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11. In the course of the running of our final experiment we were informed that H. E. Bömmel and W. F. Libby and their coworkers A. J. Darnell and B. R. Tittman of U.C.L.A. had found that InSb was superconducting. Bömmel informed us that the transition temperature that they found was 2.1°K, and therefore, our result corroborates theirs. We thank Dr. T. H. Geballe for his interest and part in the superconductivity measurements, Dr. R. L. Batdorf for the high-purity InSb crystals, and Mr. A. L. Stevens for technical assistance.

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Root Hairs, Cuticle, and Pits

Abstract. *The filamentous roots of mustard* (Raphanus sativus), *radish* (Brassica nigra), *squash* (Cucurbita pepo), *and wheat* (Triticum aestivum) *are covered throughout their length with living nucleated root hairs which may measure 1600 μ or more. The outer walls of piliferous and nonpiliferous cells consist of successive layers of mucilage, cutin, and the cellulose-pectic framework of the cell. Plasmodesmata and pits occur on all cell walls. Under the electron microscope individual pores and pits in the microfibrillar wall are evident throughout the length of the root hair. The "semipermeable membrane" of the root hair zone is thus structurally complex.*

The term "semipermeable membrane" appears to dominate current discussion of the entrance of solutes into the root-hair zone of the higher plant, while the structure of the outer epidermal wall of the root is ignored. Cutinization of root hairs is evident in the Windsor bean (*Vicia faba*) and the castor bean (*Ricinus communis*) (*1*). The outer epidermal wall of the root of the onion (*Allium cepa*) is also cutinized (*2*). Root hairs are consistently rare or lacking in onion bulbs grown in water in the laboratory. As seen under the light microscope, with the use of appropriate microchemical tests, the onion root wall consists of a pellicle of mucilage and a cuticle, beneath which lies the cellulose-pectic framework of the cell (*3*). All cell walls are pitted so that epidermal and cortical protoplasts are interconnected. Plasmodesmata are anchored in the pits in the outer tangential wall and presumably function in the transport of the precursors of mucilage and cutin.

The roots of seedlings and of mature plants of the common species, mustard (*Brassica nigra*), radish (*Raphanus sativus*), squash (*Cucurbita pepo*), and wheat (*Triticum aestivum*) provide excellent material for the study of root hairs. These species and others were grown in ordinary garden soil during the summer, fall, and winter of 1962–63.

The root system of all species consists of a primary or tap root with numerous laterals of the first, second, and higher order. In addition, however, all seedling roots and the feeder roots of the older plants possess a wealth of filamentous or transitory roots which may equal in length the tap roots and the longest laterals. In seedlings, all filamentous roots, frequently 15 cm or more long, are entirely covered with living nucleated root hairs. In contrast, as is well known, the root-hair zones of the main roots generally measure not much more than 1 cm. The root hairs of the main roots and of the filamentous roots range in length from 2 or 3 μ in the initial papillae to 1600 μ or more.

For examination under the electron microscope, the filamentous roots of mustard, radish, squash, and wheat, after brief clearing, were ultrasonically fragmented, mounted on Formvar-coated grids, and shadowed with palladium. Ultrasonic fragmentation has proved to be a useful technique in the study of the cell wall, since cell orientation and structure are demonstrated by isolated cells, three-dimensional segments, and fragments of the various layers of the cell wall. The contraction of the microfibrillar wall, inevitable in fixation, is avoided.

The root hairs of all four species are similar in submicroscopic structure. The primary wall of the youngest cells examined, piliferous and nonpiliferous, consists of a network of cellulose microfibrils. The wall of the piliferous cell, cell body and root hair, is thickened by the deposition of additional reticular microfibrils that are later generally parallel in orientation (*4*). Amorphous substances, pectins or noncellulose polysaccharides, fill the minute interfibrillar spaces except in the pit areas. Plasmodesmata occur in the outer epidermal wall and also throughout the entire length of the root hair, from tip to base (Fig. 1, *A* and *B*). They appear as the ends of minute protoplasmic strands. When plasmodesmata are removed during the preparation of material, solitary pores and pits, defined here and in previous papers as groups of pores, remain as visible perforations (Fig. 1, *A* and *B*). The cuticle of the epidermis, including the root hairs, when isolated, resembles in its ultraporous texture the cuticle of the onion leaf (*2*). In the leaf of the higher plant the plasmodesmata function in the transport of the precursors of cutin and wax, if present.

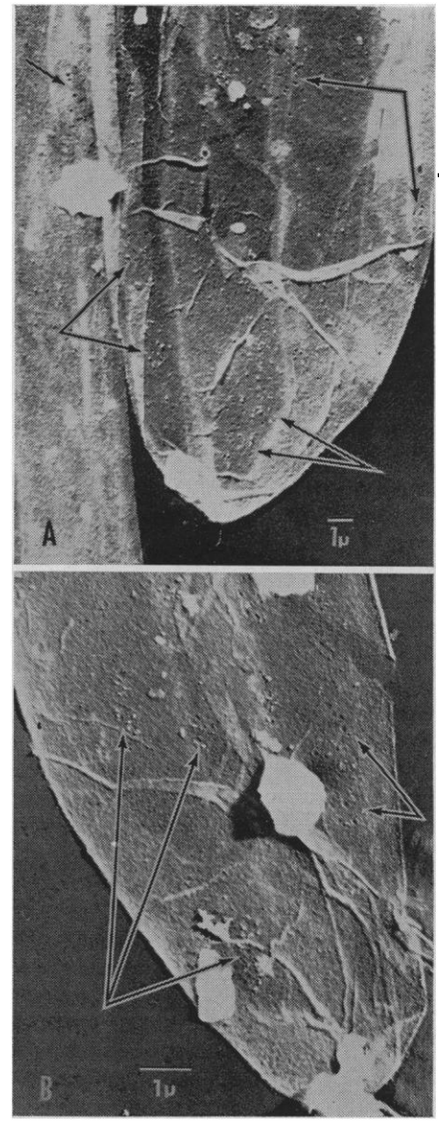

Fig. 1. *A*, Electron micrograph of squash root-hair tip and part of another root hair. *B*, Electron micrograph of wheat root-hair tip. Arrows indicate examples of pits and pores, with and without plasmodesmata.

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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28 January 1963

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

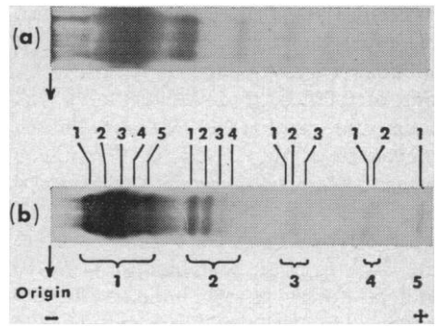

Fig. 1. Electrophoretic patterns of LDH prepared from C57BL mouse skeletal muscle on polyacrylamide gel. (*a*) Without β-mercaptoethanol; electrophoresis for 3 hours, *p*H 8.5, tris-HCl 0.05*M*. (*b*) With 0.005*M* β-mercaptoethanol; electrophoresis for 4 hours 15 minutes, *p*H 8.5, tris-HCl 0.05*M*.

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

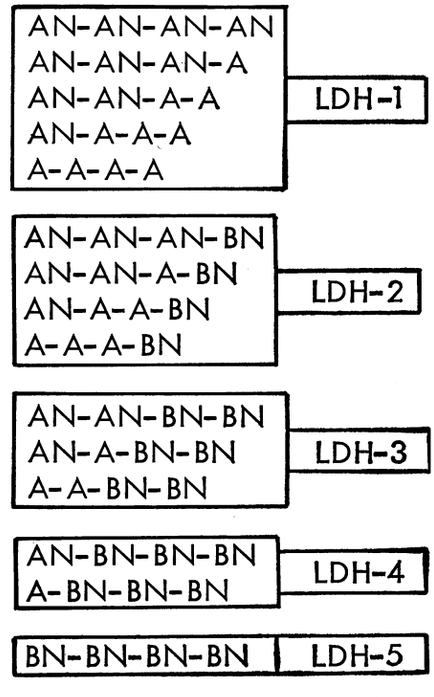

Fig. 2. Explanation for minor LDH bands appearing within five major bands. (*A*) "Muscle" type subunit; (*B*) "heart" type subunit; N, NAD molecule.