In the root they presumably function in the transport of the precursors of cutin and mucilage. The "semipermeable membrane" of the functioning region of the root-hair zone is thus a structurally complex entity.

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Lactic Dehydrogenases: Subfractionation of Isozymes

Abstract. *Electrophoresis in polyacrylamide gel of homogenates of various organs from the mouse yields five major lactic dehydrogenase bands. If the gels are treated with β-mercaptoethanol, subsequent electrophoresis produces 15 bands which show lactic dehydrogenase activity. This could be explained if one molecule of nicotinamide adenine dinucleotide (coenzyme) is attached to each of the monomeric subunits of lactic dehydrogenase and if mercaptoethanol can remove the coenzyme only from the muscle type. This is consistent with the hypothesis that intact lactic dehydrogenase is a tetramer.*

There is general agreement that lactic dehydrogenase (LDH) (*1*) from various sources exists in several enzymatically active molecular forms. Appella and Markert (*2*) found that one of the isozymes from crystalline LDH prepared from beef heart could be dissociated into four inactive subunits of equal molecular weight by treatment with 1.25*M* guanidine-HCl and 0.1*M* β-mercaptoethanol. The subunits could be separated into two classes on the basis of charge, and assorting these two kinds of subunits into all possible groups of four would theoretically yield five different molecular forms of LDH, all distinguishable by charge. Cahn *et al.* (*3*) noted that in most vertebrates there are two chief kinds of LDH, one from heart and one from muscle. These types can be distinguished in several ways, including electrophoretic mobility, substrate specificity, and immunological behavior. Cahn *et al.* noted also that sorting into combinations of four would yield five separate molecular forms of LDH. In our laboratory five major LDH bands were observed in homogenates of various mouse organs, and we now describe the splitting of four of them into minor bands to yield a total of 15 LDH bands.

The organs of various strains of mice were frozen in liquid nitrogen. The organs were thawed, weighed, homogenized with 2:1 (wt./vol.) of 0.05*M* tris-HCl buffer, *p*H 8.5 (25°C) in a Potter-Elvehjem glass homogenizer, then centrifuged for 20 minutes at 30,000*g* in a Servall refrigerated centrifuge. The polyacrylamide gels (5 percent acrylamide) were prepared by the procedure of Raymond and Wang (*4*). The gels were allowed to soak in the buffer either with or without β-mercaptoethanol for at least 24 hours. During electrophoresis the gels were cooled continuously on the top and bottom to −5°C while a potential of 16.5 volt/cm was applied. A good separation of the LDH isozymes was obtained in 3 hours. The activity of LDH was detected by the staining technique of Dewey and Conklin (*5*), except that no cyanide was used in the developing solution. The gels were placed directly in the developing solution after electrophoresis and allowed to remain for 2 hours in order for the less concentrated LDH bands to become visible.

Figure 1*a* shows the five major bands of LDH activity normally observed in homogenates of the skeletal muscle of a mouse. Figure 1*b* is the pattern produced by mouse skeletal muscle when 0.005*M* β-mercaptoethanol was added to the gel medium. Fifteen separate LDH bands are visible. The multiple bands arise from four of the major bands. Band 1, the slowest moving major band, divides into five minor bands, band 2 into four, band 3 into three, band 4 into two minor bands. Band 5 does not separate into minor bands. The separation of the major bands into minor bands is dependent on the concentration of β-mercaptoethanol; the most effective concentration is between 0.004*M* and 0.006*M* when 5 μl of the homogenates are used for electrophoresis. Even in the absence of β-mercaptoethanol some of the minor bands were occasionally observed, but never as distinctly as they were in the presence of this reagent. The splitting of the major

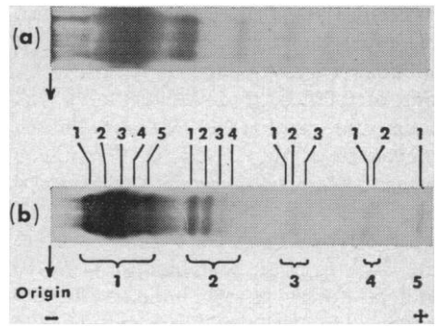

Fig. 1. Electrophoretic patterns of LDH prepared from C57BL mouse skeletal muscle on polyacrylamide gel. (*a*) Without β-mercaptoethanol; electrophoresis for 3 hours, *p*H 8.5, tris-HCl 0.05*M*. (*b*) With 0.005*M* β-mercaptoethanol; electrophoresis for 4 hours 15 minutes, *p*H 8.5, tris-HCl 0.05*M*.

bands by β-mercaptoethanol was also observed when the starch-gel electrophoresis technique of Smithies (*6*, *7*) was used; thus the effect did not result from an artifact introduced by the polyacrylamide gel.

A tentative explanation for the multiple bands is given in Fig. 2. The assumptions are made that each monomeric unit has a molecule of nicotinamide adenine dinucleotide (NAD) attached to it (*8*) and that β-mercaptoethanol can remove those NAD

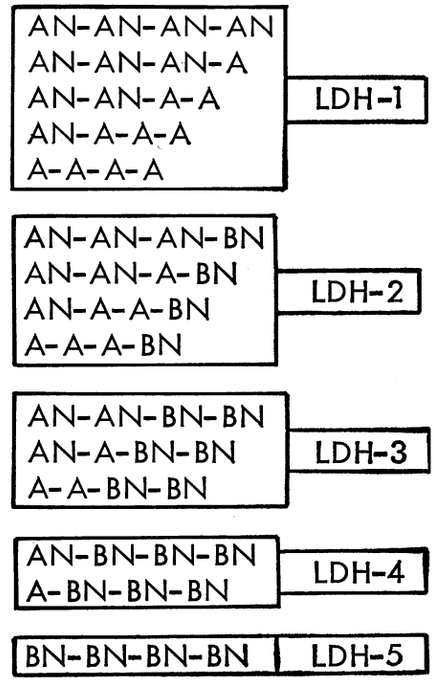

Fig. 2. Explanation for minor LDH bands appearing within five major bands. (*A*) "Muscle" type subunit; (*B*) "heart" type subunit; N, NAD molecule.

molecules attached to A subunits (muscle type) but not those attached to B subunits (heart type). At a concentration of 0.005*M* β-mercaptoethanol, the coenzyme presumably is partially removed from the A units so that in the case of band 1 the five possible combinations of the enzymes and the four coenzymes result, in the case of band 2 the four possible combinations result, and so forth. In this manner the 15 bands can be explained within the framework of the theory that the enzyme molecule consists of two dissimilar monomers (*2, 3*). On the other hand, if NAD is assumed to be completely removed from the B monomer, rather than not being removed at all, the same electrophoretic pattern would be expected (*9*).

The explanation offered for the appearance of the 15 bands of LDH leads to the prediction that a higher concentration of β-mercaptoethanol would completely dissociate the coenzymes from the enzyme, and that the latter would then migrate electrophoretically as a single band. When 0.01*M* β-mercaptoethanol was added to the gel, the multiple bands were no longer visible; each major band of LDH migrated as a compact band.

Furthermore, the presence of a sufficiently high concentration of NAD during electrophoresis should keep a full complement of coenzymes on the enzyme and again result in single bands. Indeed, when 0.01*M* NAD was presented during electrophoresis this was the case, but only in that fewer bands appeared rather than a single band. Since enzymes that degrade NAD occur in muscle homogenates, and could be expected to interfere, further experiments on the purified enzymes will be necessary to elucidate the effect of the presence of NAD on the electrophoretic behavior of LDH.

Two preparations of rabbit muscle LDH (Worthington Biochemical Corp.), recrystallized twice, have been examined, and each shows multiple bands in each of the four slowest major bands and in contrast to the mouse preparation the fastest band, number 5, is also split. The distribution of the sub-bands was the same in both a crude rabbit muscle homogenate and in crystalline preparations, but it was different from the distribution in mouse muscle (*10*).

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1. The following abbreviations are used: LDH, lactic dehydrogenase; NAD, nicotinamide adenine dinucleotide, previously known as DPN.
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Preliminary Pollen Studies at Lake Zeribar, Zagros Mountains, Southwestern Iran

Abstract. *A late Pleistocene* Artemisia *steppe, implying a cool, dry climate, changed about 13,000 years ago to an oak-pistachio savanna, as the climate became warmer. About 5500 years ago the savanna thickened to an oak forest, presumably reflecting an increase in precipitation or decrease in temperature to modern levels.*

As part of a continuing program (*1*) for the study of prehistoric environmental and climatic change in the Zagros Mountains of Iraq and Iran, sediment cores were obtained in 1960 at several lakes and marshes for pollen analysis and other paleontologic and chemical studies. This long mountain arc separates the Mesopotamian piedmont from the Iranian Plateau. Some believe it to be a principal locus for the domestication of plants and animals about 11,000 to 9000 years ago (*2*). The range consists of a series of fold ridges that are 2000 to 3000 m in elevation and separated by long valleys. It receives moisture in the winter from storms traveling from the Mediterranean Sea (*3*), and it bears a forest cover dominantly of oak between elevations of about 700 and 2000 m (*4*). The Iranian Plateau in this region has an elevation of about 1500 m. Because it lies in the precipitation shadow of the Zagros Mountains it is largely barren of trees.

The site chosen for detailed study is Lake Zeribar near the large village of Merivan, Iran, close to the Iraq border and about 160 km northwest of Kermanshah, Iran. It occupies what is probably a structural basin in the inner portion of the Zagros Mountains. The nearby ridges consist primarily of metamorphic rock. The lake basin has an elevation of about 1300 m above sea level. A few small intermittent streams enter the basin, and the lake occasionally overflows to the southeast over an alluvial divide into the headwaters of the Diyala River. The estimated annual precipitation in the region is 600 to 800 mm, and the estimated mean January and July temperatures are respectively 2° and 28°C (*3*).

The lake measures about 3 by 5 km, is almost completely surrounded by a sedge mat, the outer part of which is floating, and is only a few meters deep (*5*). The valley itself is extensively planted in wheat or barley or is used for grazing. The hills bounding the valley are covered largely with oak (*Quercus persica*), which has been reduced to a low tree by wood-cutting and grazing.

The sediment was cored from the edge of the floating mat at two sites on the west side and one site at the south end. A modified Livingstone 1-inch piston corer was used to a depth of about 13 m and a Davis corer below (*6*). The sediment ranged from peat and gyttja in the upper part to clay and silt beneath to a total thickness of 18 m. The base of the sediment was not reached.

The abbreviated pollen diagrams in Fig. 1 are preliminary in nature. About 80 pollen taxa have been identified so far (*7*). This preliminary interpretation of the pollen diagrams is supported by the chemical studies on the same sediments as those reported in the accompanying paper (*8*), and the analyses of Cladocera contained in the cores give promise of providing more information.

The pollen sum used in the design of the diagram includes all trees, shrubs, and wind-pollinated herbs except *Salix* (willow), Gramineae (grasses), and Cyperaceae (sedges). On the basis of the abrupt fluctuations in their abundance, we believe that these three latter pollen types largely represent local lake-margin plants rather than the regional vegetation. The pollen sums counted at different levels range from 47 to 465.

Of the three cores available the longest core (I-12), that used for the