bladder in the presence of aldosterone has been confirmed in several experiments. It was found that actinomycin D (10^{-6} *M*) inhibited the response to aldosterone (10^{-7} *M*) when added 10 min before or 5 min after the hormone (Figure 2). However, when added 10 min after the hormone the normal aldosterone response was developed. This failure of actinomycin D to inhibit the hormone response is in contrast to the inhibitory effect observed with 10^{-6} *M* cycloheximide.

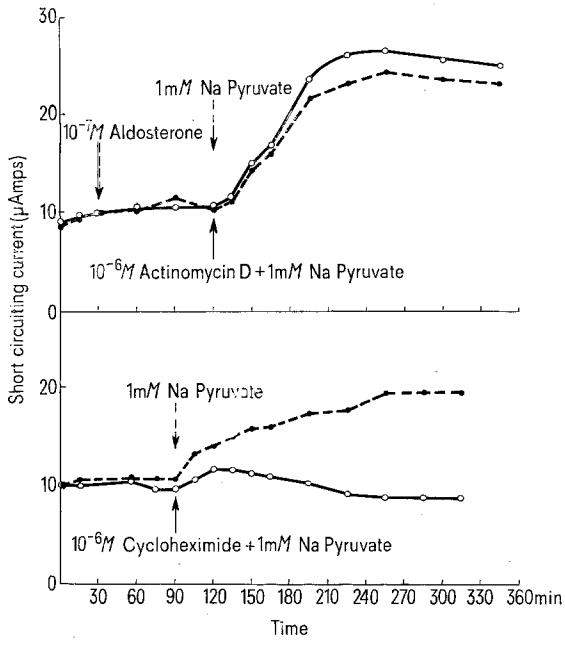

Fig. 3. (Lower) Shows the synergistic effect of 1 m*M* pyruvate added to substrate depleted bladders in the absence (--●--●--) and presence (–○—○–) of 10^{-6} *M* cycloheximide.
(Upper) Shows the synergistic effect of 1 m*M* pyruvate added to substrate depleted bladders in the absence (--●--●--) and presence (–○—○–) of 10^{-6} *M* actinomycin D. The results represent the mean of 2 experiments.

In substrate depleted bladders we fail to get an aldosterone (10^{-7} *M*) response in agreement with the observations of LEAF and SHARP[6]. We have found (Figure 3) that actinomycin D fails to inhibit the synergistic effect of pyruvate in contrast to the inhibitory effect obtained with 10^{-6} *M* cycloheximide. The pyruvate synergistic response, expressed as a percentage increase in SCC did not vary significantly over the 6 h period either in the absence or presence of 10^{-6} *M* actinomycin D added 10 min after the hormone. The simulatory effect of actinomycin D on Na^+ transport in the presence of 10^{-9} *M* aldosterone is taken as evidence for translational control of protein synthesis as part of the mechanism of aldosterone action in toad bladder. It is suggested that all transcriptional effects of the hormone may be associated with the highest affinity binding sites. The second set of binding sites characterised as cationic by agar gel electrophoresis[4] may be involved with repression of post transcriptional inhibitors.

Zusammenfassung. Die biochemischen Wirkungen von Aldosteron in der Krötenblase wurden untersucht und der Mechanismus der Hormonwirkung diskutiert.

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Intraspecific Variation of Lactate Dehydrogenase (LDH) Isoenzymes in Some *Belone belone* Populations from the Adriatic and Tyrrhenian Seas

In recent years, information on LDH isoenzymes (E.C. 1.1.1.27) in Teleost fish has greatly expanded. These studies have demonstrated that the electrophoretic pattern of this tetrameric enzyme, which is ubiquitous in all organs and tissues, is considerably more complex than that found in higher vertebrates. In fact, besides the A and B (or M and H) loci, characteristic of mammals and birds and probably homologous to these[1], 2 more loci have been described in teleosts. The first locus, postulated by MARKERT and FAULHABERT[2], designated E, has a high tissue specificity and is almost exclusively active in retina, lens and brain, although it may sometimes be active in the heart muscle[3–5]. The second locus, designated L, was described by LUSH[6] and is most active in the liver. By contrast, no description is available of a locus corresponding to the C locus, which is characteristic of the earliest spermatogenetic stages in birds and mammals[7–9].

A number of cases of intraspecific polymorphism, depending on mutation of some of the above listed loci, have been reported. Among the teleosts, which are of particular interest here, some cases of intraspecific polymorphism have been described in *Clupea arengus*[10], in various Salmonid species[11–14], in some cod populations[2, 6, 10, 15, 16] and in *Lepidorhombus whiff-iagonis*[17].

This paper describes a peculiar pattern of intraspecific LDH polymorphism in *Belone belone* (Teleostea, Belonidae) populations from different geographic areas.

Materials and methods. The electrophoretic behaviour of LDH from 2 separate *Belone belone* populations was

[1] G. S. BAILEY, A. C. WILSON, J. biol. Chem. *243*, 5843 (1968).
[2] C. L. MARKERT, I. FAULHABER, J. exp. Zool. *159*, 319 (1965).
[3] G. S. WHITT, Genetics *60*, 237 (1968).
[4] G. S. WHITT, J. exp. Zool. *175*, 1 (1970).
[5] G. S. WHITT and G. M. BOOTH, J. exp. Zool. *174*, 215 (1970).
[6] I. E. LUSH, Comp. Biochem. Physiol. *32*, 23 (1970).
[7] A. BLANCO and W. H. ZINKHAM, *Science 139*, 601 (1963).
[8] E. GOLDBERG, Science *139*, 602 (1963).
[9] E. GOLDBERG, Archs Biochem. Biophys. *109*, 134 (1965a).
[10] P. H. ODENSE, T. M. ALLEN and T. C. LEUNG, Can. J. Biochem. *44*, 1319 (1966).
[11] E. GOLDBERG, Science *151*, 1091 (1966).
[12] P. W. HOCHACHKA, Comp. Boichem. Physiol. *18*, 261 (1966).
[13] W. J. MORRISON and J. E. WRIGHT, J. exp. Zool. *163*, 259 (1966).
[14] H. O. HODGINS, W. E. AMES and F. M. UTTER, J. Fish. Res. Bd. Can. *26*, 15 (1969).
[15] I. E. LUSH and C. B. COWEY, Biochem. J. *110*, 33 (1968).
[16] F. M. UTTER and H. O. HODGINS, J. exp. Zool. *172*, 59 (1969).
[17] P. R. DANDO, J. mar. biol. Ass. U.K. *50*, 801 (1970).

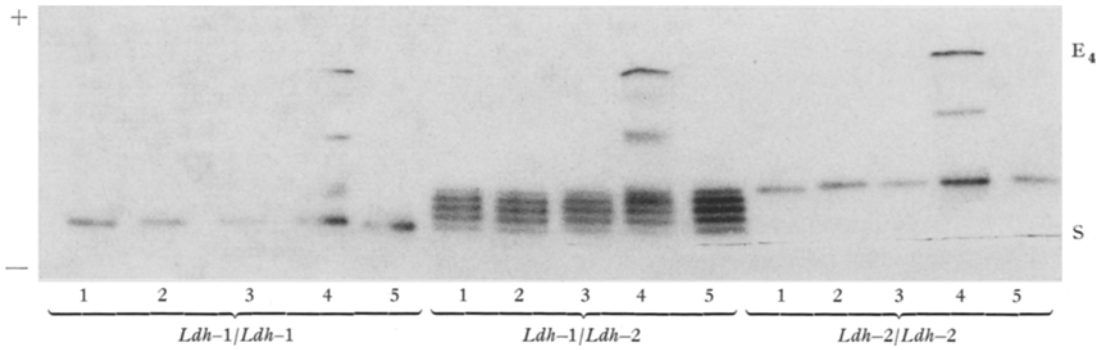

Fig. 1. Lactate dehydrogenase isoenzyme patterns from *Belone belone* organs and tissues of the 3 genotypes. 1, heart; 2, white skeletal muscle; 3, red skeletal muscle; 4 retina; 5, liver; S, start.

analyzed. One population was collected from the Adriatic Sea in September of 2 consecutive years, and the other, from the Tyrrhenian Sea, again in September. The number of specimens and collection sites are shown in the Table. Immediately after capture, the fish were kept on ice, but once in the laboratory they were stored at −20 °C until analyzed. The time between capture and analysis was never more than a fortnight.

Initially the LDH isoenzymes from heart, white skeletal muscle, red skeletal muscle, retina and liver were separated; but after identification of polymorphism pattern, only the isoenzymes of the whole eye were examined. The various organs and tissues were carefully washed in 0.1 *M tris* buffer, adjusted to pH 7 by means of HCl, and cooled to 4 °C. All the samples were then homogenized with 2 or 3 times the sample weight of this buffer. Homogenates were centrifuged for 30 min at 100,000 × *g* at 0 °C in a refrigerated M.S.E. centrifuge. The supernatant was either used for electrophoresis at once, or stored at −20 °C until analyzed.

Electrophoresis was run on starch gel. The slightly modified POULIK[18] system of discontinuous buffers was found to be the best. Buffers of 0.076 *M tris* and 0.5 *M* citric acid at pH 8.6 were used for gels and the buffers for the electrode cuvettes were 0.3 *M* borate and 0.5 *M* sodium hydroxide at pH 8.6. Electrophoresis was carried out for 2 h at 2 °C with a voltage drop of 20 V/cm across the gel.

For molecular hybridization in vitro, the whole eye was used. One eye was removed from each specimen and homogenized. Electrophoresis of this material permitted the identification of LDH phenotype and hence the presumptive genotype (see Figure 1). Then the remaining eyes were excised and the 2 presumptive homozygotes were homogenized together. In this procedure the same buffer was used, though made up to 0.5 *M* NaCl. The homogenate was repeatedly subjected to freezing (−30 °C) and thawing to facilitate hybridization.

The gels were stained for LDH activity using the procedure of WHITT[4]. The control for nothing dehydrogenase or alcohol dehydrogenase was accomplished by incubating the gels in all the staining components except L-lactate.

Results and discussion. Electrophoretic plates (Figure 1) clearly show that multiple and polymorphic LDH isoenzymes were encountered in *Belone belone*. 3 phenotypes can readily be recognized: 2 are distinct from each other, and the third one clearly displays an intermediate pattern. The occurrence of 3 phenotypes could be explained as 2 homozygous genotypes which in crossing produce a hybrid with intermediate characteristics. Let the 2 presumptive homozygous genotypes be designated *Ldh*-1/*Ldh*-1 and *Ldh*-2/*Ldh*-2, and the presumptive heterozygous genotype *Ldh*-1/*Ldh*-2 (Figure 1). The *Ldh*-1/*Ldh*-1 genotype produced a single band, apparently corresponding to a LDH homotetramer, in all the organs and tissues, except the retina in which the E_4 homotetramer was also found. This was true for the *Ldh*-2/*Ldh*-2 genotype as well, for the only homotetramer occurring in all the organs and tissues but with a different migration. The heterozygous genotype *Ldh*-1/*Ldh*-2 was composed of 5 LDH-isoenzymes, apparently corresponding to 2 LDH homotetramers and 3 LDH heterotetramers, as would be expected from random assortment of 2 structurally different LDH polypeptides into tetrameric enzyme molecules, in all the organs and tissues except the retina, in which, obviously, the E_4 homotetramer also occurred. Moreover, some bands of uncertain significance also appeared from the retina of all 3 genotypes; they were probably heterotetramers consisting of type E subunits, which combined with the subunits produced by *Ldh*-1 and *Ldh*-2 loci. Molecular hybridization, obtained through simultaneous homogenization of presumptive homozygotes in the presence of 0.5 *M* NaCl, seems to confirm that the *Ldh*-1/*Lhd*-2 genotype is actually the heterozygous one (Figure 2).

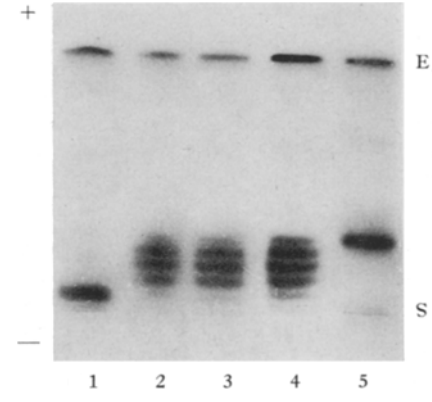

Fig. 2. Lactate dehydrogenase isoenzyme patterns of the eye illustrating the in vitro molecular hybridization. 1, genotype *Ldh*-1/*Ldh*-1; 2,4, genotype *Ldh*-1/*Ldh*-2; 5, genotype *Ldh*-2/*Ldh*-2; 3, eyes of the 2 homozygous genotypes homogenized together; S, start.

[18] M. D. POULIK, Nature *180*, 1477 (1957).

Sample locality	Date	No. of specimens	Frequency of genotypes				Allele frequency	
			Ldh-1/*Ldh*-1	*Ldh*-1/*Ldh*-2	*Ldh*-2/*Ldh*-2	X^2	*Ldh*-1	*Ldh*-2
Volano Beach (Adriatic Sea – 100 km south of Venice)	September 1971	476	Obs. 165 Exp. 166.8	234 239.9	77 79.2	0.22	0.592	0.408
	September 1972	421	Obs. 149 Exp. 152. 5	209 201.7	63 66.6	0.53	0.602	0.398
The Gulf of Naples (Tyrrhenian Sea)	September 1972	706	Obs. 286 Exp. 279.3	317 329.5	103 97.1	0.98	0.629	0.371

Our hypotheses were also confirmed by the good fit between the observed and expected gene frequencies (see the Table). The gene frequency was nearly identical in the various populations. This is not surprising in view of the wide range and high rate of migration of *Belone belone* populations living in the Mediterranean Sea.

The foregoing observations show that both Adriatic and Tyrrhenian populations of *Belone belone* contain 2 panmictic homozygous genotypes of LDH isoenzyme (*Ldh*-1/*Ldh*-1 and *Ldh*-2/*Ldh*-2), which through interbreeding, yield a hybrid (*Ldh*-1/*Ldh*-2) displaing intermediate features.

Riassunto. Gli isoenzimi della LDH di *Belone belone* si presentano multipli e polimorfi all'analisi elettroforetica. Nelle popolazioni studiate si mettono in evidenza 3 genotipi diversi, 2 dei quali a se stanti ed 1 con caratteristiche intermedie. L'ibridazione molecolare in vitro ed il confronto fra le frequenze genotipiche osservate e teoriche dimostrano che si tratta di 2 genotipi omozigoti che incrociandosi danno un ibrido con caratteristiche intermedie.

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Effects of Glycine and Serine on Serine Hydroxymethyltransferase Levels in Logarithmic Cultures of *Neurospora crassa* Wild Type and *Ser-1* mutant

From recent detailed investigations it is clear that the formation and metabolism of one-carbon units in microorganisms is finely regulated. This may be achieved by repression[1–8] of certain key pteroylglutamate[9] dependent enzymes and by feedback inhibition of their activity[8,10–13]. As the serine hydroxymethyltransferase reaction is generally considered to be the major physiological route for synthesis of C-1 units it is conceivable that the formation as well as metabolism of 5,10-CH_2-H_4PteGlu will be controlled in these systems. In *Escherichia coli* 113-3 such control is exerted by exogenous L-methionine[7] which regulates the availability of C-1 units by repression and derepression of serine hydroxymethyltransferase. In an amethopterin-resistant strain of *Streptococcus faecium*[3] high levels of L-serine in the culture medium cause some repression of this enzyme's synthesis. In *Saccharomyces cerevisiae*, where the β-carbon of serine is a direct precursor of the methyl carbon of methionine[14], it is clear that synthesis of 5-CH_3-H_4PteGlu is regulated by this latter amino acid[8,13] and that glycine stimulates formation of serine hydroxymethyltransferase[15].

As part of a continuing study[8,13] of C-1 metabolism in fungi, the present investigation has examined the possible regulation of serine hydroxymethyltransferase by glycine, serine and methionine in *Neurospora crassa* wild type. Comparative studies with a *N. crassa* serine-glycine auxotroph (*Ser-1*), known to be deficient in synthesis of serine from phosphohydroxypyruvate[16], are also reported.

Materials and method. Neurospora crassa Lindegren A (FGSC no. 853) and *Ser-1* mutant strain H605a (FGSC no.118) were cultured aerobically at 25°C in the minimal medium of Westergaard and Mitchell[17] modified to include ammonium citrate[18]. 6 l aliquots of

[1] A. M. Albrecht and D. J. Hutchison, J. Bact. *87*, 792 (1964).
[2] O. Ohara and R. Silber, J. biol. Chem. *244*, 1988 (1969).
[3] A. M. Albrecht, F. K. Pearce and D. J. Hutchison, J. Bact. *95*, 1779 (1968).
[4] J. J. Burchall and G. H. Hitchings, Adv. Enzyme Regul. *6*, 323 (1967).
[5] R. J. Rowbury and D. D. Woods, J. gen. Microbiol. *24*, 129 (1961).
[6] R. Silber and A. Mansouri, Ann. N. Y. Acad. Sci. *186*, 55 (1971).
[7] A. Mansouri, J. B. Decter and R. Silber, J. biol. Chem. *247*, 348 (1972).
[8] K. L. Lor and E. A. Cossins, Biochem. J. *130*, 773 (1972).
[9] Abbreviations for pteroylglutamate derivatives are those of the IUPAC-IUB Commission as listed in Biochem. J. *102*, 15 (1967).
[10] F. R. Dalal and J. S. Gots, Biochem. biophys. Res. Commun. *22*, 340 (1966).
[11] F. R. Dalal and J. S. Gots, J. biol. Chem. *242*, 3636 (1967).
[12] R. T. Taylor, H. Dickerman and H. Weissbach, Biochem. biophys. Res. Commun. *16*, 593 (1964).
[13] G. Combepine, E. A. Cossins and K. L. Lor, FEBS Lett. *14*, 49 (1971).
[14] J. L. Botsford and L. W. Parks, J. Bact. *94*, 966 (1967).
[15] J. L. Botsford and L. W. Parks, J. Bact. *97*, 1176 (1969).
[16] A. Meister, Adv. Enzymol. *16*, 185 (1955).
[17] M. Westergaard and H. K. Mitchell, Am. J. Bot. *34*, 573 (1947).
[18] G. Turian, Nature, Lond. *202*, 1240 (1964).