bules could precipitate enough uric acid to clog the tubules (*3*, *6*). Actually, though it has not been suggested previously, I propose that the structure of the avian kidney makes clogging unlikely in the countercurrent multiplier method of urine concentration which depends on reabsorption of water from the collecting tubules. As the collecting tubules course toward the ureter and become ureteral branches, they fuse successively and thus gradually increase in size (*3* and Fig. 1, *C* and D_{1-3}). In effect this means that, unlike mammalian ureters, avian ureters are continuous with the collecting tubules (*3*). Thus the pronounced milking action of a bird's ureters (*7*) can pull a sludge of uric acid out of the collecting tubules. Also, the mucus produced by the glands of the collecting tubules (*3*) facilitates the passage of this uric acid through the collecting tubules.

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18 February 1965

Lactate Dehydrogenases in Trout: Evidence for a Third Subunit

Abstract. *Various tissues of the brook trout contain as many as nine forms of lactate dehydrogenase, an indication that at least three polypeptide subunits synthesized under the control of three nonallelic genes take part in the lactate dehydrogenase composition of this species. There was no evidence of a spermatozoan-specific lactate dehydrogenase.*

The occurrence of five molecular forms of lactate dehydrogenase (LDH) in tissues of most vertebrate classes has been well documented (*1*). Synthesis of the catalytically active protein requires the assembly of two classes of polypeptide subunits (A and B) into a tetramer. Since the polypeptide monomers are separable on the basis of charge, their random association would be expected to produce as many as five electrophoretically distinguishable isozymes of lactate dehydrogenase. On the basis of monomeric composition, LDH-1 (A^0B^4) and LDH-5 (A^4B^0) would thus represent two different proteins whose synthesis was controlled by two separate genetic loci. One gene regulates the synthesis of polypeptide A and the second of polypeptide B. This hypothesis is supported by chemical analysis of the amino acid composition of LDH-1 and LDH-5 (*2*), immunochemical analysis of antigenic properties of the two isozymes (*3*), and dissociation in vitro of tetramers and recombination of monomers to form LDH-2, LDH-3, and LDH-4 (*4*). Additional evidence is provided by the observations of variant isozymes in deer mice (*Peromyscus maniculatus*) and in human red blood cells, presumably produced by mutation of the gene at the B-locus (*5*), and similarly by a report of an A-locus mutation in human erythrocytes (*6*).

There is now compelling evidence for a third genetic locus active only in the testes of sexually mature mammals and birds (*7*) and responsible for the synthesis of the spermatozoan-specific LDH which was described independently by Blanco and Zinkham and by me (*8*). Thus, there is contained in spermatozoa a third class of subunit, C, which confers distinctive properties on the LDH it composes and which can combine with B monomers from LDH-1, at least in vitro (*9*). Presumably, control of synthesis of polypeptide C is associated with repression of the C-locus in immature testes as well as in the various other cell types in the animal. I now present evidence for the occurrence of an active C gene locus in all tissues of the speckled trout, *Salvelinus fontinalis*.

Multiple forms of LDH were separated by electrophoresis on polyacrylamide gels and localized on the gels as previously described (*9*). Organs were removed from the freshly killed fish, washed in ice-cold 0.1*M* sodium phosphate buffer to remove excess blood, blotted with filter paper, weighed, and then disrupted in a glass homogenizer in an amount of buffer sufficient to make a 10-percent homogenate. Spermatozoa were treated in

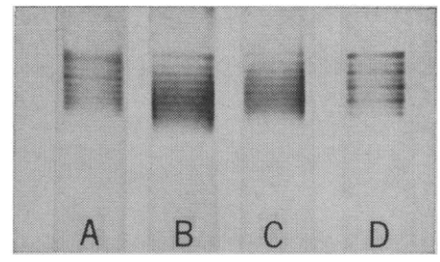

Fig. 1. LDH isozyme patterns in speckled trout tissues. The sample was applied to the top of the gel and the proteins migrated toward the anode. *A*, muscle; *B*, testes or sperm; *C*, heart; *D*, ovary.

approximately the same way except that the cells were washed by centrifugation and disrupted by high-frequency sound. Testes were homogenized and then disrupted by high-frequency sound. Skeletal muscle was first disrupted in a blender and then homogenized. All of the extracts were centrifuged at 4°C for 20 minutes at 10,000*g*, and the supernatants were used for assay. The LDH activity in the extracts was assayed spectrophotometrically by measuring the oxidation of reduced nicotinamide adenine dinucleotide (NAD) at 340 mμ with pyruvate as substrate. On this basis, it was possible to add to each gel samples of the various preparations containing the same LDH activity as measured by the changes in absorbancy per minute. Usually an amount of extract causing a decrease in absorbance of 0.10 per minute was added to a gel. Extracts were prepared from heart, muscle, testes, sperm, and ovary.

Most of the tissues examined had as many as nine isozymes of LDH but none of these isozymes were unique to the sperm cell. Tissue specificity of LDH components is reflected only by differences in relative activity of the isozymes (Fig. 1). The most rapidly migrating anodal forms were most active in the heart preparation as compared to the approximately equal activity of the testicular isozymes. The muscle LDH pattern was comparable to that of the heart, which suggests that there is no predominant muscle or heart form of the enzyme in this species (*3*). Perhaps the most distinctive pattern was observed in extracts of ovary where there were five bands indicating intense activity, with four bands of lesser enzyme activity interspersed. The mobilities of corresponding isozymes from each tissue were coincident as indicated by the appear-

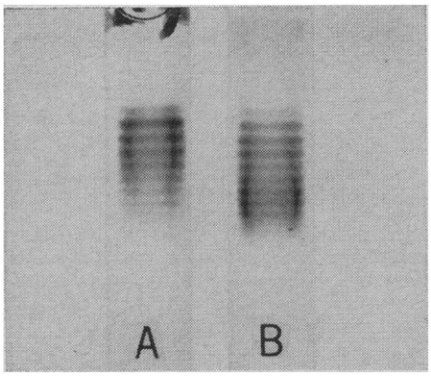

Fig. 2. LDH isozyme patterns in splake muscle (*A*) and heart (*B*).

ance of only nine bands on gels to which extracts from the various tissues were added together.

It was not possible to discriminate between the isozymes on the basis of differential activity with α-hydroxybutyrate, α-hydroxyvalerate, and the acetylpyridine analogue of NAD (*3*, *8*, *9*). Each form of LDH showed approximately the same response to these agents as judged by the deposition of formazan at the appropriate site on the gel. In all cases, lactate was the preferred substrate (five times more active as measured by the time required for bands to appear on gels), and NAD the preferred coenzyme (three times more active than with acetylpyridine adenine dinucleotide). All of the isozymes were inhibited by $2.5 \times 10^{-2}M$ sodium oxalate and by $1 \times 10^{-3}M$ sodium pyruvate (*9*). Activity of each isozyme was decreased by about 50 percent in the presence of 2*M* urea (*10*).

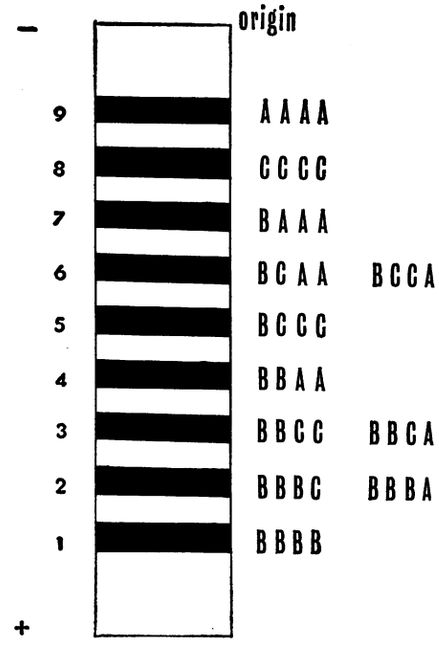

Fig. 3. Diagrammatic representation of LDH isozymes showing proposed subunit composition of LDH-1 to LDH-9.

Two other members of the family Salmonidae, *Salvelinus namaycush* (lake trout) and *Salmo gairdneri* (rainbow trout), were examined for LDH isozyme pattern. *Salvelinus namaycush* showed LDH bands with mobilities similar to those from tissues of the closely related species *S. fontinalis*. Extracts of lake trout testes contained LDH forms coincident with LDH-1 to -4, heart LDH coincident with LDH-1 to -5, and muscle LDH coincident with LDH-1, -2, -5, -6, and -7 of brook trout tissues. The hybrid (splake) obtained by crossing a lake trout female with a speckled trout male had LDH components in heart and muscle extracts comparable to those of the speckled trout male (Fig. 2). Embryos obtained from a cross between the female splake and the male speckled trout also contained nine forms of LDH. Rainbow trout tissues contained a completely different isozyme pattern in heart, muscle, and ovary, with two, or at best three, forms of LDH poorly resolvable in this system.

In order to fit these data within the framework of the subunit hypothesis of LDH structure it is necessary to postulate the activity of a third gene participating in the synthesis of the multiple forms of this enzyme. The tissue differences in activity of the isozymes is an argument in favor of such a subunit scheme. If there is an interaction of three subunits to form tetramers, one would predict the formation of 15 forms of LDH. Therefore, the fact that only nine forms are resolved in this system must be accounted for. There are at least three possibilities: (i) A-C or A-B-C combinations of monomers are not formed; (ii) A-C or A-B-C combinations are enzymatically inactive, perhaps due to problems of conformation; or (iii) the charge difference between A and C subunits is too small for electrophoretic resolution of their constituent tetramers. The third proposal seems most likely, with the scheme presented in Fig. 3 as one possibility to account for the patterns obtained. However, no matter what the monomer composition, the suggestion of a third genetic locus seems reasonable, particularly in view of the observations cited concerning the control of the spermatozoan-specific LDH in mammalian and avian testes.

One may speculate that the mechanism by which this C gene is repressed is not operative, or perhaps has not evolved in the fish species studied here. My data require further refinement before the results obtained with the lake trout and the hybrids can be interpreted, except that the data do support my observations on speckled trout since there is some indication of nine isozymes in *S. namaycush* (*11*).

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12. Supported by NSF grant GB-1771. I thank J. P. Cuerrier, chief limnologist, Canadian Wildlife Service, for providing the speckled trout and splake.

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Convection Plumes from Ulmus americana L.

Abstract. *An unusual example of convection was recently seen above large elm trees shortly after sunset. The condensation of transpirational moisture in air that was cooler than that within the tree crowns is believed to account for the readily visible convective motion above the trees.*

Although exchange processes between plants and air occur throughout the biosphere (*1*), rarely can we see such phenomena with the unaided