Differences in the Number of Molecules Produced by Two Allelic Electrophoretic Enzyme Variants in *D. melanogaster*

Natural populations of *Drosophila melanogaster* are often polymorphic for genetically determined fast (F) and slow (S) electrophoretic variants of alcohol dehydrogenase (Adh). Within each of 5 populations investigated[1-3] flies homozygous for the fast allele had higher enzyme activity than flies homozygous for the slow allele and the heterozygotes were intermediate in activity. The data described below suggest that for the alleles extracted from one population flies homozygous for the fast allele produce more enzyme molecules than flies homozygous for the slow allele.

Antisera were prepared in New Zealand white rabbits against crude extracts of flies homozygous for either GRELL'S[4] AdhF or AdhS alleles on a common genetic background. 3 i.m. injections of the extracts homogenized with Freund's adjuvant were given at weekly intervals following a control bleeding. The rabbits were bled 1 week after the final injection.

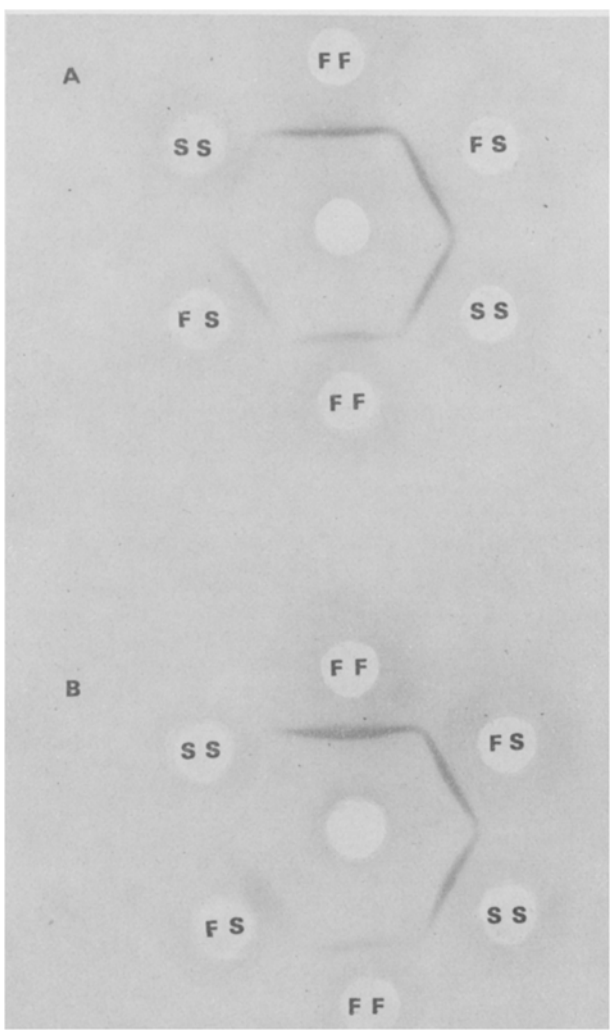

Fig. 1. Photograph of double diffusion plate containing in the central wells A) anti AdhF/F and B) anti AdhS/S. In both plates the uppermost outside wells and the next 2 wells to the right contain undiluted crude extract of the genotypes indicated. The next 3 wells contain crude extract diluted 1:2 with distilled water.

The precipitin lines of the alcohol dehydrogenase-antiserum complex on double diffusion plates[5] were stained specifically for Adh activity using a solution containing per ml of *Tris* HCl pH 9.0; NAD 0.5 mg; Nitro Blue 0.25 mg; phenazine methosulphate 0.1 mg and iso propanol 0.01 ml.

It can be seen in Figure 1 that the precipitin lines produced by AdhF/F, AdhS/S and AdhF/S crude enzyme extracts against both AdhF/F and AdhS/S antisera are the same indicating that the 3 genotypes produce immunologically undistinguishable enzymes.

Crude enzyme extracts of each of the 3 Adh genotypes were prepared by homogenizing 48 mg of 4-day-old female flies in 2 ml buffer. A replicated set of tubes were set up in each of which 0.1 ml AdhS/S extract was incubated at 25°C for $^1/_2$ h with 0.9 ml of 0.05*M* orthophosphate buffer (pH 7) and various volumes of AdhS/S antiserum. After 48 h at 4°C the tubes were centrifuged at 4,000 rpm for 20 min. Both the supernatant and the resuspended precipitate were assayed for alcohol dehydrogenase activity in a Hitachi Perkin Elmer spectrophotometer using nicotinamide adenine dinucleotide (NAD) and isopropanol as substrates. Total protein content of each initial extract was determined by the Folin method as modified by LOWRY et al.[6]. The experiment was repeated using extracts from the other 2 Adh genotypes and in one series control serum was used.

Whilst confirming that extracts of AdhF homozygotes have a higher enzyme activity than extracts of the other 2 genotypes, the data (Figure 2) also show that roughly twice as much antiserum is required to remove all detectable enzyme activity from the supernatant of the extract of the AdhF homozygote than from the supernatant of the AdhS extract. The extract of AdhF/S heterozygote was intermediate in both enzyme activity and in the amount of antiserum required.

The experiments were repeated in the same way except that the crude extracts of the AdhF homozygote and AdhF/S heterozygote were diluted to give similar initial enzyme activities to that of the AdhS homozygote. The results (Figure 2) show that in this case there are no significant differences in the amounts of antisera required to remove all detectable enzyme activity from the supernatants prepared from extracts of any of the 3 genotypes. In all cases assays of enzyme activity in the precipitates gave complementary results.

As it seems likely that the enzyme molecules produced by the 3 genotypes have the same number of antibody binding sites I conclude that the AdhF homozygote produces more enzyme molecules than the AdhS homozygote. WARD[3] has consonant results for he has been unable to detect significant differences in the Kms for both NAD and iso-propanol in crude enzyme extracts from any of the 3 genotypes.

COVE[7] has recently drawn attention to cases suggestive of autoregulatory mechanisms in both prokaryotes

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and eukaryotes. An example similar to the present one is that reported by SIROTNAK[8] in which mutations in the dihydrofolate synthetase structural gene of *Diplococcus pneumoniae* increase the rate of production of the altered enzyme product.

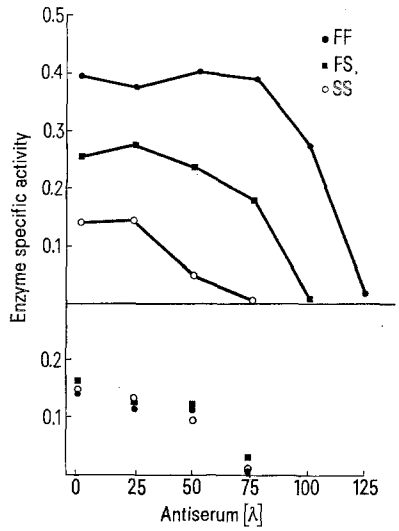

Fig. 2. Alcohol dehydrogenase activity in the supernatant after incubation with antiserum. In the upper figure the crude extracts of the 3 genotypes were undiluted whilst in the lower figure the 3 genotypes were diluted to give similar initial activities.

There is considerable variation in enzyme activity within both fast and slow Adh alleles extracted from natural populations and the variation is susceptible to artificial divergent directional selection[9]. It would be interesting to find out to what extent the variation in enzyme activity within an electrophoretic form is due to changes in the structural gene or background modification which results in changes in the rate of production of the enzyme molecule rather than to changes solely in the affinity of the enzyme molecule for its substrates. The results presented here suggest that some modifiers at least may prove to affect alcohol dehydrogenase activity by changing the rate of enzyme production.

Résumé. Les techniques immunologiques ont démontré que chez *Drosophila melanogaster* une forme électrophorétique «Fast» d'alcool déshydrogénase produit plus de molécules d'enzyme qu'une forme électrophorétique «Slow». On expose les résultats en les comparant à d'autres suggérant des mécanismes autorégulatoire.

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2 February 1972.

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Duplication of the Gene Loci Coding for the Supernatant Aspartate Aminotransferase by Polyploidization in the Fish Family *Cyprinidae*[1]

Aspartate aminotransferase (AAT; E.C.: 2.6.1.1.) is a widely distributed enzyme in plant, animal, and human tissues. It catalyses the reversible reaction L-aspartate + α-ketoglutarate ⇌ oxaloacetate + L-glutamate using pyridoxal phosphate as the coenzyme. Two distinctly different forms of AAT have been described[2,3]. This finding has subsequently been confirmed by several other authors[4–10]. It has been shown that 1 of these 2 forms of AAT is found in the mitochondrial fraction (M-form), while the other one occurs in the supernatant of cell homogenates (S-form). Electrophoretic separation at neutral pH revealed that the M-form migrates towards the cathode and the S-form in anodal direction.

Among the vertebrates studied so far, genetically determined polymorphisms have been found for the S-form in herring[11], for the M-form in mouse (*Mus musculus*)[12], and for the M- and S-form in man[13,14]. A trans-specific variability for both forms has recently been described in primates[15]. The findings in mouse, man, and primates can be interpreted under the assumption of one gene locus existing in different alleles. The data on genetic polymorphism confirm investigations[16,17], suggesting a dimeric structure of either form of AAT.

The supernatant form of this enzyme was introduced as a genetic marker in the course of our studies designed to closer elucidate the diploid–tetraploid relationship established among members of the fish family *Cyprinidae*[18–24], order *Ostariophysi*. The present paper reports our findings in 3 diploid and 3 tetraploid species of cyprinid fish.

Materials and methods. The following species were examined: *Barbus tetrazona* (obtained from a local pet shop), *Rutilus rutilus* (from the Rhine river), *Tinca tinca* (Rhine river and a local fish store), *Barbus barbus*, *Cyprinus carpio*, and the hybrid *Carassius carassius* × *Carassius auratus* (all 3 species from the Rhine river). A number of different tissues were analysed (heart, liver, kidney, muscle, brain, gills, eye, and gonads). In the majority, however, the investigations were limited to heart and liver. In the case of *Barbus tetrazona*, because of its small size, the entire fish was used.

The tissues were homogenized 1:1 in 0.01 *M* PO_4-buffer, pH 7.4, frozen and thawed twice, and centrifuged at 20,000 × *g* until the supernatant was clear, which was subjected to electrophoresis. Gels were made of 0.01 *M* *Tris*–0.0028 *M* citric acid buffer, pH 5.5 in a 14% starch gel. The bridge buffer consisted of a 0.155 *M* *Tris*–0.043 *M* citric acid solution, pH 5.5. Electrophoresis was performed at 12 V/cm for 5 h, and afterwards the gels were sliced and stained in the following solution: 460 mg L-aspartic acid, 200 mg α-ketoglutaric acid, 10 mg pyridoxal phosphate, and 400 mg fast blue BB salt, suspended in 150 ml 0.05 *M* *Tris*-HCl, pH 7.6.

In order to identify the 2 respective forms of AAT, mitochondria were isolated (method according to HEN-