH variants in this study of 1200 patients is of the same order of magnitude as the frequency of 0.009 obtained by Kraus and Neely (*4*) in an investigation of 940 apparently normal hospital employees from Memphis, Tennessee. In this study, in agreement with Kraus and Neely, a higher frequency of variants was observed among Negroes; also, a greater number of A than B variants occurred. Frequencies are best calculated individually for each electrophoretically distinguishable variant. Since the primary structures of isozymes with identical electrophoretic mobilities may differ, it may prove incorrect to assume that two unrelated individuals possess identical point mutations because their corresponding variant lactic dehydrogenase isozymes migrate similarly. Likewise, LDH variants may exist with normal electrophoretic mobility so that the total number of different LDH variants may far exceed that determined solely by electrophoretic screening.

Additional LDH variants have been observed by electrophoretic screening of erythrocytes from various populations (*8*). The populations screened so far have been too small and the number of LDH variants discovered have been too few to determine whether differences in the frequency of variants exist among populations. A greater frequency of variants among Negroes may eventually be established.

The nature of the LDH polymorphism, particularly whether it might confer a selective advantage, such as has been suggested in the case of the sickle-cell heterozygote, is not known. However, if the mutation were neutral, the current frequency of LDH variants in the order of 1 percent in some populations might result solely from the accumulation of mutations over many previous generations. Neutrality is becoming increasingly accepted with the growing number of inherited protein variations which, like the LDH polymorphism, appear to be unassociated with disease. Even if disadvantageous, the LDH polymorphism could still be explained as arising solely from mutation, were it only mildly disadvantageous, and selection against it consequently would be negligible. Alternatively, the observed frequency of variants is also compatible with the notion of transition from an environment in which the mutation had been adaptively advantageous to one in which it no longer was so.

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9 February 1965

Cellular Segregation and Heterocytic Dominance in Hydra

Abstract. *Hydra heterocytes—that is, hydra containing cells from both normal and mutant animals, were used for studying some developmental properties of mixed cell populations. Cells which initially had originated from a nonbudding mutant, and which subsequently became part of a heterocyte of normal appearance, were able to proliferate, to segregate, to come off as buds, and to express their phenotype in those buds. Furthermore, the heterocytes underwent a transformation in which the mutant phenotype eventually became dominant in all instances.*

Somatic cells that spontaneously mutate may profoundly influence adjacent normal tissues. To investigate such influences, I am studying progeny originating from heterografts made between normal and mutant hydra of the same species. Hydra offer experimental advantages for this type of study in that, unlike most other metazoa, hydra can be made to grow continuously and to reproduce solely by asexual means. Consequently, the mutant somatic cells do not die with the host, but are distributed indefinitely among the progeny where interactions with normal cells may be allowed to express themselves.

Developmental mutants of hydra, which rarely have been reported, were obtained in the following manner. We induced mass cultures (*1*) of *Chlorohydra viridissima* (Florida strain, 1961) to form eggs and sperm by maintaining them in "M" culture solution (*2*) for a week without washing. Hatching from the resultant fertilized eggs were a number of mutant hydra having morphological aberrancies. One such animal was used in the experiments described in this report. This hydra was unusual