examined with an electron microscope.

Fossil pollen grains previously studied by electron microscopy were picked out of the sediments by capillary tube (*11*) or by needle (*12*). The present development of the technique makes it possible to work directly from pollen residues regularly prepared from sediments. By this method over ten known or unknown fossil pollen replicas are encountered in each 200-mesh grid, and all pollen from 2.0 cm^3 of clay-gyttja sediment from the Cuscachapa core or of peat from the Tinte core can be accommodated on about 45 grids. Electron-microscopic pollen analysis is thus technically feasible and promises to solve many serious identification problems in paleoecology.

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Enzymatic Identification of Fish Products

Abstract. *Comparative enzymological techniques were used to distinguish between the muscle lactate dehydrogenases of 26 fish species. Intergeneric differences in enzymatic properties were frequently encountered. The techniques revealed, in addition, that some commercial samples of frozen fish fillets, labeled "haddock," contained cod lactate dehydrogenase.*

During a comparative study of lactate dehydrogenases (*1, 2*), we examined certain properties of these enzymes in fishes of the family Gadidae. This group of fishes includes haddock (*Melanogrammus aeglefinus*) and cod (*Gadus callarias*) and others commonly used for food. At one point in the study we used haddock muscle from a package of frozen fillets labeled "haddock." A cell-free extract prepared from this commercial sample was subjected to starch-gel electrophoresis (*3*). The gel was treated with a stain specific for lactate dehydrogenase (*4*). Surprisingly, the LDH (*5*) of this sample was electrophoretically identical with LDH from a sample of authentic cod muscle,

Table 1. Electrophoretic mobility and thermostability of muscle LDH from various fish species.

Species	Distance moved * (cm)	Inactivation temperature † (°C)
Teleosts		
Gadidae		
Atlantic cod, *Gadus callarias*	5.3	52
Haddock, *Melanogrammus aeglefinus*	1.7‡	43
Pleuronectidae		
Pacific halibut, *Hippoglossus stenolepis*	0.0	56
Petrale sole, *Eopsetta jordani*	2.0	50
Scombridae		
Atlantic bluefin tuna, *Thunnus thynnus*	−1.5	48
Pacific yellowfin tuna, *Thunnus albacares*	−1.5	
Mackerel, *Scomber scombrus*	0.3	56
Scorpaenidae		
Hawaiian scorpionfish, *Scorpaenopsis gibbosa*	2.5	47
Ocean perch, *Sebastes marinus*	3.0	48
Anoplopomatidae		
Sablefish, *Anoplopoma fimbria*	2.2	55
Sparidae		
Scup, *Stenotomus chrysops*	1.0	48
Cyprinidae		
Carp, *Cyprinus carpio*	2.6	67
Clupeidae		
Herring, *Clupea harengus*	0.2	39
Albulidae		
Bonefish, *Albula vulpes*	.2	54
Elopidae		
Tenpounder, *Elops saurus*	.1	54
Chanidae		
Milkfish, *Chanos chanos*	.8	55
Salmonidae		
Brook trout, *Salvelinus fontinalis*	.3‡	51
Osmeridae		
Smelt, *Osmerus mordax*	5.0	48
Esocidae		
Chain pickerel, *Esox niger*	0.5	50
Nonteleosts		
Garpike, *Lepisosteus spatula*	3.5	§
Sturgeon, *Acipenser transmontanus*	0.6‡	49
African lungfish, *Protopterus* species	1.7	50
Atlantic spiny dogfish, *Squalus acanthias*	−2.0	61
Pacific spiny dogfish, *Squalus acanthias*	−2.0	61
Seven-gill shark, *Notorhynchus maculatum*	0.6	49
Lamprey, *Petromyzon marinus*	0.4	51
Pacific hagfish, *Eptatretus stouti*	−0.9	64

* Distance moved toward the anode by the major form of LDH present in a muscle extract. Extracts were made by grinding a 1-g portion of white (epaxial) muscle in 5 ml of cold 0.25*M* sucrose in a glass homogenizer. The extracts were clarified by centrifugation and stored at −10°C. Electrophoresis was carried out with 0.02-ml samples of extract under standard conditions (*3*); phosphate-citrate buffer at *p*H 7.0 was used. The gel was then treated with a nitroblue tetrazolium staining mixture specific for lactate dehydrogenase (*3, 4*). A sample of chicken H_4 LDH was used as a standard on each gel; under these conditions, this enzyme moved 2.2 cm. † Temperature required for 50 percent inactivation in 20 minutes (see *1*). Muscle extracts were diluted tenfold or or 100-fold in a buffer, *p*H 7.5, containing 0.14*M* NaCl, 0.0005*M* $MgSO_4$, 0.00015*M* $CaCl_2$, 0.01*M* tris, and 0.1 percent bovine serum albumin, and then heated at various temperatures for 20 minutes. The heated samples were quickly cooled to 0°C and later assayed at 23°C for LDH activity (*3*). ‡ Five closely spaced spots of LDH activity were observed in these extracts. The mobility value given is for the central spot, which was also the most intensely staining spot (*9*). § The muscle LHD of this species was unstable at 0°C when diluted tenfold or 100-fold in the standard buffer.

but distinctly different from that of authentic haddock muscle LDH (*6*). According to the electrophoretic test and the two other tests described below, it appeared that the commercial sample was cod mislabeled as haddock. Although an extensive survey of packaged fish was not undertaken, enough samples of frozen fillets and breaded fish sticks were examined to reveal that mislabeling of cod as haddock was not an isolated incident at the time (1964).

To identify species from frozen tissues we made further studies with electrophoresis. Table 1 gives the mobilities of muscle LDH's from a variety of teleost and nonteleost species (*7*). Species such as bluefin and yellowfin tuna, which belong to the same genus (*Thunnus*) did not seem to differ in muscle LDH mobility. More distantly related species, such as cod and haddock, or halibut and sole, commonly differ from each other in this respect. Such intergeneric variation in electrophoretic mobility of fish muscle LDH has been observed with other species (*8*).

The sensitivity of enzymes to heat inactivation is a property that may vary among species (*1, 3*). We therefore examined the thermostability of LDH in fish muscle extracts under the standard conditions used earlier (*1, 3*). The authentic haddock enzyme was rapidly inactivated at 46°C, while commercial "haddock" and cod enzymes were stable. Table 1 summarizes the results of thermostability experiments with the muscle LDH's of other species. Several examples of variation between genera were encountered.

Crystalline preparations of LDH's from cod and haddock were recently made (*10*). Antiserums to such purified enzymes were then produced in rabbits. These antiserums and the sensitive immunological method of quantitative microcomplement fixation (*11*) provided a further means of distinguishing between the two enzymes. The antiserum directed against haddock muscle LDH reacted less strongly with the cod enzyme than with the haddock enzyme (Fig. 1). The reverse was true of the antiserum directed against cod muscle LDH.

The techniques described above require trained personnel and relatively expensive equipment. It is of interest that Levine and Weston have developed a cheaper and faster immunological procedure for distinguishing between cod and haddock muscle LDH's based on hemagglutination; their procedure is similar to those used for blood typing and pregnancy tests (*12*). In principle, the use of enzymatic properties as a means of species identification is not restricted to a single enzyme nor to fish products alone (*13*).

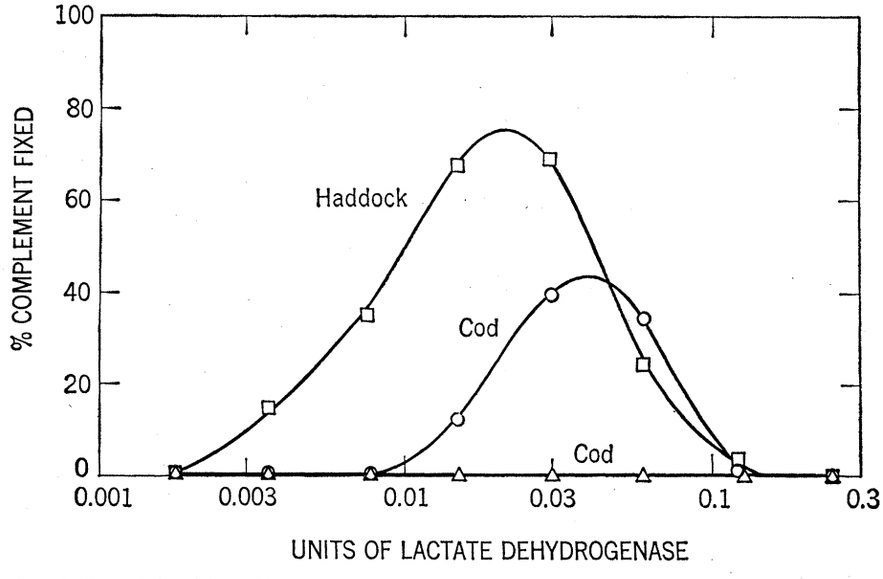

Fig. 1. Reactivity of purified haddock and cod muscle LDH's with a rabbit antiserum directed to haddock muscle LDH. The rabbit was immunized by an initial injection into the toepads and thigh muscles with 5 mg of crystalline haddock muscle LDH emulsified in Freund's adjuvant; a series of intravenous 1-mg injections was given during the 2nd and 3rd month. Eight days after the last injection, the rabbit was bled. A 1/2000 dilution of the resulting antiserum (547-B4) was tested for reactivity with serial dilutions of haddock or cod muscle LDH by the microcomplement fixation procedure with 7-ml reaction volumes. The units of LDH activity given on the abscissa are defined elsewhere (*3*); one unit is approximately equivalent to 1 μg of LDH. All reagents (antiserum, enzyme, and complement) were diluted in the buffer whose composition is given in Table 1. The haddock enzyme reacted (□—□) with a 1/2000 dilution of antiserum but the cod enzyme did not (△—△). A reaction with the cod enzyme was detected (○—○) when the antiserum concentration was raised to 1/700.

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