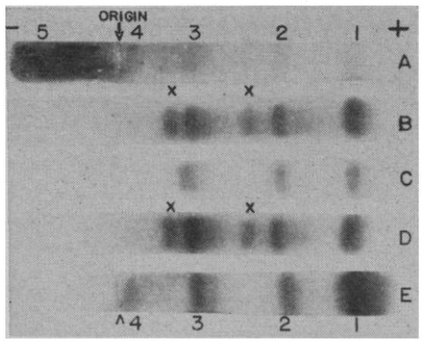


Fig. 1. Starch-gel electrophoretic pattern of lactate dehydrogenase in homogenates from adult bat tissues. (*A*) liver; (*B*) active testis; (*C*) inactive testis; (*D*) active testis; and (*E*) heart. All homogenates were prepared with one part of tissue and five parts of distilled water (weight to volume) and subjected to electrophoresis simultaneously in the same starch block. Numbers and **x** indicate the position of the corresponding isozymes.

tissues, subbanding was a common feature, especially in the LDH-1, LDH-2, and LDH-3 areas.

Homogenates from active testis exhibited two additional bands. One with an electrophoretic mobility intermediate between those of LDH-2 and LDH-3, and another migrating close behind LDH-3 (Fig. 1). These bands were detected only in testes and epididymides containing mature spermatozoa. None of the subbands revealed in most tissues showed the same mobility. The relative rates of migration of all the fractions did not change when different buffer systems [tris(hydroxymethyl)amino methane-borate-ethylenediaminetetraacetate (*8*) and borate (*7*)], and different *p*H's (from 7 to 8.6) were used. Staining with $\alpha$-hydroxybutyrate and $\alpha$-hydroxyvalerate as substrates instead of lactate gave better activity at the site of the two "extra" bands than at the other LDH areas. Such affinity for those $\alpha$-hydroxy acids is a characteristic frequently observed with LDH-X from different species (*1–3*). These findings indicate that the additional bands in active testes from bats can be regarded as LDH-X.

Multiple bands "X" have been previously observed. They could be formed if more than one additional polypeptide is being synthesized, or if the extra polypeptide C is able to recombine in vivo with any of the chains (A or B) from the common isozymes. Both possibilities have been demonstrated in pigeons (*2*). At present, we do not have data to establish which mechanism is responsible for the appearance of two LDH-X's in bat testis. In other animals showing two additional bands, evidence indicates that one of the molecular forms is a homopolymer of C polypeptides, and the other is a heteropolymer of C and B (pigeons) (*2*) or C and A chains (guinea pigs) (*9*).

It has not been determined which of the cells in seminiferous tubules begins to synthesize the new polypeptide. Testes with cells in stages of differentiation up to primary spermatocytes did not possess the additional isozymes in demonstrable amounts; they must appear in a later stage. Although studies of the isozymic complement of separated sperm could not be performed, data presented here and observations accumulated on other species permit the assumption that LDH-X of bats is a lactate dehydrogenase fraction of mature spermatozoa.

An interesting phenomenon is the cyclic appearance of the enzyme. There is an extraordinary timing in the activation of the gene responsible for the synthesis of additional polypeptides. The factors involved in that activation are unknown. Probably, hormonal changes accompanying sexual cycles are related to the "turning on" of genes for LDH-X.

The demonstration of periodic isozymic changes like these shown in bat testes provides material for studying the mechanisms regulating genetic expression.

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## Senckenberg Lignite: A Lignitized Wood with Apparently Original Cellulose and Lignin

Abstract. *A lignitized wood of Miocene derivation has been identified to the genus* Taxodium. *The cell walls of latewood tracheids are Mäule positive. A structurally intact lignin residue is obtained after incubation of the wood in 72 percent sulfuric acid. Cellulose persists as a structural polysaccharide in the lignitized wood.*

The layers of plant cell walls are variously resistant to degradation. Thus, in peat and other sediments associated with the Boylston Street Fishweir of Boston, Massachusetts, cellulose of the primary cell wall is well preserved in a wide range of primary and secondary tissues (*1*). In the secondary wall of tracheary elements from the same source, cellulose is considerably degraded; the secondary wall in many instances persists largely as a lignin residue. However, in tracheids of a hardwood specimen in peat (Pleistocene) from Griffin Hill, Massachusetts, both the lamellation of cellulose and the distribution of lignin in the cell wall closely resemble that characteristic of tracheids of living material (*2*).

Small specimens of a Miocene lignite from Senckenberg, Germany (*3*), about 1 by 1 by 2 mm were deminer-

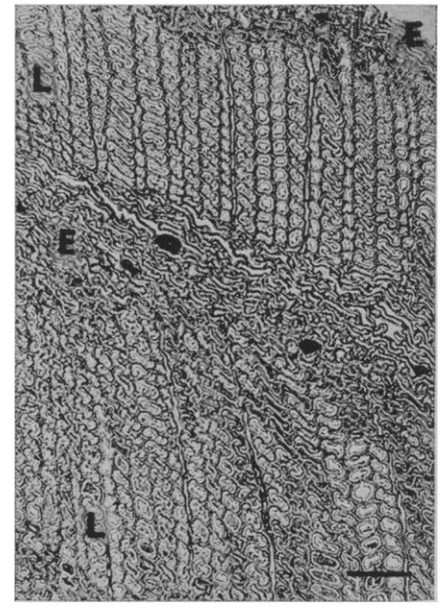


Fig. 1. Bright-field micrograph of an unstained transverse section of lignitized wood from Senckenberg, Germany. The earlywood (*E*) is compressed whereas well-defined radial files of tracheids persist in the latewood (*L*) of each annual increment. Scale line represents 100 $\mu$.

alized in concentrated hydrofluoric acid (4 to 14 days), washed in running tap water (2 days), and deaerated for 2 hours in a vacuum oven. Tissue specimens were dehydrated in an ethanol series and embedded in a polymerized methacrylate mixture consisting of butyl methacrylate and methyl methacrylate (5:1), and 2 percent benzoyl peroxide. Embedded lignite was sectioned (1 to 3 $\mu$) on a sliding microtome, and sections were dried on glass slides. The methacrylate matrix and extractives were removed with xylene and a mixture of ethanol and benzene (1:1), respectively. Coherent tissue residues were either processed microchemically or mounted in Permount for anatomical study, or both.

The tissue residue of the axial and horizontal systems of cells in the latewood (Fig. 1) is structurally well preserved. The relatively thin-walled cells of the earlywood (Fig. 1) are by contrast considerably compressed and unidirectionally sheared.

Following several standard keys (*4*), we identified the lignitized wood as *Taxodium* or as a genus very similar to *Taxodium*. Thus in the radial walls of earlywood tracheids, which in a few instances are not compressed, large bordered pits occur in opposite arrangement and in short vertical files. Axial parenchyma in latewood is diffusely distributed, and rays are uniseriate and wholly parenchymatous. *Taxodium* is highly represented in the brown coal of Central Europe (*5*).

Cellulose was detected with the polarizing microscope and by staining responses to zinc-chlor-iodide in this lignitized wood. Between crossed nicols in transverse sections of latewood tracheids two birefringent zones are observed both in undelignified and partly delignified tissue (Fig. 2). The outer bright birefringent layer corresponds to the primary wall plus the $S_1$ layer, the outer layer of the secondary wall. The thicker and more diffusely birefringent inner zone is the $S_2$ wall layer. Examination of sections of this wood from which the lignin had not been removed in a graded series of liquids of known refractive indices between 1.500 and 1.700 indicates further that the birefringence observed here is intrinsic and not derived from the form birefringence of a lignin residue.

In bright-field the initially yellow-tan wall substance of latewood tracheids becomes differentially stained in zinc-chlor-iodide. In this cellulosic reagent (*6*) wall layers which are otherwise

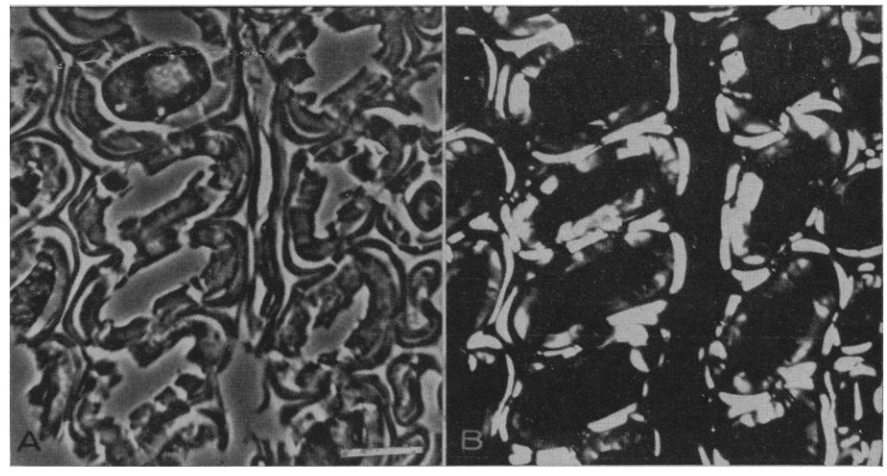


Fig. 2. Transverse section of latewood, partially delignified by repeated chlorination and extraction in ammonia (10 percent by weight). (A) Phase-contrast micrograph of a largely holocellulose residue. (B) Same section between crossed nicols, showing birefringence of wall layers. Scale line represents 20 $\mu$.

birefringent are variously stained brown, brown-red and several shades of light gray-blue. The typical blue coloration of cellulose in zinc-chlor-iodide is never obtained. It is significant that isotropic wall layers remain yellow-tan throughout prolonged incubation in zinc-chlor-iodide.

The evidence then suggests that cellulose persists in Senckenberg Lignite as a structural polysaccharide. However, the abnormal reactivity of lignitized cellulose to zinc-chlor-iodide may be indicative of incipient degradation of this cell-wall substance in the coal-producing sediment. On the other hand, it cannot be excluded that the necessary but rather drastic measures used in demineralization and delignification of the specimen are causal in the abnormal response of the cellulose in zinc-chlor-iodide.

Latewood was incubated for 16 hours in $H_2SO_4$ (72 percent by weight) to remove the holocellulose cell-wall fraction (Fig. 3). The lignin residue is coherent without noticeable tendency toward swelling; this possibly indicates a low polysaccharide content. However, in gymnospermous woods, the normally slight tendency toward swelling in 72 percent $H_2SO_4$ is reduced by extended previous treatment in hydrofluoric acid (*7*). Pits with reduced borders and intact membranes (Fig. 3) are observed in the tangential and radial walls of latewood tracheids.

Microchemical tests indicate that in transverse sections of late wood the cell-wall material is Mäule positive, a color reaction to show the presence of lignin, regardless of whether chlorination is by permanganate–hydrochloric acid (*8*) or directly by chlorine water (*9*) and regardless of whether the holocellulose is present or extracted previously. Similar tissue is Wiesner negative, another color reaction for lignin, a result not surprising for previous treatment of softwoods with hydrofluoric acid prevents the development of the red coloration usually obtained when fresh material is

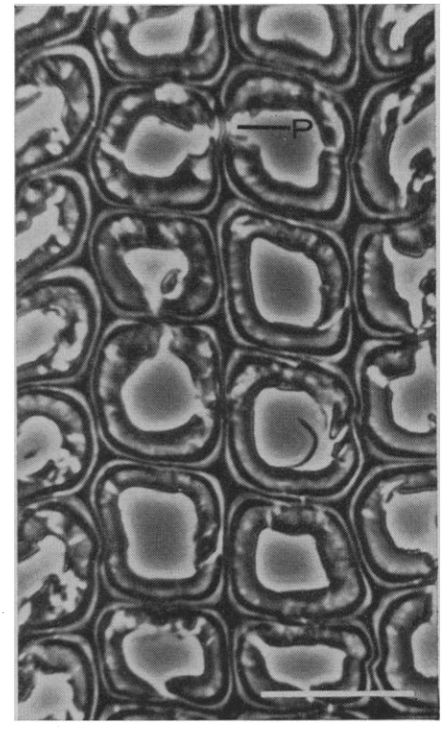


Fig. 3. Phase-contrast micrograph of a transverse section of latewood from which holocellulose is removed by extraction for 16 hours in sulfuric acid (72 percent by weight). The structural characteristics of the wood at the cellular level are retained in the lignin residue; *P*, pit membrane. Scale line represents 23.5 $\mu$.

incubated in phloroglucinol–hydrochloric acid (*7*).

After demineralization of this lignite a coherent tissue residue is obtained which retains much of the structural pattern of the original wood. Included in the preserved organic wall substance are substantial amounts of cellulose and lignin or a lignin-like material.

This lignitized wood thus consists of a mineralized phase in which much of the original cell-wall substance is retained in a highly organized state. The mineralization of this specimen may have slowed or arrested the degrading forces active upon the organic substance of the cell wall at an early stage in the deposition of the original plant material. By contrast, in a ground thin section of *Taxodium* also of Tertiary age, there was considerably more degradation of the secondary cell wall (*1*). It appears that the preservation of the organic substance of the cell wall in mineralized woods may depend upon the time at which mineralization begins relative to the degradative changes which occur during the initial stages in the development of a sediment.

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## L-Tyrosine-3,5-$^3$H Assay for Tyrosinase Development in Skin of Newborn Hamsters

Abstract. *Tyrosinase in hamster skin, measured by the production of $^3$HOH from* L*-tyrosine-3,5-$^3$H, increases about sevenfold in specific activity from the 14-day fetus to day 6 of life. Albino hamsters exhibit no activity, but enzyme is present in the ventral (white) areas of the Syrian hamster. 3,4-Dihydroxy-*L*-phenylalanine is required as a cosubstrate.*

The low levels of tyrosinase in skin have been difficult to measure in small samples because there has been no sensitive assay. The enzyme was detected in mouse skin and in fetal guinea pig skin by manometry (*1*) and in the skin of mice, goldfish, reptiles, frogs, and rats (*2*, *3*) by measurement of the incorporation of L-tyrosine-$^{14}$C into melanin, by the method of Kim and Tchen (*2*). This latter method, although more sensitive than manometry, is inconvenient because it requires incubations of 16 to 24 hours. It may also lead to errors because the reaction rate is probably not constant for that period, and because melanin, the product measured, is far removed from the substrate and is itself of variable composition. The melanin assay has been used by Coleman (*2*) and by Chen and Chavin (*3*) to follow development of tyrosinase in skin of young pigmented mice and rats, respectively.

A sensitive assay (*4*), which measures $^3$HOH released from L-tyrosine-3,5-$^3$H as a result of the tyrosine hydroxylase activity of tyrosinase, was introduced to study the enzyme in melanoma. Hall and Okazaki (*5*) used this method to demonstrate tyrosinase in the feather tracts of male weaver birds, and more recently it was applied to the assay of tyrosinase in adult rat skin (*6*). The method requires only a 1-hour incubation, during which the reaction rate is constant. It is used to investigate skin tyrosinase changes occurring during late fetal and newborn life in hamsters.

Randomly bred Syrian golden hamsters (Lakeview Hamster Colony, Newfield, New Jersey) have a gestation period of 16 days. In some instances dated pregnant hamsters were killed by ether anesthesia and the fetuses removed on days 14, 15, and 16 of gestation. For newborn animals the day of littering was designated as day 1 of life. Animals were decapitated and, after removal of the tail and limbs at the first joint, the body skin was peeled off and scraped to remove visible fat. It was then frozen for later assay or used immediately. The skin was blotted, weighed, minced, and then homogenized in a threefold volume of 0.02*M* sodium pyrophosphate buffer, *p*H 7.4, in the micro attachment of the VirTis homogenizer. For animals of day 15 of gestation to 4 days of life, two or three skins were pooled for each homogenization, while single skins of older animals were used. Skin from 14-day fetuses was very fragile, and it was not possible to remove it in one piece or to obtain a wet weight. Consequently, skin from 14-day fetuses in several litters was pooled in groups, and each was homogenized. The DNA contents of homogenates were measured (*7*) with calf thymus DNA as standard and the enzyme activity was expressed as micro-

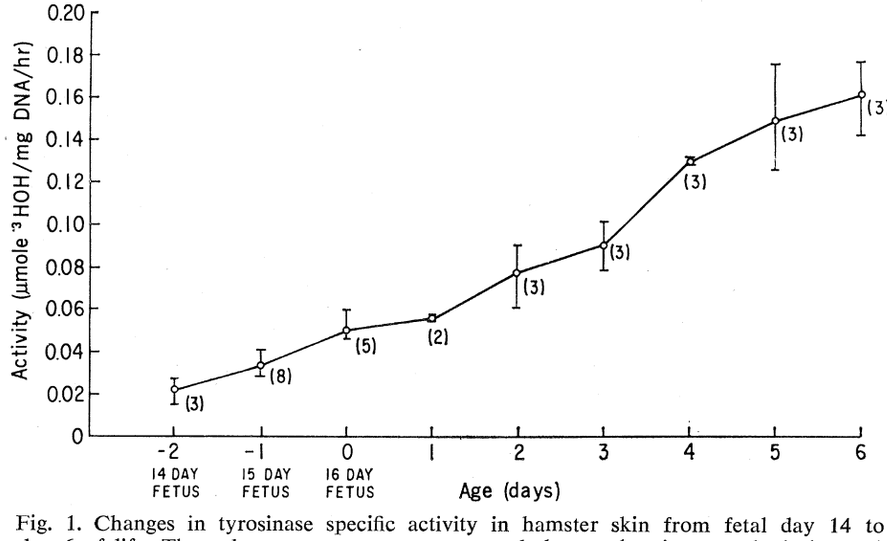


Fig. 1. Changes in tyrosinase specific activity in hamster skin from fetal day 14 to day 6 of life. The values are means ± range, and the number in parenthesis by each value is the number of homogenates for that age.