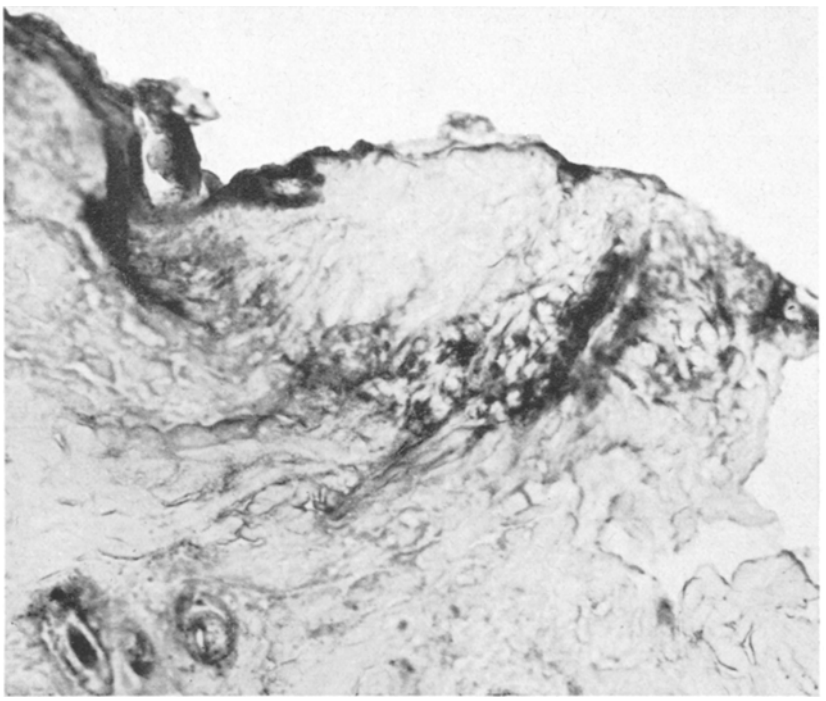

Fig. 2. Histochemical demonstration of ICDH activity in a one-day-old wound. ×100.

Zusammenfassung. Nach einem Absinken während der zwei ersten posttraumatischen Stunden stieg die fluorimetrisch bestimmte Aktivität der NADP-abhängigen Isocitrat-Dehydrogenase bis zu 120 h. Histochemisch erkannte man eine Aktivitätsverminderung in unmittelbarer Nähe der Wundfläche. Die Aktivität nahm zwischen der 8. bis 48. h in der äusseren, die nekrotische Wundnähe umgebenden Zone zu.

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Evolution of a Retinal Specific Lactate Dehydrogenase Isozyme in Teleosts

L-Lactate dehydrogenase (LDH) exists in multiple molecular forms (isozymes[1]) and serves as an excellent model for the study of evolution of proteins[2-7]. The lactate dehydrogenase polypeptide monomers, designated A and B, of vertebrates are encoded in at least 2 genetic loci[8,9]. The random assortment of these 2 subunits generates 5 tetrameric isozymes in higher vertebrates[10], however a smaller number is commonly observed in fish because of restricted assembly[7,11-13].

The tissue and developmental specific synthesis of the LDH isozymes has been attributed to their distinctive kinetic properties, which permit them to operate more efficiently during different conditions of anaerobiosis in the cell[14,15].

A third LDH locus (the E locus) has been postulated to function in the nervous system of teleosts[11,16,17]. The existence of this E locus in teleosts has since been established by genetic, immunochemical, physical, and kinetic comparisons of the teleost LDH isozymes[5-7,13,18,19]. The E gene is activated at the time of retinal differentiation[7,18,20] and is mainly functional within the photoreceptor cells of the neural retina which suggests that the E_4 isozyme plays a unique role in the visual metabolism of teleosts[7,21].

Immunochemical, kinetic, and physical analyses indicate that the LDH E polypeptide is more closely related to the B subunit than to the A subunit[5-7,13,18,19,22]. The E gene probably arose by gene duplication at the B locus

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which had earlier originated from the ancestral LDH A locus[5–7].

Our present study was designed to determine which orders of fish, representing the evolutionary spectrum, possess the functional E gene. On the basis of our investigations, this enzyme appears limited to the teleosts and is not present in more primitive fish.

The frozen eyes, heart, brain, and dorsalis trunci muscle from each fish examined were homogenized with a Potter-Elvehjem motorized homogenizer or with a Sorvall omni-mixer with microattachment at 4 °C in one volume of 0.1 *M* *Tris*-HCl buffer, pH 7.1. The homogenate was centrifuged twice at 48,200 *g* at 4 °C for 30 min. The clear supernatant was used for electrophoresis.

2 buffer systems were used. The EBT stock buffer contained 0.001 *M* EDTA (tetra-sodium), 0.025 *M* boric acid, and 0.045 *M* *Tris* and had a pH of 8.6. A 1:20 dilution of stock was used for the starch gel; and a 1:7 dilution and 1:5 dilution was made for the anode and cathode vessel buffer respectively. The concentrated *Tris* citrate stock buffer contained 0.75 *M* *Tris* and 0.25 *M* citrate (mono-hydroxy) adjusted to pH 6.9. A 1:10 dilution of stock was used in the gel and a 1:5 dilution in both electrode vessels. This buffer has proven excellent for the resolution of the E_4 isozyme[6,7,13].

Electrophoresis was performed using an 11% vertical starch gel for 12–16 h at 4 °C with voltage gradients of 13 V/cm for the EBT gels and 7.7 V/cm for the *Tris*-citrate gels. After the gels were sliced, staining was performed as described previously[7,19]. The E_4 homopolymer was readily distinguished from the other LDH isozymes by its unique occurrence in eye tissue and its characteristically rapid anodal migration upon electrophoresis.

The eye specific isozyme pattern characteristic of most orders of teleosts is exhibited by the Atlantic midshipman (*Porichthys porosissimus*) (Figure A). The eye is the only tissue possessing the E_4 isozyme. On the basis of our investigation and the previously published data[5–7,11,13,16–19,21–31] it has been established that the following orders of teleosts contain species exhibiting a functional E gene: Clupeiformes, Beloniformes, Myctophiformes, Cyprinodontiformes, Gasterosteiformes, Gadiformes, Perciformes, Pleuronectiformes, Percopsiformes, and Batrachoidiformes. However, at least one order of teleosts, the Cypriniformes, appears to be lacking the E gene function. 18 species representative of 5 families of this order (Characidae, Cyprinidae, Catastomidae, Ariidae, and Ictaluridae) were examined during the course of our investigation and all lacked the E_4 isozyme. The Golden shiner (*Notemigonus crysoleucas*), a typical Cypriniform, is shown in Figure B. In this fish, no isozymes composed of E subunits are detectable. Immunochemical confirmation of these results for teleosts will be published in detail elsewhere.

The tissue specific isozyme pattern of the lamprey (*Petromyzon marinus*), representative of the primitive Class Agnatha, is shown in Figure C. This is typical of all non-teleostean fish examined (Table) in lacking the

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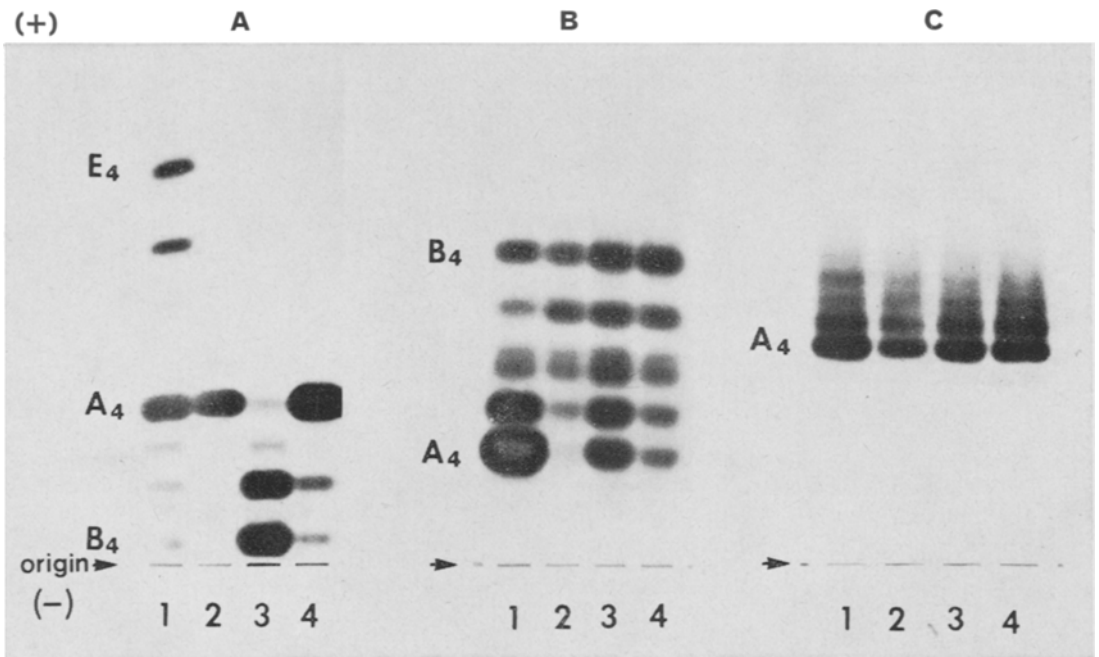

Fig. 1. Lactate dehydrogenase isozyme patterns in fish tissues. The subunit compositions of the homopolymeric LDH isozymes are indicated to the left of each zymogram. The subunit compositions were determined by immunochemical procedures[6,7,13] and by the predominance of the A_4 isozyme in skeletal muscle and the B_4 isozyme in the heart.

A) Atlantic midshipman. 1. eye, 2. brain, 3. heart muscle, 4. skeletal muscle.

B) Golden shiner. 1. skeletal muscle, 2. heart muscle, 3. brain, 4. eye. This species of teleost lack the E_4 isozyme.

C) Sea lamprey. 1. skeletal muscle, 2. heart muscle, 3. brain, 4. eye. This Agnathan (a primitive fish) also lacks the E_4 isozyme.

E_4 isozyme. The lamprey may differ from other fish in possessing only the A_4 isozyme in the adult tissues[2].

It is not possible, from our data, to exclude the hypothesis that the E gene is present but its product is undetectable in some fish. Future work should reveal whether the existence of a retinal specific LDH E_4 isozyme is indicative of a fundamental difference in the visual metabolism between teleosts and other vertebrates.

During the mid-Triassic, the teleosts began to successfully compete with the fresh water holosteans, and then underwent adaptive radiation in both fresh water and marine environments[32]. The E gene may have arisen in teleosts prior to this diversification through a duplication of the B gene[5–7]. Because the fossil record is incomplete, there is some disagreement about the phylogenetic arrangement of teleost orders and whether they arose monophyletically[33,34] or polyphyletically[35]. Nevertheless, the orders Cypriniformes, Anguilliformes, and some Clupeiform families are regarded as primitive teleosts[33,34]. The absence of the LDH E_4 isozyme in species of these orders may be due to their being in an evolutionary line which never possessed the E locus. Alternatively, these orders may have lost the E gene function which they once possessed.

This present study also suggests that the use of isozymes as genetic markers is a powerful procedure for the investigation of evolutionary relationships[36].

Primitive fish which lack a functional LDH E locus

	Scientific name	Common name
Class Agnatha		
Order Myxiniformes		
Family Myxinidae	*Myxine glutinosa*	Atlantic hagfish
Family Petromyzontidae	*Polistotrema stoutii*	Pacific hagfish
	Petromyzon marinus	Sea lamprey
Class Chondrichthyes		
Order Squaliformes		
Family Heterodontidae	*Heterodontus francisci*	Horn shark
Family Scyliorhinidae	*Cephaloscyllium uter*	Swell shark
Family Carcharhinidae	*Mustelus canis*	Smooth dogfish
	Triakis semifasciata	Leopard shark
Order Rajiformes		
Family Rajidae	*Raja eglanteria*	Clearnose skate
Family Myliobatidae	*Myliobatis freminvillei*	Bullnose ray
Class Osteichthyes		
Subclass Crossopterygii		
Order Dipnoi	*Protopterus annectens*	African lungfish
	Lepidosiren paradoxa	South American lungfish
Subclass Actinopterygii		
Superorder Chondrosteii		
Order Acipenseriformes		
Family Polyodontidae	*Polyodon spathula*	Paddlefish
Superorder Holosteii		
Order Semionotiformes		
Family Lepisosteidae	*Lepisosteus platostomus*	Shortnose gar
Order Amiiformes		
Family Amiidae	*Amia calva*	Bowfin

Résumé. Un troisième locus génétique indique la présence d'un unique isozyme de déshydrogénase lactique ne fonctionnant que dans les tissus neuraux des Téléostéens, surtout dans la rétine neurale. La fonction de gène ne se manifeste pas dans les ordres de poissons primitifs. Ces résultats suggèrent une différence de métabolisme rétinien entre les Téléostéens et les classes de poissons plus primitifs.

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Über die Speicherung von Lipiden durch Knallgasbakterien

Dass höhere Organismen unter günstigen Ernährungsbedingungen Lipide, insbesondere Triglyceride, als Reservestoffe speichern können, ist seit langem bekannt. Auch Bakterien vermögen unter speziellen Ernährungsbedingungen Reservestoffe zu bilden und intrazellulär anzuhäufen. Viele Bakterien akkumulieren ein ungewöhnliches Lipid, Poly-β-hydroxybuttersäure (PHBS); es ist nur bei Prokaryonten gefunden worden. Die Frage, ob Bakterien Triglyceride oder andere Ester langkettiger Fettsäuren als Speicherstoffe anhäufen können, ist meist verneint worden. Die Frage nach einer Speicherung von Estern langkettiger Fettsäuren durch Bakterien ist allerdings noch nicht systematisch bearbeitet worden. Zur experimentellen Beantwortung dieser Frage bieten sich Knallgasbakterien an.

Knallgasbakterien können chemolithoautotroph wachsen, d.h., sie verwenden die Knallgasreaktion (Oxydation von molekularem Wasserstoff durch molekularen Sauerstoff) als Energiequelle zur Assimilation von CO_2; sie können jedoch auch von geeigneten organischen Verbindungen heterotroph leben, z.B. von den unter günstigen Ernährungsbedingungen gespeicherten Stoffen. Da Knallgasbakterien aus leicht zugänglichem anorganischem Material organisches Material aufbauen können und weil sie eine günstige Bilanz ihres Gasstoffwechsels aufweisen, diskutiert man ihre Verwendung in der Raumfahrttechnik[1].

Die verwendeten Bakterienstämme Hydrogenomonas H 16 und Stamm 11/x speichern unter günstigen Ernäh-

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