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Dietary Ethanol and Lipid Synthesis in *Drosophila melanogaster*

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When cultured on a defined diet, ethanol was an efficient substrate for lipid synthesis in wild-type Drosophila melanogaster *larvae. At certain dietary levels both ethanol and sucrose could displace the other as a lipid substrate. In wild-type larvae more than 90% of the flux from ethanol to lipid was metabolized via the alcohol dehydrogenase (ADH) system. The ADH and aldehyde dehydrogenase activities of ADH were modulated in tandem by dietary ethanol, suggesting that ADH provided substrate for lipogenesis by degrading ethanol to acetaldehyde and then to acetic acid. The tissue activity of catalase was suppressed by dietary ethanol, implying that catalase was not a major factor in ethanol metabolism in larvae. The activities of lipogenic enzymes,* sn-*glycerol-3-phosphate dehydrogenase, fatty acid synthetase (FAS), and ADH, together with the triacylglycerol (TG) content of wild-type larvae increased in proportion to the dietary ethanol concentration to 4.5% (v/v). Dietary ethanol inhibited FAS and repressed the accumulation of TG in ADH-deficient larvae, suggesting that the levels of these factors may be subject to a complex feedback control.*

KEY WORDS: ethanol; alcohol dehydrogenase; lipid; *Drosophilia;* carbohydrate; nutrition.

INTRODUCTION

Several short-chain alcohols, especially ethanol, occur in the natural habitats of *Drosophila melanogaster* (Gibson *et al.,* 1981; McKechnie and Morgan,

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1982) and alcohol dehydrogenase (ADH; alcohol:oxireductase; EC 1.1.1.1) plays a key role in the detoxification and metabolic utilization of ethanol and other alcohols by the insect (David *et al.*, 1976, 1978, 1981; Van Herrewege *et al.*, 1980; Parsons, 1980). In general, primary alcohols are converted to aldehydes, and secondary alcohols to ketones, by ADH in this species. The ketones formed from secondary alcohols are toxic to the animal and are largely metabolically inert. In contrast, ethanol is transformed into acetaldehyde by ADH and acetaldehyde is subsequently converted to acetic acid, then to acetyl-CoA. Acetyl-CoA may serve as a substrate for fatty acid synthesis or be oxidized by the Krebs cycle.

Exposure to dietary ethanol increases the activities of the lipogenic enzymes *sn*-glycerol-3-phosphate dehydrogenase (GPDH) and fatty acid synthetase (FAS), as well as increasing the triacylglycerol (TG) content of *D. melanogaster* larvae (Geer *et al.*, 1983). Because these diet-induced changes may be parts of a physiological mechanism for increasing the capacity of the individual to convert dietary ethanol to lipid, we investigated (1) the utilization of ethanol as a substrate for lipogenesis and (2) the involvement of ADH in lipogenesis.

MATERIALS AND METHODS

Animals and Dietary Conditions

The Canton-S wild-type strain of *D. melanogaster* was employed for experiments unless otherwise indicated. The Canton-S strain is homozygous for the Adh^F allele and had been maintained under continuous axenic culture on modified Sang's medium C for more than 4 years before the initiation of the current investigation. A strain lacking ADH, Adh^{n2}, was obtained from the *Drosophila* Stock Center at Bowling Green State University and was employed in this study. Two other wild-type strains used in a part of this study, one homozygous for Adh^F and one for Adh^S, were acquired from Dr. Douglas Cavener, Department of Molecular Biology, Vanderbilt University. The ethanol-conditioned populations, E1 and E2, and the control populations, C1 and C2, were also provided by Dr. Cavener.

Test cultures were set up by placing 10 to 15 female–male pairs of adults in 6-dram shell vials containing 5 ml of modified Sang's medium C for 24 hr. After 4 days of growth, larvae were transferred to different test diets by the method of Geer *et al.* (1976) and cultured 2 days before analysis. The test cultures contained 20–50 larvae per vial and were maintained at 22.8°C and 45% relative humidity with a 15-hr light:9-hr dark lighting schedule. Cultures contaminated with microorganisms were discarded.

Modified Sang's medium C was prepared according to Geer *et al.* (1983). The contents of ethanol, sucrose, and 2-propanol in the test diets were as indicated in the Results. Alcohol and sucrose concentrations are given as the percentage of the total diet. For example, a 428 mM ethanol concentration is 2.5% (v/v); a 14.6 mM sucrose concentration is 0.5% (w/v).

To add ethanol and other volatile nutrients to the test diet, the medium was prepared and autoclaved without the additive. The mixture was allowed to cool to a temperature close to solidification, then the test compound was added and the medium was dispensed into vials. ^{14}C-labeled ethanol and glucose were added to the medium with unlabeled ethanol and sucrose at the concentrations indicated in the Results. Larvae were transferred into the test cultures immediately after the food was prepared. The final steps of medium preparation and the handling of adults and larvae were conducted in a uv-sterilized bacteriological glove box.

Enzyme Assay Methods

Enzymes were assayed in whole larval homogenates by following the change of extinction of the reaction mixture at 30 °C with a Gilford Model 222 spectrophotometric system. The ADH activity was monitored at 340 nm using the reaction mixture of McKechnie and Geer (1984) and either 0.1 M 2-propanol or 0.1 M ethanol as the substrate. The aldehyde dehydrogenase activity of ADH was assayed according to Heinstra *et al.* (1983). Aldehyde oxidase (AO; EC 1.2.1.3) and pyridoxal oxidase (PO) were measured by the method of Cypher *et al.* (1982) using 25 mM 2,4,5-trimethoxybenzaldehyde and 25 mM heptaldehyde as substrates, respectively. Catalase (EC 1.11.1.6) was determined by the method of Lubinsky and Bewley (1979), FAS was assayed by the method of Horie (1968), and *sn*-glycerol-3-phosphate dehydrogenase (GPDH; EC 1.1.1.8) was assayed according to Beenakkers (1969).

Whole larval homogenates were prepared by grinding 10 larvae in 0.16 ml of buffer with a hand-operated Teflon-pestle homogenizer. The homogenate was allowed to stand for 20 min and then was centrifuged at 15,000 *g* for 15 min. The supernatant was removed, and aliquots were taken for enzyme activity determination and protein assay. All operations were conducted at 4°C. Protein assays were performed by the method of Bradford (1976) using bovine serum albumin as the standard. For the assay of AO and PO, the homogenates were prepared using a basic homogenizing buffer of 0.01 M K_2HPO_4–KH_2PO_4, *p*H 7.4, and 0.07 mM phenylthiourea (PTU); to measure the ADH, ALDH, and GPDH activities, 2 mM Na_2H_2EDTA and 0.2 mM dithiothreitol (DTT) were added to the homogenizing buffer; for the

determination of FAS activity, the homogenizing buffer contained 2 mM Na_2H_2EDTA and 2 mM DTT; and to assay the catalase activity, 0.2% Triton X-100 was added to the homogenizing buffer.

Lipid Methods

Larvae were homogenized in glass-distilled water and the lipids extracted by the method of Folch *et al.* (1957). To trace label into total larval lipids ^{14}C-labeled ethanol or glucose was fed to larvae and the radioactivity of the total lipid extract was determined by scintillation counting methods according to Geer and Downing (1972).

The TG content of larvae was assayed by the method of Pinter *et al.* (1967) using Sigma Chemical Company Kit 320-UV (Sigma Chemical Co., St. Louis, Mo.). Homogenates were prepared with 0.07 mM PTU and extracted without centrifuging. The protein contents of the 15,000 *g* supernatants of homogenates were measured according to Bradford (1976).

Statistical Analysis

Unless otherwise indicated the data were analyzed using the *t* test. Two-way analysis of variance was employed as indicated.

Reagents

Vitamin-free casein for the test diets was obtained from the United States Biochemical Corp., Cleveland, Ohio. Other biochemicals were purchased from the Sigma Chemical Co., St. Louis, Mo. Inorganic compounds and solvents were reagent grade.

RESULTS

Degradation of Ethanol by ADH

The contribution of ADH to the degradation of ethanol in *D. melanogaster* larvae was examined by comparing the capacities of wild-type and ADH-null larvae to incorporate label from dietary ethanol into lipid (Table I). ADH-null larvae, which are killed by 2% ethanol, incorporated only 7–9% as much label into lipid as wild-type larvae when fed three different test diets. This indicated that the biochemical pathway represented by ADH metabolized 91–93% of the dietary ethanol when 1.5% ethanol was fed. The inclusion of 2 mM palmitic acid in a diet with 0.5% sucrose and 1.5% ethanol repressed the flux from ethanol to lipid by 57% in wild-type larvae and by 47% in the mutant

Table I. Incorporation of Label from [^{14}C]Ethanol into the Lipids of *Adh*^{n2} and Wild-Type Larvae[a]

Diet	*Adh*^{n2}	Canton-S (CS)	*Adh*^{n2}/CS
0.5% sucrose + 1.5% ethanol (control)	241 ± 16*	3612 ± 118	0.07
0.5% sucrose + 1.5% ethanol + 2 mM palmitic acid	137 ± 22**	1554 ± 42*	0.09
5% sucrose + 1.5% ethanol	123 ± 16***	1700 ± 70*	0.07

[a]Tabulated values are means ± SE of five determinations of the nmol of ethanol incorporated into lipid/mg of protein. The medium contained 7.7 μCi of [1-^{14}C]ethanol (Amersham) per 100 ml.

*Significantly different from the mean of Canton-S larvae fed the control medium ($P < 0.001$).

**Significantly different from the mean of *Adh*^{n2} larvae fed the control medium ($P < 0.01$).

***Significantly different from the mean of *Adh*^{n2} larvae fed the control medium ($P < 0.001$).

larvae compared to the control diet. This result confirms the use of ethanol for fatty acid synthesis since the inclusion of palmitic acid under these dietary conditions is known to repress lipid synthesis (Geer *et al.*, 1976). That this decline in the incorporation of label into lipid occurred in both mutant and wild-type larvae when palmitic acid was fed indicates that the lower level of label found in the lipid of the null larvae was not due to contamination during preparation; otherwise, the same level of incorporation of label into lipid would have been expected with or without dietary palmitic acid. The data imply that 7–9% of the dietary ethanol may be degraded to substrate for fatty acid synthesis in larvae by metabolic systems other than the one represented by ADH.

The Interaction of Dietary Sucrose and Ethanol

Larvae were examined to determine how sucrose and ethanol interact as substrates for lipid synthesis. While maintaining dietary ethanol at 1.5%, the elevation of the dietary sucrose level from 0.5 to 5% reduced the flux of label from ethanol to lipid by 49% in mutant larvae and by 53% in wild-type larvae, indicating that dietary carbohydrate and ethanol interact in both these types of larvae in similar ways (Table I). These findings also suggest that the repression of ethanol utilization for lipid synthesis by dietary sucrose was not dependent on a functional ADH. Nonetheless, the degree of repression of

Table II. The Interaction of Dietary Glucose and Ethanol in Lipid Synthesis in Canton-S Larvae[a]

Diet	Source of label	Label into lipid
0.5% sucrose	[^{14}C]Glucose	814 ± 10
0.5% sucrose + 2.5% ethanol	[^{14}C]Glucose	318 ± 10*
0.5% sucrose + 2.5% ethanol	[^{14}C]Ethanol	4545 ± 23
5% sucrose + 2.5% ethanol	[^{14}C]Ethanol	3895 ± 216**

[a]Mean ± SE of four determinations of the nmol of labeled substrate incorporated/mg of soluble protein. Labeled [U-^{14}C]glucose (New England Nuclear) was added at 12.8 μCi per 100 ml of medium. [1-^{14}C]Ethanol (Amersham) was added at 12.9 μCi per 100 ml of medium. The medium contained 12.9 μCi of [1-^{14}C]ethanol (Amersham) per 100 ml.
*Significantly different from the mean of larvae fed the 0.5% sucrose medium ($P < 0.001$).
**Significantly different from the mean of larvae fed the 0.5% sucrose + 2.5% ethanol medium ($P < 0.05$).

ethanol utilization for lipid synthesis by sucrose was dependent on the dietary ethanol concentration. When the ethanol concentration was 2.5%, the flux of label from ethanol to lipid was reduced only 14% in wild-type larvae by an increase of sucrose from 0.5 to 5% (Table II). Conversely, dietary ethanol suppressed the flux of label from carbohydrate into lipid. The addition of 2.5% ethanol to a 0.5% sucrose diet reduced the incorporation of label from

Table III. The ADH and ALDH Activities of Adh^F and Adh^S Larvae Fed Diets with Varied Concentrations of Sucrose and Ethanol[a]

Diet	ADH		ALDH	
	Adh^F	Adh^S	Adh^F	Adh^S
0.5% sucrose	205.5 ± 9.0	124.6 ± 11.7	18.0 ± 4.3	15.9 ± 2.8
0.5% sucrose + 2.5% ethanol	835.7 ± 92.8* (406)	651.0 ± 35.8* (522)	68.6 ± 7.3* (381)	48.5 ± 9.1* (305)
5% sucrose	330.7 ± 35.4* (161)	240.2 ± 29.1* (193)	29.4 ± 4.0* (163)	20.1 ± 5.3 (129)
5% sucrose + 2.5% ethanol	479.0 ± 25.3* (233)	386.9 ± 58.7* (311)	41.0 ± 4.7* (228)	38.3 ± 5.1* (241)

[a]Mean ± SD of four determinations. The percentage of the control larvae that was fed a 0.5% sucrose diet is indicated in parentheses for each test value.
*Significantly different from the mean of larvae fed the 0.5% sucrose diet ($P < 0.01$).

carbohydrate into lipid by 62% (Table II). These observations indicate that both ethanol and sucrose are good substrates for lipid synthesis in larvae, and at certain dietary levels each may suppress the utilization of the other compound.

The Aldehyde Dehydrogenase Activity of ADH

Because ADH has been reported to possess aldehyde dehydrogenase (ALDH) activity (Heinstra *et al.*, 1983), the ALDH activity of ADH was examined under a number of experimental conditions. The ADH and ALDH activities were assessed in homogenates of homozygous Adh^F and Adh^S larvae that were fed different combinations of sucrose and ethanol, and the ADH and ALDH activities of ADH in the two experimental lines were observed to parallel each other (Table III). The inclusion of 2.5% ethanol in a 0.5% sucrose diet elevated both the ALDH and the ADH activities of the enzyme three- to fourfold, and the addition of 2.5% ethanol to a 5% sucrose diet had a parallel but lesser effect on both ADH activities. The ADH and ALDH activities of the Adh^F strain were somewhat higher than the corresponding activities of the Adh^S strain. Both the ADH and the ALDH activities of ADH were modulated normally by ethanol and sucrose in two strains lacking AO and PO (*mal* and $aldox^n$ lpo^n), indicating that the observed ALDH activity was not due to AO or PO. ADH-null larvae did not possess a pyrazole-sensitive ALDH activity. These observations confirm that ADH has both ADH and ALDH activities and that both activities are modulated by dietary ethanol.

Table IV. The Effects of Dietary Ethanol and Sucrose on the Activities of Aldehyde Oxidase (AO), Pyridoxal Oxidase (PO), and Catalase in Canton-S Larvae[a]

Diet	Catalase	AO	PO
0.5% sucrose	477.9 ± 80.1	77.1 ± 8.6	112.0 ± 11.9
0.5% sucrose + 2.5% ethanol	157.7 ± 29.6**	42.4 ± 5.3**	88.8 ± 6.7*
5% sucrose	185.4 ± 20.0**	71.3 ± 8.8	95.4 ± 9.4
5% sucrose + 2.5% ethanol	159.9 ± 24.1**	61.3 ± 4.8**	78.1 ± 5.7**

[a]Mean ± SD of four determinations of the substrate metabolized/min/mg of protein at 30°C.
*Significantly different from the mean of larvae fed the 0.5% sucrose diet for the same enzyme ($P < 0.05$).
**Significantly different from the mean of larvae fed the 0.5% sucrose diet for the same enzyme ($P < 0.01$).

Table V. Catalase and ADH Activities in Larvae from Ethanol-Conditioned (E1, E2) and Control (C1, C2) Lines on Diets Containing Sucrose and Ethanol[a]

Diet	Alcohol dehydrogenase				Catalase			
	C1	C2	E1	E2	C1	C2	E1	E2
0.5% sucrose (control)	176.4 ± 9.1	279.5 ± 21.0	362.7 ± 35.5	321.1 ± 21.9	635.5 ± 18.0	572.5 ± 33.8	625.5 ± 57.3	382.5 ± 21.7
0.5% sucrose + 2.5% ethanol	620.5 ± 25.3 (352)[b]	865.6 ± 48.5 (310)	1157.9 ± 56.5 (319)	1000.3 ± 38.3 (311)	436.8 ± 18.7 (69)	396.1 ± 25.8 (69)	297.4 ± 33.3 (47)	178.4 ± 16.1 (47)
5% sucrose	304.1 ± 18.5 (172)	436.0 ± 20.4 (156)	529.1 ± 36.6 (146)	528.2 ± 29.7 (164)	337.8 ± 18.7 (53)	348.7 ± 27.7 (61)	152.1 ± 16.3 (24)	152.5 ± 9.1 (40)
5% sucrose + 2.5% ethanol	531.1 ± 36.5 (301)	635.5 ± 47.4 (227)	730.2 ± 34.9 (201)	602.9 ± 20.3 (188)	363.3 ± 26.2 (57)	338.1 ± 15.3 (59)	135.7 ± 28.8 (22)	119.1 ± 11.0 (31)

[a] Mean activities of five replicates ± SE as nmol of substrate metabolized/min/mg of protein at 30°C.
[b] Activity level expressed as a percentage of the activity of the same line on control medium.

The Effect of Ethanol on Catalase, Aldehyde Oxidase, and Pyridoxal Oxidase

Because catalase may be involved in ethanol degradation in the mammalian liver, the effects of ethanol on catalase and related enzymes were assessed in *D. melanogaster* larvae. Unlike ADH activity, catalase activity was reduced in Canton-S wild-type larvae by both dietary ethanol and dietary sucrose (Table IV).

To ascertain whether the reciprocal relationship of catalase and ADH is a general phenomenon, the ethanol-conditioned and control lines of Cavener and Clegg (1981) were examined. E1 and E2 had been exposed to environmental ethanol for more than 100 consecutive generations and shifts in the frequencies of several pairs of allelozymes had been noted. The tissue activity of ADH was greater in the ethanol-conditioned lines than in the control lines under all dietary conditions, and the degree of ADH induction by dietary ethanol was about the same in the ethanol-conditioned and control lines (Table V). The tissue activity of catalase in the lines varied in larvae fed the control 0.5% sucrose medium, but the addition of 2.5% ethanol reduced the catalase activity 54% in the ethanol-conditioned lines and 30% in the control lines. Supplementation of the diet with 5% sucrose reduced the catalase activity, but the reduction in the ethanol-conditioned lines was greater than the reduction in the control lines. Apparently, exposure of the test populations to environmental ethanol exerted some selection pressure for a greater repression of catalase activity by ethanol and sucrose.

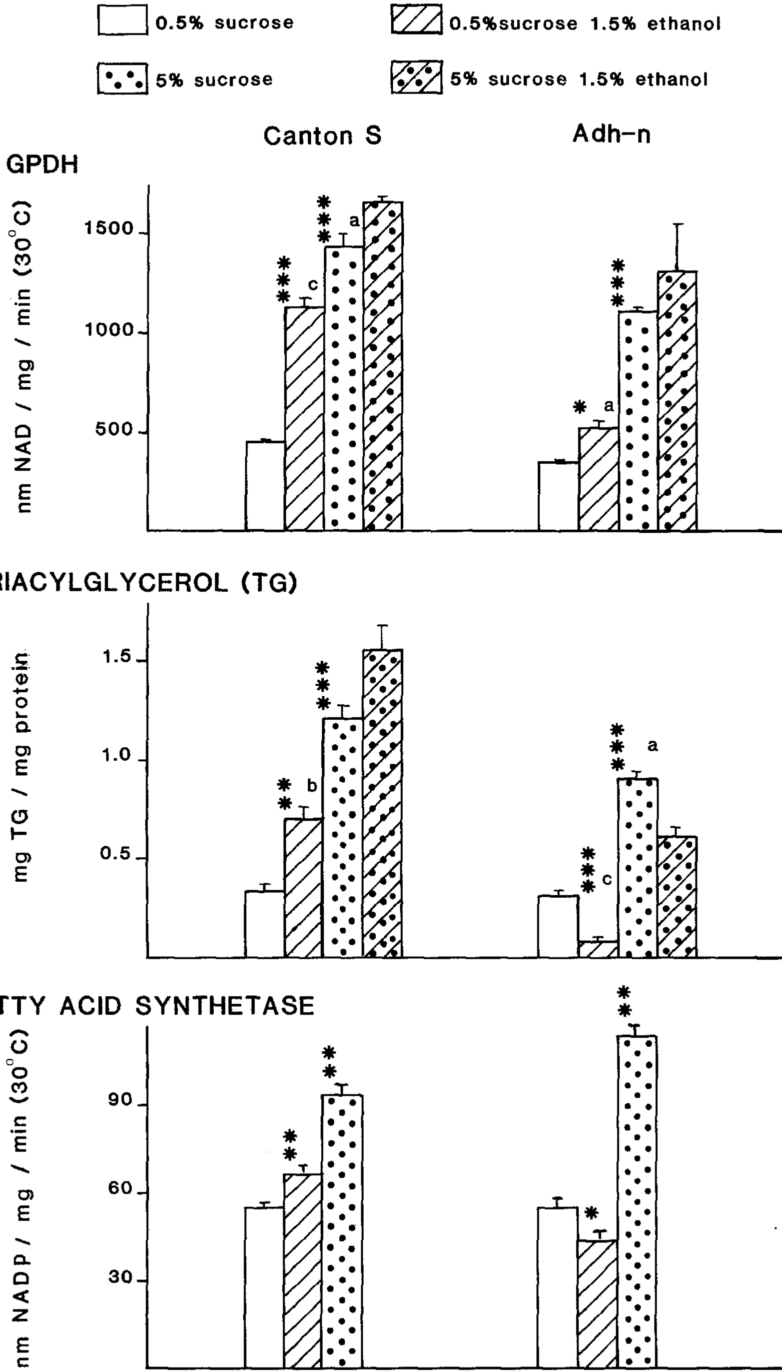
Catalase is frequently metabolically linked to oxidases that generate

Table VI. Metabolic Traits Associated with Lipogenesis in Canton-S Larvae Fed Different Concentrations of Ethanol[a]

Ethanol supplement	TG	FAS	GPDH	ADH	ALDH
None	444 ± 23	72.9 ± 23.0	559.1 ± 59.2	70.0 ± 16.3	27.3 ± 6.0
2.5%	724 ± 99*	113.7 ± 8.9*	1113.3 ± 134.8*	206.7 ± 16.1*	72.7 ± 18.2*
	(163)	(155)	(199)	(295)	(266)
4.5%	922 ± 34*	166.2 ± 26.8*	2143.3 ± 310.0*	271.2 ± 46.7*	125.0 ± 19.3*
	(208)	(227)	(385)	(387)	(458)
5.5%	1097 ± 100*	102.3 ± 16.5*	1437.5 ± 207.3*	225.6 ± 38.5*	101.9 ± 14.8*
	(247)	(140)	(257)	(322)	(374)

[a]For FAS, GPDH, ADH, and ALDH the mean ± SD of five determinations as nmol/mg of protein/min at 30°C is given. The TG values are the mean ± SD of four determinations as μg of TG/mg of protein. The percentage of the control larvae that was fed an unsupplemented diet is indicated in parentheses for each test value. For the determination of ADH activity, 0.1 M ethanol was employed as the substrate.

*Significantly different from the mean of Canton-S larvae on the 0.5% sucrose diet ($P < 0.01$).


0.5% sucrose
0.5%sucrose 1.5% ethanol
5% sucrose
5% sucrose 1.5% ethanol
Canton S
Adh-n
GPDH
nm NAD / mg / min (30°C)
1500
1000
500
TRIACYLGLYCEROL (TG)
mg TG / mg protein
1.5
1.0
0.5
FATTY ACID SYNTHETASE
nm NADP / mg / min (30°C)
90
60
30

hydrogen peroxide. In this experiment AO and PO, two such oxidases, declined in activity in wild-type larvae in synchrony with catalase when ethanol or sucrose was added to the diet (Table V). This suggests that the three enzymes may be functionally linked. However, the type of regulation exerted by ethanol on catalase, AO, and PO would be counterproductive to a major involvement of the enzymes in ethanol metabolism.

Lipogenic Enzyme Activities and Ethanol

Several aspects of lipogenesis were measured in wild-type larvae fed different levels of ethanol. Canton-S larvae tolerate 5.5% ethanol in a 0.5% sucrose diet relatively well, but higher concentrations are increasingly lethal (McKechnie and Geer, 1984). The TG content of larvae reflected the dietary ethanol content over the entire test range (Table VI). The TG levels were increased 1.6-, 2.1-, and 2.5-fold by 2.5, 4.5, and 5.5% dietary ethanol concentrations, respectively. The activities of GPDH and FAS, two enzymes directly involved in lipogenesis, and ADH approximated the TG content to 4.5% ethanol, but the enzyme activities were lower at 5.5 than at 4.5% ethanol. The activities of GPDH and ADH were stimulated more than FAS by dietary ethanol, but the FAS activity most closely reflected the TG content. The ALDH activity of ADH followed the same pattern as the ADH activity of the enzyme in larvae fed different levels of ethanol. These observations indicate the ADH is closely associated with lipogenesis in larvae.

Lipid Synthesis in ADH-Deficient Larvae

Three traits of lipogenesis in ADH-null larvae were compared with those in wild-type larvae (Fig. 1), the tissue levels of TG, GPDH, and FAS. When ADH was present in larvae, ethanol was an efficient precursor of TG. The concentration of TG was 2.1 times greater in wild-type larvae fed a 0.5% sucrose–1.5% ethanol diet than in larvae fed a 0.5% sucrose control diet (Fig. 1). In contrast, regardless of the sucrose level in the medium, the presence of ethanol led to a decrease in the TG content in ADH-null larvae. Two important effects of ethanol on lipid synthesis in wild-type larvae were

Fig. 1. Traits associated with lipogenesis in Canton-S and *Adh*^{n2} larvae on diets supplemented with sucrose and ethanol. Means ± SE for GPDH are based on five determinations, those for TG are based on four determinations, and those for FAS are based on five determinations. The GPDH activity is given as the nmol of NAD^+ formed/mg of protein/min at 30°C. The FAS activity is indicated as the nmol of $NADP^+$ formed/mg of protein/min at 30°C. Means different from that of larvae fed a 0.5% sucrose diet are indicated by (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$. Means different from that of larvae fed 5% sucrose + 1.5% ethanol are indicated by (a) $P < 0.05$, (b) $P < 0.02$, and (c) $P < 0.001$. An ANOVA on the TG data is shown in Table VII.

apparent: (1) a reduction of the utilization of sucrose-derived substrate, detected as a decrease in the incorporation of label from glucose into total lipid (Table II); and (2) an ethanol-stimulated increase in the TG content. In ADH-null larvae only the ethanol-stimulated increase in the TG content was blocked (see Table I). Also, the TG experiment showed that when ethanol is not present in the environment (and the above effects are not at work), an active ADH may enhance the conversion of sucrose to TG in larvae. This result is revealed by the two-way strain–sucrose interaction shown in Table VII. The sucrose-stimulated increase in TG in larvae lacking ADH (2.9-fold) was significantly less than the sucrose-stimulated increase in TG in larvae possessing ADH (3.6-fold). Although this phenomenon needs to be examined in other ADH-null and wild-type strains, the possibility exists that ADH may aid in the production of TG from carbohydrate in some way.

The activity of GPDH, which supplies *sn*-glycerol-3-phosphate for lipid synthesis and facilitates the continuing oxidation of ethanol via the action of the *sn*-glycerol-3-phosphate shuttle, was increased 1.5-fold by the addition of 1.5% ethanol to the control diet of ADH-null larvae. However, the same ethanol-supplemented diet elicited a 2.5-fold increase in wild-type larvae (Fig. 1). The lower GPDH activity in ADH-null larvae apparently reflected a lower demand for GPDH activity due to low rates of ethanol oxidation and TG synthesis. Sucrose was a potent stimulator of GPDH activity in both mutant and wild-type larvae.

The activity of FAS, one of the two enzyme complexes that function in fatty acid synthesis, was lower in ADH-null larvae fed an ethanol-containing diet than in larvae fed the control diet (Fig. 1). This contrasted with the ethanol-stimulated increase in FAS activity noted in wild-type larvae. A 5% sucrose diet promoted FAS activity about twofold greater than the control levels in larvae of both genotypes. This result implies that an ethanol derivative, and not ethanol itself, was responsible for the upward modulation

Table VII. Two-Way ANOVA of the Triacylglycerol Content of Canton-S and Adh^{n2} Larvae Fed 0.5 and 5% Sucrose Diets Lacking Ethanol[a]

Source of variation	df	Mean square	F
Main effects			
Strain	1	0.109	12.7*
Sucrose	1	2.097	245.0*
Interaction			
Strain–sucrose	1	0.084	9.81*
Error	12	0.0086	

[a]The triacylglycerol data are from Fig. 1.
*Significant variation ($P < 0.01$).

of FAS in wild-type larvae. Ethanol may also accumulate in the tissues of ADH-null larvae and inhibit FAS and other lipogenic enzymes, resulting in lower TG levels. Dietary ethanol itself appears to be the repressor of FAS activity in ADH-null larvae.

DISCUSSION

At least three metabolic systems have the potential to convert ethanol to acetaldehyde in higher animals: (1) the catalase system, (2) the microsomal ethanol-oxidizing system (MEOS), and (3) the ADH system (reviewed by Rognstad and Grunnet, 1979). In normal circumstances the catalase and MEOS systems contribute less than 10% to ethanol oxidation in mammals. An analysis of ethanol oxidation by homogenates of wild-type and mutant *D. melanogaster* adults suggested that the MEOS system was not very active, but the catalase system was more important than anticipated (Deltombe-Lietaert *et al.*, 1979). In the current investigation the ADH system accounted for more than 90% of the carbon flux from ethanol to lipid in *D. melanogaster* larvae at a dietary ethanol concentration approximating those encountered in some natural habitats. We found that catalase, AO, and PO were prominent in larvae fed a low-sucrose diet, but feeding ethanol or additional sucrose diminished the activities of these enzymes. Since catalyase, AO, and PO responded to dietary ethanol in a manner opposite to the responses of ADH, GPDH, FAS, and the TG content, the role of the catalase system in ethanol metabolism in wild-type *D. melanogaster* larvae appears to be limited.

In mammals the major enzyme for the conversion of acetaldehyde to acetic acid is a mitochondrial NAD-dependent ALDH (reviewed by Weiner, 1979a), and an enzyme with these characteristics has been partially purified and characterized for *D. melanogaster* adults (Garcin *et al.*, 1981, 1983). We have also detected a mitochondrial ALDH in *D. melanogaster* larvae (Geer and McKechnie, unpublished). Indirect evidence indicates that the ALDH may operate in ethanol degradation in *D. melanogaster*. In the current study ADH-null larvae degraded 7–9% as much labeled ethanol as did their wild-type counterparts, and Garcin *et al.* (1985) have observed that ADH-null adults have ALDH activity. ALDH may be a part of the ethanol-metabolizing system(s) that gives ADH-null *D. melanogaster* a limited ability to utilize dietary ethanol.

AO, PO, and xanthine dehydrogenase (XDH) have broad-range substrate specificities and are able to convert acetaldehyde to acetic acid (Dickinson and Gaughan, 1981; Cypher *et al.*, 1982), but *mal* mutants of *D. melanogaster* which lack these three enzymes have a high tolerance to ethanol (Finnerty, 1976; O'Brien and MacIntyre, 1978). And as observed in the current study, dietary ethanol depresses the activities of AO and PO in *D.*

melanogaster larvae. Consequently, AO, PO, and XDH do not appear to be major factors in the conversion of acetaldehyde to acetic acid in the ethanol degradative pathway of *D. melanogaster*. Because ADH has a relatively high ALDH activity under conditions that are physiologically compatible with its ADH function (Heinstra *et al.*, 1983; Geer, B. W., unpublished), it is possible that part of the acetaldehyde derived from ethanol in *D. melanogaster* is converted to acetic acid by ADH. Nevertheless, the relative roles of ADH and ALDH in the conversion of acetaldehyde to acetate in *D. melanogaster* have not yet been defined.

Heinstra *et al.* (1983) have proposed that the ADH and ALDH activities of *Drosophila* ADH are conducted at a single active site. The parallel ADH and ALDH activities of ADH^F and ADH^S and the concurrent dietary modulation of the ADH and ALDH activities of ADH in larvae observed in the present study are consistent with this proposal. If ethanol is converted to acetic acid at a single active site of *Drosophila* ADH, it is questionable whether the acetaldehyde-to-acetic acid conversion is the limiting step in the ADH system as it is in mammalian ethanol oxidation (Weiner, 1979b). Because of the extreme toxicity of acetaldehyde (David *et al.*, 1978; Deltombe-Lietaert *et al.*, 1979), it is often assumed that the toxicity of environmental ethanol to *D. melanogaster* is due to the accumulation of acetaldehyde in tissues. The coordinately regulated ADH and ALDH activities of *D. melanogaster* noted in the current investigation may help to minimize the accumulation of acetaldehyde.

Dietary ethanol is very toxic to ADH-deficient larvae. Ethanol simultaneously repressed the accumulation of TG and the activity of FAS in ADH-null larvae in the present study. Whether the suppression of lipid synthesis was due to a direct effect of ethanol on the enzymes of lipogenesis is uncertain, but the activities of GPDH and FAS were repressed by 1.5% ethanol (v/v) in reaction mixtures in tests in our laboratory. Whether the sensitivity of FAS to ethanol *in vivo* limits lipogenesis in *D. melanogaster* remains to be determined.

Ethanol is an excellent substrate for lipogenesis in wild-type *D. melanogaster*, and the current radiotracer studies indicate that ethanol and carbohydrate are interchangeable sources of substrate for lipogenesis at appropriate dietary concentrations. Interestingly, the present study suggests that ADH may play a role in the sucrose-stimulated accumulation of TG in wild-type larvae. That is, the increase in TG when the dietary sucrose level was raised from 0.5 to 5% was significantly greater in wild-type larvae than in ADH-deficient larvae. This observation is also consistent with the positive modulation of ADH activity and cross-reacting material by sucrose that was noted in a number of wild-type strains (McKechnie and Geer, 1984; Geer and

Laurie-Ahlberg, 1984). We plan to examine the possibility of a non-alcohol-related ADH function in future studies.

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