RNA and polyU, is the same for both classes. Thus, while association with the viral RNA in vitro distinguished "active" from "inactive" ribosomes, it did not distinguish a class particularly responsive to that messenger. If these messengers are representative, then ribosomes are nonspecific with respect to the attachment of messengers.

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6. Omission of supernatant and the 5-minute incubation period prior to centrifugation is a significant difference in the two experiments. The initial attachment of TYMV-RNA to ribosomes is immediately reversed by polyU, whereas, after incubation of the viral RNA with ribosomes in the presence of supernatant, the RNA is displaced less effectively, and only after a lag of several minutes.
7. The substitution of glycerol for sucrose in the gradients gave greater recovery of activity. In separate runs containing P^{32}-labeled TYMV ($S_{20,w} = 116S$) as a marker, the peak in the absorbancy profile corresponded with $S_{20,w} = 70S$.
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Alcohol Dehydrogenase in Drosophila melanogaster: Isozymes and Genetic Variants

Abstract. *Alcohol dehydrogenase, in* Drosophila melanogaster *homozygous for the alleles* Adh^F *or* Adh^S, *is found in three electrophoretically different forms.* Adh^F *differs from* Adh^S *in that isozymes with faster electrophoretic mobilities are present. In* Adh^F/Adh^S *heterozygotes, hybrid isozymes as well as the parental isozymes are present, indicating that the dehydrogenase may exist as a dimer of two polypeptide subunits. The gene,* Adh, *is located on the second chromosome, with a map position of 50.1 and a cytological position between 34E3 and 35D1.*

In multicellular organisms an enzyme sometimes occurs in several separable forms called isozymes. In the case of lactate dehydrogenase the enzyme is considered to be a tetramer composed of either or both of two individual polypeptide subunits. Presumably each subunit is a gene product, but evidence for two genes has not been obtained. Although lactate dehydrogenase is virtually absent in *Drosophila melanogaster*, another isozyme system was discovered which is under genetic control and for which the genetic locus has been identified. This enzyme is alcohol dehydrogenase (ADH), and the existence of isozymes has been independently discovered and reported by Johnson and Denniston (*1*). Instead of the two isozymes of ADH reported, we find that there are three. Other electrophoretic variants of enzymes and proteins have also been reported to occur in *Drosophila melanogaster* (*2*).

For electrophoresis, polyacrylamide gels (5 percent acrylamide) were prepared according to the procedure of Raymond and Wang (*3*). The gels were stored in 0.05*M* tris buffer adjusted with H_3PO_4 to *p*H 8.5 at 25°C. The gels were cut, and filter paper, containing the sample, was inserted between the cut ends. In general, a sample consisted of one or more *Drosophila* crushed on the filter paper with a glass rod. A potential of 18 to 27 volt/cm was applied. The gels were cooled on top and bottom to at least 20°C during electrophoresis. A complete separation of ADH isozymes was obtained in 8 hours; however, most isozymes are separated within 2 hours. The ADH activity on the gels was detected by a modified staining technique for lactate dehydrogenase (*4*). The developing solution contained 90 ml of tris buffer (0.05*M*, *p*H 8.5), 4 ml of nicotinamide adenine dinucleotide solution (10 mg/ml), 4 ml of phenazine methosulfate solution (2 mg/ml), 2 ml of nitro blue tetrazolium (10 mg/ml), and 0.75 ml of ethanol (95 percent) or *sec*-butanol. Purple spots of the reduced formazan were usually visible within 1 hour after start of incubation at 37°C, but longer incubation was necessary to observe areas of low ADH activity. To demonstrate that the assay is dependent on added ethanol it was necessary to evaporate the two indicator dyes to dryness before preparation of the above developing solution. *Iso*-propanol and *sec*-butanol are more effective substrates than ethanol for the gel assay.

Inbred strains of *Drosophila melanogaster* regularly have three ADH isozymes in either of two types of patterns. One pattern is a slow type, is found in Canton-S wild type (Fig. 1*a*), and consists of bands 1, 4, and 7; the other pattern is a fast type, and is found in Samarkand, Swedish-b, and many other wild types, and consists of bands 3, 6, and 9 (Fig. 1*b*). In their respective patterns the slowest band is most active enzymatically, and the fastest band is least active. Ordinarily, adult flies were examined, but the isozymes are also all present in the fully grown larvae and in the pupae. Larvae were actually the best source of bands 7 and 9.

Examination of over 100 laboratory strains has not revealed further variety among inbreds. Of course, it may not be assumed that enzymes of two stocks are identical merely because they have the same electrophoretic mobility. In fact, many differences would not be revealed by this technique.

A cross of a strain containing bands 1, 4, and 7 with a strain containing bands 3, 6, and 9 yields hybrid F_1 progeny (Fig. 1*c*) that contain the isozymes of both parents plus three additional isozymes. One band unique to hybrids is band 2. It belongs in the group of what may be termed primary ADH isozymes and has a mobility intermediate between the two parental primaries, bands 1 and 3. Band 5 is

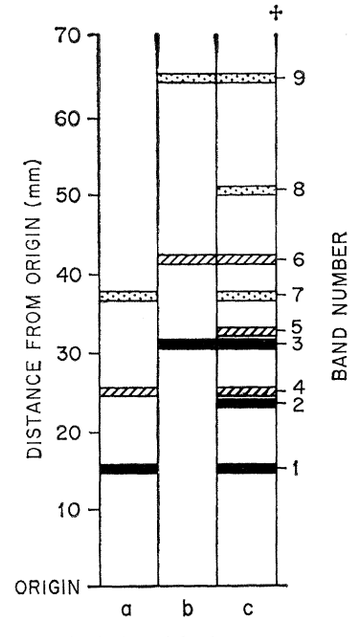

Fig. 1. Alcohol dehydrogenase zymograms of Canton-S (*a*), Samarkand (*b*), and F_1 hybrid between the two (*c*).

another additional band in hybrids and belongs to the group of secondary ADH isozymes. Its mobility is intermediate between the two parental secondaries, bands 4 and 6. The tertiary pattern, bands 7, 8, and 9, is not readily demonstrable in hybrid adults but is better seen in larvae.

The F_1 flies from reciprocal matings contain the hybrid isozymes 2, 5, and 8, and male and female F_1 progeny are indistinguishable in this respect. Thus sex-linked inheritance of differences between strains may be immediately eliminated.

For further study of the genetic control of ADH, a stock *Cy/Bl; Ubx*^{130}*/Vno* (*Cy*=Curly wings; *Bl*=short bristles; *Ubx*^{130}=enlarged halteres; *Vno*=veins missing) was tested and found to contain the fast pattern of ADH isozymes (bands 3, 6, and 9). *Cy* and *Bl* are dominant mutations on the second chromosome; *Ubx*^{130} and *Vno* are dominant mutations on the third chromosome. Females of this stock were crossed with Canton-S males, which contain the slow pattern of isozymes (bands 1, 4, and 7). Male F_1 progeny were selected that were *+/Cy*; *+/Ubx*^{130}. Some were tested and were found to contain the six-banded pattern (bands 1–6 were followed in this testing) of heterozygotes as expected; others were back-crossed to Canton-S females. Progeny of the back cross were selected, categorized by the presence of the dominant markers, and tested for their isozymes. The presence of *Cy* was always associated with the six-banded pattern, whereas in the absence of *Cy* the slow isozymes only (bands 1 and 4) were found. The *Ubx*^{130} and the six-banded enzyme patterns segregated independently. Thus, the factor which specifies the ADH isozymes must be located on the second chromosome with *Cy*.

In order to obtain an approximate location of the factor on the second chromosome, we crossed Canton-S (bands 1 and 4) females to males with seven marker mutations spaced along the entire length of the second chromosome and with ADH bands 3 and 6. The F_1 females were back-crossed to males homozygous for the marked chromosome. From among the progeny, single crossovers in each of the six regions defined by the seven markers were selected and tested for their ADH isozymes. From this experiment it was concluded that the locus which specifies the electrophoretic mobility of ADH is located between the markers *b* (black body color; map position 48.5) and *pr* (purple eye color; map position 54.5). Furthermore, it is closer to *b* than to *pr*.

Table 1. Distribution of alcohol dehydrogenase alleles among crossovers from females heterozygous for *b el Adh*^{F} *rd*^{s} *pr* and + + *Adh*^{S} + +.

Crossover type	*Adh* allele	No.
b + + +	slow	10
b + + +	fast	0
+ *el rd*^{s} *pr*	slow	0
+ *el rd*^{s} *pr*	fast	6
b el + +	slow	3
b el + +	fast	25
+ + *rd*^{s} *pr*	slow	17
+ + *rd*^{s} *pr*	fast	2
b el rd^s +	slow	0
b el rd^s +	fast	5
+ + + *pr*	slow	5
+ + + *pr*	fast	0

For the precise location, the *b* to *pr* region of the second chromosome was divided into regions bounded by the recessive markers, *b*, *el*, *rd*^{s}, and *pr* (*el*=elbow wings, map position 50.0; *rd*^{s}=scraggly bristles, map position 51.0). Females homozygous for *b el rd*^{s} *pr* and fast ADH were crossed to Canton-S males; F_1 females were back-crossed to *b el rd*^{s} *pr* (fast ADH) males. Single crossovers in each of the three regions were selected and tested for their ADH isozymes. From the results (Table 1), it is concluded that a locus, symbolized *Adh*, is located between *el* and *rd*^{s}. Of 47 crossovers between *el* and *rd*^{s}, five were crossovers between *el* and *Adh*. Thus, *Adh* is located about one-tenth (5/47) of the distance between the two markers from *el*. Since the standard locations (*5*) of *el* and *rd*^{s} are 50.0 and 51.0, *Adh* is placed at 50.1 on the genetic map of the second chromosome.

A cytological location of the *Adh* gene was made in the following way. Canton-S males were irradiated with 4000 roentgens of x-ray and mated to *b el rd*^{s} *pr cn* females. Among the progeny were two males which were phenotypically *black* and *elbow*. On appropriate testing, the irradiated Canton-S second chromosomes appear to lack any allele of *Adh*. That is, heterozygotes with *Adh*^{F} have the fast pattern and heterozygotes with *Adh*^{S} have the slow pattern. Thus, these chromosomes are apparently deficient for *b*, *el*, and *Adh*. Examination of the salivary gland chromosomes revealed that bands are missing. The missing section has a left break between 34E3 to 34F1 and a right break between 35C3 to 35D1 on Bridges's salivary chromosome map (*5*). The Adh gene is then located somewhere between 34E3 and 35D1. Thus by crossing-over experiments and by observation of a chromosomal deletion, the location of the *Adh* gene is fixed in a very restricted region of chromosome number two.

The unique feature of this system is the regulation of three isozyme groups by a single gene, *Adh*. In *Drosophila* homozygous for the allele *Adh*^{F}, the dimer forms of the enyzme could be AA, AB, and BB (bands 3, 6, and 9). In flies homozygous for *Adh*^{S} the dimer forms could be CC, CD, and DD (bands 1, 4, and 7). This is based on an assumption that two genes are contributing polypeptide subunits in each strain, as has been postulated for lactate dehydrogenases (*6*). The genetic evidence indicates that the two genes would have to be extremely closely linked unless the evidence presented pertains to the presence of a regulator gene which controls the expression of the genes for subunits A, B, C, and D. On the other hand, if a single gene, *Adh*, does control the multiple bands of alcohol dehydrogenase found in inbred strains, a chemical explanation of the genesis of these bands may be considered. We may postulate a simple alteration of one or more of the amino acids in the polypeptide subunit, such as the loss of the amide nitrogen of asparagine, which would result in an altered charge. Actually this change could occur at any stage after the genetic information was transcribed, but it is not a mutation, of course. To illustrate the result of an altered polypeptide subunit we shall designate the subunit of ADH from Samarkand as F and the altered form as F′; similarly the subunit from Canton-S is S and the altered form is S′. If, in the synthesis of F, F′ is produced 20 percent of the time and if, in the synthesis of S, S′ is produced 20 percent of the time, then a binomial expansion shows the following ratios of ADH forms for the inbred lines: 0.64FF : 0.32FF′ : 0.04F′F′ and 0.64SS : 0.32SS′ : 0.04S′S′. The hybrids would have either 9 or 10 bands of ADH, depending on whether or not FS′ has the same electrophoretic behavior as F′S.

The observed patterns are not very dissimilar from the patterns predicted by this assumption. When the assay for ADH can be performed quantitatively on the gel this hypothesis can be tested directly. Experiments are under way to distinguish between the two hypotheses.

In Adh^F/Adh^S heterozygotes, the parental and intermediate bands form an interdigitating trio of patterns. Bands 1, 2, and 3 form the primary group and contain most of the ADH activity; bands 4, 5, and 6 form the secondary group and comprise a minor portion of the activity; bands 7, 8, and 9 form another group and contain a very small percentage of the total alcohol dehydrogenase of the fly. Bands 2, 5, and 8 are present only in the heterozygote, indicating that *Drosophila* alcohol dehydrogenase contains two subunits. Thus it is apparent that these hybrid bands may be formed by the association of one slow and one fast subunit, as postulated for hybrid bands of maize esterases by Schwartz (*7*). Johnson and Denniston (*1*) reported that alcohol dehydrogenase isozymes exist in *Drosophila* in "strong zones" and "weak zones," each consisting of a three-banded pattern in the hybrid condition. These correspond to the primary and secondary groups described above. They also postulate a dimer structure for the alcohol dehydrogenases of *Drosophila* to explain their observations.

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Abrogation of Allogeneic Inhibition by Cortisone

Abstract. *Tumor cells from homozygous mice grow better upon transplantation to syngeneic mice than to F_1 hybrids between the tumor strain and a foreign strain. The inhibition of cell growth in the hybrids (called allogeneic inhibition), which is detected by tumor transplantation into mice, could be abrogated by treatment of the recipient mice with cortisone acetate. Cortisone also abolished allogeneic inhibition in vitro; abolition was detected by treating tumor cultures with cell extracts containing foreign isoantigens of the H-2 type.*

Tumor cells from homozygous mice regularly grow better upon transplantation to syngeneic mice than to various allogeneic hosts, including F_1 hybrids between the tumor strain and a foreign strain, the homozygous mice developing tumors in a higher frequency and after a shorter latency period (*1, 2*). This phenomenon has been termed syngeneic preference or allogeneic inhibition, the latter term referring to the deficient growth in the F_1 hybrids (*3*).

The mechanism of allogeneic inhibition was studied by explanting tumors of C57BL and A/Sn × A.CA F_1 hybrid origin in culture and exposing the cultures either to a cell-free homogenate or to an antigenic extract, which was prepared from A/Sn or C57BL mice and which contained either foreign or matching histocompatibility antigens of the H-2 type. The experiments showed that homogenates and extracts containing antigens which were mismatched possessed a specific cytotoxic or growth-inhibitory effect when compared to similar but matching preparations, an indication that allogeneic inhibition, as detected by tumor transplantation to mice, probably depends on exposure of the grafted cells to foreign histocompatibility antigens of the hosts (*4*).

An incidental finding was that cortisone decreased allogeneic inhibition, as detected by tumor transplantation to syngeneic and F_1 hybrid animals. Subsequent related experiments have shown that allogeneic inhibition in tissue culture could also be abolished by cortisone. An account of these results is given here.

Two tumors were used for the experiments in vivo, MC57S and MLG; both were fibrosarcomas originally induced by methylcholanthrene in strains C57BL and C57L. They were kept by serial transplantation in syngeneic animals for 27 and 23 passages, respectively, prior to the present tests. For each experiment, syngeneic animals were used as well as F_1 hybrids between the tumor strain and a foreign strain (C3H × C57BL F_1 and A × C57L F_1, respectively). Female mice, aged 1 to 2 months, were used. Mice of each type were divided into two similar groups, one being an untreated control and the other being given one intramuscular injection of 2.5 mg cortisone acetate (Upjohn) 2 days before tumor inoculation. Suspensions of tumor cells were prepared by treating the tumors with trypsin; 10^4 of the trypsin-treated cells were injected subcutaneously in the backs of the mice. The mice were inspected every 3rd to 5th day after inoculation.

Table 1. Effect of cortisone on allogeneic inhibition in vitro, as detected by exposing sarcoma MACD of A × A.CA F_1 hybrid origin to antigenic extracts from strains A and C57BL, respectively. Findings are presented from three different experiments, where cells were cultivated in the absence or presence of cortisone.

Cortisone (μg/ml)	Living cells ($\times 10^6$) after exposure to antigen from:*		Inhibition by allogeneic antigen‡ (Av. No.)
	A†	C57BL†	
0	2.10	1.32	
0	1.80	1.10	
0	1.78	0.91	1.57
0	1.74	1.29	
0	1.56	1.11	
2.5	1.92	1.49	
2.5	1.74	1.60	
2.5	1.54	1.60	1.05
2.5	1.50	1.53	
2.5	1.38	1.46	
0	1.88	1.12	
0	1.84	0.64	2.01
0	1.56	0.96	
0	1.48	0.64	
2.5	2.32	2.04	
2.5	2.00	1.67	1.16
2.5	1.68	1.36	
2.5	1.53	1.40	
0	2.56	1.80	
0	2.46	1.41	1.51
0	1.93	1.39	
2.5	2.46	1.73	
2.5	2.04	1.98	1.15
2.5	1.92	1.86	

*Antigenic preparations were made according to the technique of Haughton (*5*). Pooled antigenic material was diluted 1:2 in tissue culture medium, the final concentration being 0.3 mg antigenic material per milliliter culture medium. † Each entry represents the average number of cells unstained by trypan blue in a single culture tube after 48 hours growth in the presence of antigen. ‡ Number of cells in tubes given syngeneic antigen divided by number of cells in tubes given allogeneic antigen.