CHEMICAL SELECTION OF ALCOHOL DEHYDROGENASE NEGATIVE MUTANTS IN DROSOPHILA[1,2]

WILLIAM H. SOFER AND MARK A. HATKOFF[3]

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

Manuscript received May 26, 1972

ABSTRACT

We describe a selection procedure which utilizes the vapor from an unsaturated alcohol, 1-pentene-3-ol, for the detection and isolation of mutant flies with little or no alcohol dehydrogenase activity. ADH-negative flies are unaffected by exposure to the unsaturated alcohol, but ADH positives (wild-types) die after short exposure. The technique can be used to select rare ADH-negative individuals from large populations of wild-type flies.

WE are interested in investigating the genetic circuitry which controls the expression of the enzyme alcohol dehydrogenase (ADH) in various tissues of *Drosophila melanogaster*. Our approach has been to generate a very large number of mutations which affect alcohol dehydrogenase activity and then to try to find control mutations among these.

However, getting great numbers of ADH mutants can be quite laborious for three reasons. First, mutation frequencies at any one locus are low, even after treatment with potent mutagens (e.g., BRINK 1970). Typically, thousands of flies must be examined before such mutants are detected. Second, each fly must be individually homogenized and then assayed for ADH activity in order to detect the mutant phenotype. GRELL, JACOBSON and MURPHY (1968) were able to bypass this second difficulty when they found a behavioral difference between wild-type flies and those with greatly reduced ADH activity. Flies with low ADH were unable to survive overnight on medium containing 15% ethanol. This eliminated the necessity of doing numerous individual enzyme assays to detect mutants. Notice, however, that any mutants discovered by the procedure of GRELL *et al.* (1968) or by enzyme assay are killed in the course of the analysis. This introduces a third difficulty. In order to recover the mutation, some arrangement must be made prior to analysis so that either the progeny or the siblings of the prospective mutant flies can be separated from nonmutant flies. In practice this means that a separate vial must be established to house the progeny or siblings of every tested fly in order to recover any rare mutations.

These three difficulties are avoided in the method described here. The method utilizes an unsaturated secondary alcohol to selectively kill those flies which ex-

[1] This is Contribution No. 694 from the Department of Biology, Johns Hopkins University.
[2] Supported by NSF Grant No. GB 7803 and NIH Grant No. GM 18254.
[3] NIH Predoctoral Fellow under Grant No. GM-57.

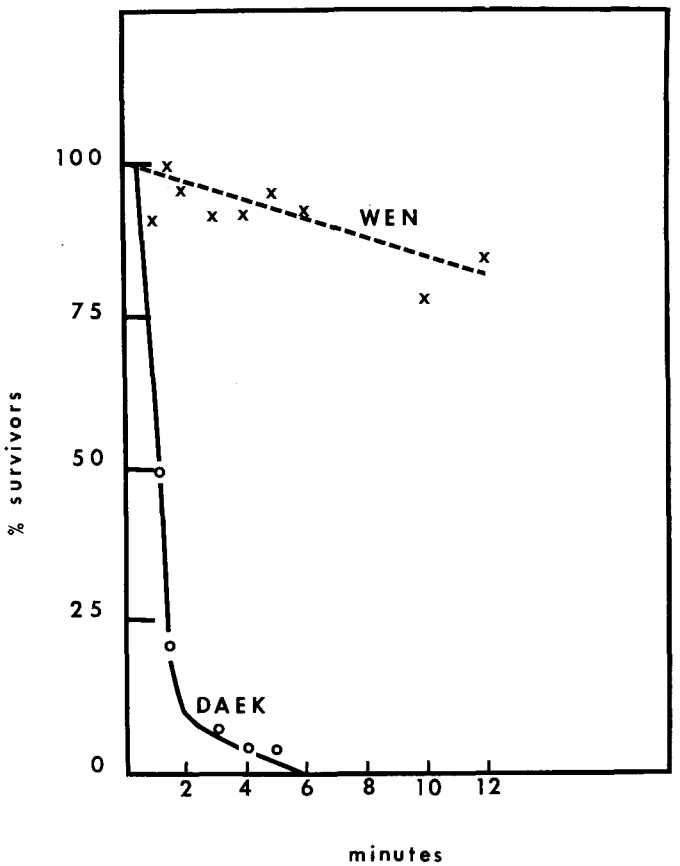

FIGURE 1.—Pentenol sensitivity as a function of dose. Flies were exposed for varying times (minutes) to the vapors from a 5% solution of pentenol. The ordinate represents the percentage of flies which survived for more than 16 hr after treatment. ADH-negative flies have white eyes (WEN). Daekwanryung (DAEK) is a wild-type strain which has high ADH activity.

hibit wild-type levels of ADH (ADH positives). Apparently, these flies oxidize the alcohol into a poisonous ketone. Flies with little or no ADH activity (ADH negatives) cannot carry out the oxidation, and consequently survive. A similar procedure has been used by MEGNET (1967) to select for mutants which affect the ADH of yeast.

The selection procedure is carried out in a chemical hood. A solution containing 5 ml of 5% 1-pentene-3-ol (pentenol) is freshly prepared in a 25 ml sidearm flask. Air is bubbled through this solution by a piston-type aquarium pump for two minutes. The vapor resulting from forcing air through the pentenol solution is then directed into a one-half pint milk bottle containing up to 500 flies. The flies are kept from escaping by means of a rubber stopper fitted with two small glass tubes. After exposure, the bottle is flushed with a stream of air for two minutes. The flies are then maintained on a tissue moistened with a solution of yeast in water.

RESULTS AND DISCUSSION

In Figure 1 the results of exposing wild-type ADH-positive flies (Daekwanryung = DAEK) and white-eyed ADH-negative flies (WEN) to pentenol for differing times in the same vial is shown. After about two minutes the ADH-positive flies became hyperactive, and usually died within an hour after treatment. The ADH-negative flies did not appear to be significantly affected.

Several other strains were tested for their ability to survive the pentenol treatment. In Table 1 their susceptibility to pentenol is correlated with the presence or absence of ADH activity. In each case, all ADH positive flies were killed after exposure to pentenol for six or more minutes. The ADH negative strains showed better than 75% survival under the same conditions. ADH^{n5} flies proved exceptional, but it is known (GRELL *et al.* 1968), and we have confirmed, that these flies show significant, although very low ADH activity.

ADH activity increases dramatically during the first few days after ecdysis (URSPRUNG, SOFER and BURROUGH 1970; DUNN, WILSON and JACOBSON 1969). Therefore, we thought there might be a relationship between the sensitivity of flies to pentenol and their age. Accordingly ADH-negative and positive strains of increasing age were treated with pentenol for one and one-half minutes. This dose was chosen because it killed about one half of the flies of an unselected wild-type population (Figure 1). As shown in Figure 2, ADH-positive individuals were relatively resistant to the effects of low doses of pentenol at one and two days post-ecdysis. However, they became increasingly pentenol sensitive, so that at four days and beyond (we have carried out the experiments for up to 8 days)

TABLE 1

Pentenol sensitivity and ADH activity

Stock	ADH activity	Killed by pentenol
Daekwanryung (DAEK)	yes	yes
Chieti-v	yes	yes
Öreboro	yes	yes
Pacific	yes	yes
Jaslow o.c.	yes	yes
Bisignano	yes	yes
Woodbury	yes	yes
Yangdong	yes	yes
Canton-s	yes	yes
w; Adh^{n1} (WEN)	no	no
w	yes	yes
mal	yes	yes
b,cn,vg	yes	yes
Adh^{n2}	no	no
Adh^{n3}	no	no
Adh^{n4}	no	no
Adh^{n5}	very low	yes
DAEK ♀ × WEN ♂ progeny	yes	yes
DAEK ♂ × WEN ♀ progeny	yes	yes

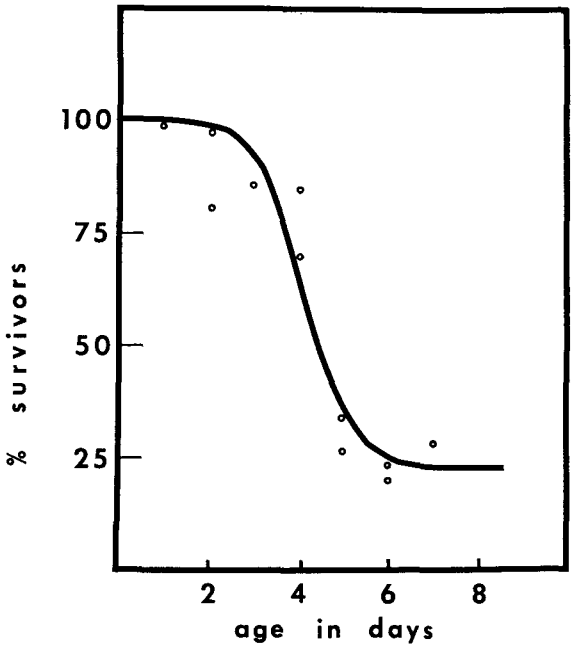

FIGURE 2.—Pentenol sensitivity as a function of age. Flies of the Daekwanryung strain were allowed to age for the times indicated in the abscissa. They were then exposed to pentenol for 1.5 min. The ordinate represents the percentage of flies which were alive 16–18 hr after treatment.

over 75% of the flies died under these conditions. Their behavior correlates well with the change in activity of ADH with age; activity increasing to a maximum in about four days at 25° (DUNN *et al.* 1969). ADH-negative flies were apparently unaffected by the treatment at all ages.

A simple interpretation of the data presented so far is that pentenol is oxidized in the presence of active ADH into some highly toxic substance(s) (probably 1-pentene-3-one). If so, pentenol should be a good substrate for fly ADH. In order to show this, crude homogenates of Drosophila adults were electrophoresed in agar and ADH activity was localized histochemically using ethanol and pentenol as substrates. Under these conditions ADH can be unequivocally demonstrated due to its characteristic banding pattern (URSPRUNG and LEONE 1965). These experiments showed that pentenol was indeed utilized as a substrate by fly ADH; the banding pattern was the same whether ethanol or pentenol was used as substrate. No additional bands were observed with pentenol nor were any bands seen in extracts from ADH negative flies (ADH^{n1-n4}).

In addition, cuvette assays for fly ADH confirmed that pentenol, like other secondary alcohols, was an excellent substrate for the enzyme. In fact when crude extracts from the Daekwaryung strain were used as a source of enzyme, the initial rates of ADH activity with 0.1 M pentenol as substrate were found to be nearly 6 times that with 0.1 M ethanol.

We have used this selection procedure in a pilot study to detect and isolate new

ADH-negative mutants after ethyl methanesulfonate mutagenesis. For this program, we aged prospective mutant flies for four days or more and then exposed them to the pentenol vapor for three minutes. Of 100,000 aged flies treated with pentenol, 14 survived. Twelve of these were ADH negative. Their characteristics will be discussed in a future publication. Only two flies proved to be "false negatives." One was homogenized and was shown to have ADH activity. In addition, its progeny were ADH positive and pentenol sensitive. The other had only ADH-positive, pentenol-sensitive progeny. It was not assayed for ADH activity.

In summary, we have developed a method whereby flies which have normal levels of ADH are killed in the presence of pentenol and flies which lack enzyme activity survive. This method may allow isolation of a large number of mutants which may help in understanding the regulation of ADH activity in Drosophila.

We are most grateful to Dr. E. GRELL for providing us with the Adh-negative stocks.

LITERATURE CITED

BRINK, N. G., 1970 Complete and mosaic visible mutations produced by ethyl methanesulfonate in *Drosophila melanogaster*. Mutation Res. **10**: 227–236.

DUNN, G. R., T. G. WILSON and K. B. JACOBSON, 1969 Age-dependent changes in alcohol dehydrogenase in Drosophila. J. Exptl. Zool. **171**: 185–190.

GRELL, E. H., K. B. JACOBSON and J. B. MURPHY, 1968 Alterations of genetic material for analysis of alcohol dehydrogenase isozymes of *Drosophila melanogaster*. Ann. N. Y. Acad. Sci. **151**: 441–455.

MEGNET, R., 1967 Mutants partially deficient in alcohol dehydrogenase in *Schizosaccharomyces pombe*. Arch. Biochem. Biophys. **121**: 194–201.

URSPRUNG, H., W. H. SOFER and N. BURROUGH, 1970 Ontogeny and tissue distribution of alcohol dehydrogenase in *Drosophila melanogaster*. Wilhelm Roux' Arch. Entw. Mech. **164**: 201–208.

URSPRUNG, H. and J. LEONE, 1965 Alcohol dehydrogenases: A polymorphism in *Drosophila melanogaster*. J. Exptl. Zool. **160**: 147–154.