group of animals had significantly higher levels of activity than the older group.

Inasmuch as sulfatase is known to be present in some leukocytes (*4*), and because some blood was present in the tissues used, we were concerned whether the sulfatase present was due to contamination with blood. The metaphysis, which also contains appreciable amounts of myeloid tissue and blood, showed greater activity per unit weight than bone marrow alone, in the case of the younger animals. Thus the presence of high sulfatase levels in the metaphysis could not be attributed solely to the presence of marrow. The epiphyseal, articular, and costal cartilages had relatively little blood, and it is not likely that this would affect the results appreciably.

The mechanisms by which sulfatase acts in growing bone is not known. Yamagata *et al.* (*5*) found that sulfatase by itself does not have any effect on chondroitin sulfate. In preliminary experiments, we have found no release of sulfate from proteinpolysaccharide incubated with sulfatase. Saito *et al.* (*5, 6*) have shown that a release of sulfate from chondroitin sulfate prepared from cartilage does occur if the chondroitin sulfate is degraded with other enzymes prior to treatment with sulfatase.

The presence of sulfatase as well as a protease and perhaps other degradative enzymes in the growing region of bone, and particularly the metaphysis, suggests that desulfation of the sulfated glycosaminoglycan part of the proteinpolysaccharide may occur here, but the function of the sulfatase in this region has not yet been demonstrated.

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25 March 1969

Testicular Lactate Dehydrogenase Isozyme: Cyclic Appearance in Bats

Abstract. *In male bats* Tadarida brasiliensis *spermatogenesis occurs only during winter and spring. Two additional lactate dehydrogenase isozymes appear in testes containing mature spermatozoa. This type of isozymic change implies cyclic activation of genes and affords valuable markers for study of the factors involved.*

An additional isozyme of lactate dehydrogenase (LDH) (L-lactate: nicotinamide-adenine dinucleotide oxidoreductase, E.C. 1.1.1.27) occurs in homogenates of mature testes from many species (*1*). This isozyme, designated LDH-X, appears at the time of sexual maturation and is a tetramer of polypeptides (C) different from the A (M, muscle type) and B (H, heart type) monomers composing the five isozymes common to most tissues. Evidence obtained in a study of pigeon testes clearly indicated that the synthesis of the additional polypeptide is controlled by a third genetic locus (*2*). The LDH-X isozyme is the predominant LDH fraction in spermatozoa and has some peculiar catalytic properties suggesting a very specialized role (*3*).

The tissue specificity and close relationship of LDH-X with spermatogenesis raise the question of whether this isozyme shows a cyclic appearance in those species with a seasonal pattern of sexual activity. Males from some species of bats follow a seasonal sexual cycle. Copulation takes place only during a defined period of the year. The season and length of this period vary from one species to another and it also varies from one region to another within the same species (*4*).

To study the correlation between sexual activity and testicular LDH, we investigated histological and isozymic changes in testis of *Tadarida brasiliensis*, a species of bats widely distributed in the American continent, from the United States to Argentina and Chile (*4, 5*). Females are monoestrous and fecundation occurs only at the beginning of spring (*4*).

Throughout a period of 2 years, 133 adult males were collected at two different sites in the province of Córdoba (Argentina). Histological sections of testes did not show spermiogenic activity in animals obtained between the end of spring (November to December in Argentina) and early autumn (March to April). Testes were atrophic (average weight, 9.6 mg); seminiferous tubules had only Sertoli cells and quiescent spermatogonia. Spermatogenesis started during the fall (April to May); there were frequent mitoses and numerous spermatocytes, but no spermatozoa. Animals caught in winter and the beginning of spring (June through October) exhibited enlarged testes (average weight, 37 mg) with reduction of interstitial tissue and wider tubules showing the complete sequence of gametogenesis, including mature spermatozoa. Epididymides were full of sperm in specimens taken in September and October.

In 63 females captured during the same period, pregnancy was observed only from October to December. No pregnant animals were obtained at any other time.

This sexual cycle of *Tadarida brasiliensis* in Argentina is similar to that reported by Sherman for *T. cynocephala* in Florida and by Davis *et al.* for *T. brasiliensis* in Texas (*6*).

Animals were killed with ether, and heart, liver, kidney, stomach, intestine, skeletal muscle from several regions, uterus, testis, and epididymis were removed immediately and processed at once or stored at −20°C for a few days. Results were the same with fresh or frozen organs. Tissues were ground in a Potter-Elvejhem homogenizer with distilled water. Extracts were separated by centrifugation at 20,000*g* for 20 minutes at 4°C. Supernatants were subjected to electrophoresis on starch gel (*7*) made with 12 g of hydrolyzed starch for each 100 ml of 0.008*M* citrate-phosphate buffer, *p*H 7.0. The electrode vessels contained 0.15*M* citrate-phosphate, *p*H 7.0. A voltage gradient of 6 volt/cm was applied for 14 hours at 4°C. After electrophoresis, the gels were stained for identification of lactate dehydrogenase by the method of Zinkham *et al.* (*1*).

In most tissues, five lactate dehydrogenase isozymes were observed. Their relative distribution was similar to that observed in preparations of the same tissues from other species. The isozyme closest to the anode, LDH-1, predominates in heart- and breast-muscle extracts; LDH-5, closest to the cathode, is the dominant band in liver and leg muscle. Inactive testis showed only LDH-1, LDH-2, and LDH-3. In many

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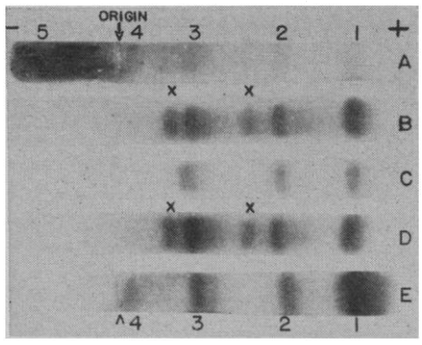

Fig. 1. Starch-gel electrophoretic pattern of lactate dehydrogenase in homogenates from adult bat tissues. (*A*) liver; (*B*) active testis; (*C*) inactive testis; (*D*) active testis; and (*E*) heart. All homogenates were prepared with one part of tissue and five parts of distilled water (weight to 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 α-hydroxybutyrate and α-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 α-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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28 January 1969; revised 11 March 1969

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-

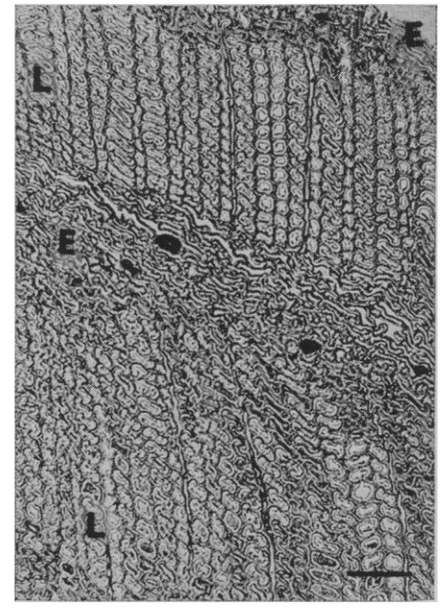

Fig. 1. Bright-field micrograph of an unstained transverse section of lignitized wood from Senckenberg, Germany. The earlywood (*E*) is compressed whereas well-defined radial files of tracheids persist in the latewood (*L*) of each annual increment. Scale line represents 100 μ.