

Ringo, J.M. University of California, Davis, California. The effects of anesthetization upon survival and behavior of *D. grimshawi*.

To study some elements of behavior of *D. grimshawi* Oldenberg, it is necessary to mark and mutilate individuals, which in turn requires that the flies be lightly anesthetized. In order to find an anes-

thetization procedure which interferes the least with subsequent behavior, the effects of three agents (CO_2 , cold, and ether) were evaluated.

To assess the effects of treatment upon survival, one control group ($n=30$) was maintained and each agent was applied to five other groups of flies ($N=20$ in each group) for different lengths of time (1/2, 1, 2, 4, and 8 mins.) Flies were chosen at random from a population of PK9 *D. grimshawi* adults, aged 1 to 22 days. CO_2 was administered by suspending flies in a plastic tube over dry ice in a one lb. coffee can. The tube was fitted through a hole in a cardboard top, and the bottom of the tube was covered by a piece of bolting silk. This apparatus was modified from Seecof (1963). The temperature at the bottom of the tube was approximately 4°C . Anesthetization with cold was attained by placing flies in an aluminum cigar tube immersed in ice; the temperature was about 0°C . Ether was used in an ordinary small plastic etherizer. About two ml of ether was placed on the gauze at the bottom of the etherizer, and a few drops were added between treatment groups. The temperature was approximately 20°C . After treatment, each group was placed in a half pint bottle containing fresh food and maintained at $20^\circ\text{C}\pm 1^\circ$. Dead flies were removed and counted at 24, 48, 72, and 96 hours after treatment. The results are summarized in the following table and graphs:

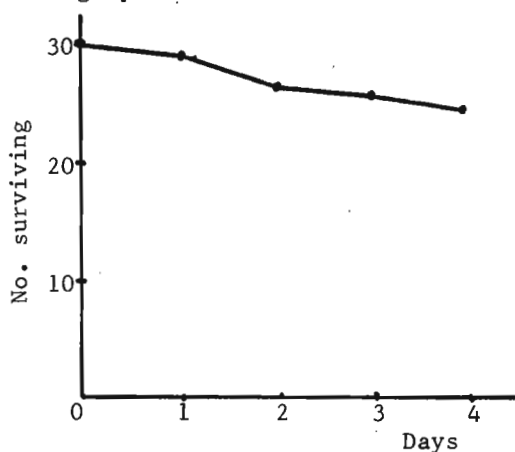


Figure 1.

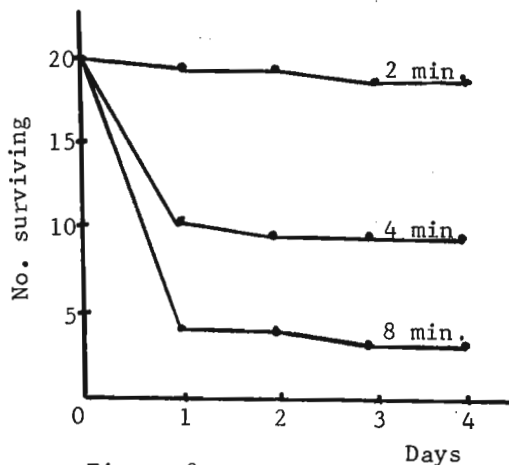


Figure 2.

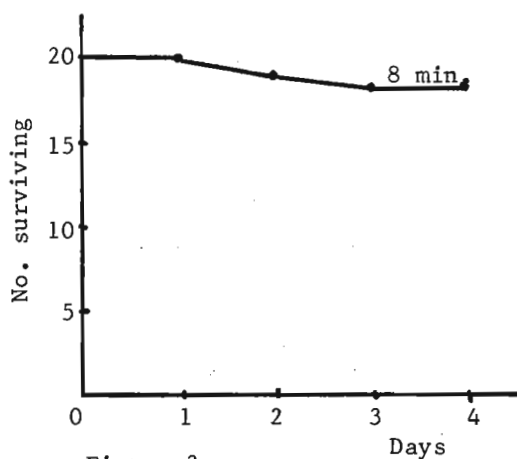


Figure 3.

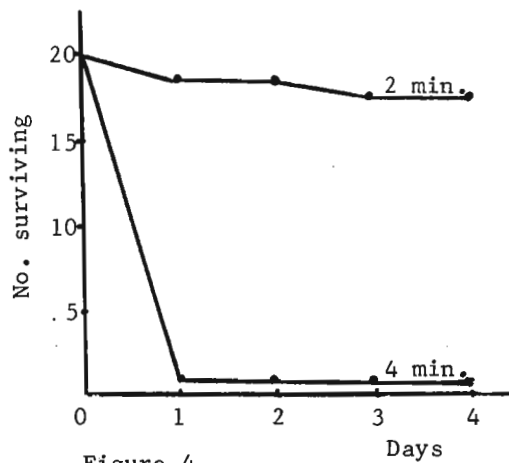


Figure 4.

Survival curves for six treated groups and control group. Fig. 1, controls; fig. 2, CO_2 treatment for 2, 4, and 8 mins.; fig. 3, cold treatment for 8 min.; fig. 4, ether treatment for 2 and 4 mins.

Table 1

No. surviving after 24 hours	Min. with CO ₂					Min. with cold					Min. with ether					Controls
	1/2	1	2	4	8	1/2	1	2	4	8	1/2	1	2	4	8	
" 48 "	19	19	19	10	4	20	18	19	20	20	19	20	18	1	0	29
" 72 "	19	19	19	9	4	19	14	19	18	19	18	19	18	1	0	26
" 72 "	18	18	18	9	3	17	14	18	18	18	18	18	17	1	0	25
" 96 "	18	18	18	9	3	17	14	18	18	18	18	18	17	1	0	24

We accept the hypothesis that the proportion of survivors among the controls and all flies treated for 30 sec. were equal ($\chi^2=1.43$, $df=2$, $p>.20$)

A second experiment sought to determine differences in behavior attributable to these three methods of anesthetization. The phenotype of greatest interest is jousting, a type of behavior found only in males of this species. Subjects were drawn at random from a population of adult PK9 males aged 19 to 25 days. $N=30$ for each treatment group. Ss were anesthetized for 30 sec., their wings were marked with nail polish containing non-toxic dyes; they were isolated in individual half pint bottles containing fresh food and were maintained at $20^\circ\text{C}\pm 1^\circ$. Allowing at least two hours for recovery, Ss were observed in batches ($N=10$) in plexiglass cells ($2\times 5\times 9$ cm) with moist sponge at one end. Their interactions were observed for 20 minutes and recorded; the exact time spent jousting was recorded for each subject using an Esterline Angus 10-channel event recorder. The observations were repeated four more times for each S.

There were marked behavioral differences between treatments. Aggression and courting were very much reduced in cold-treated Ss, and somewhat reduced in CO₂-treated Ss relative to etherized Ss. The quantitative results for jousting show a similar pattern:

Table 2

Treatment	Total of all scores	No. of Ss
CO ₂	834.3	28
cold	932.9	25
ether	1842.1	30

The data can be analyzed in two ways. One can simply record whether or not a subject jousted during a given observation period, or one can consider the relative amount of jousting for each test period. An ordinary analysis of variance is impossible, since the scores have a J-shaped distribution. Out of 415 observations (7 Ss died) or scores,

271 were zero. Using $271/415 = .653$ as the expected proportion of zero scores among treatments and testing $H_0: \theta_1 = \theta_2 = \theta_3$ against the alternative that the proportions are not equal, we reject H_0 ($\chi^2=10.37$, $df=2$, and $p<.01$). The large number of zero scores in all groups of Ss indicates that a simple dichotomous measure has as much biological significance as the amount of time spent jousting. The simplest non-parametric test using the scores is the Friedman two-way analysis of variance by ranks (Siegel 1956). The Friedman test requires equal sample sizes, but 7 Ss died during the experiment and could not be replaced so we averaged the scores for each batch. We reject the hypothesis that treatments do not differ in their effects ($\chi^2_{F}=6.50$, $df=2$, $p<.05$).

The results of these experiments show that light etherization is a better method of anesthetization for behavioral studies in *D. grimshawi* than the use of either CO₂ or low temperature.

References: Seecof, R.L. 1963 DIS 37:145; Siegel, S. 1956 Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill, Inc., New York.

Hunt, D.M. University College London, England. A haemolymph protein anomaly associated with the lethal-giant-larvae mutant in *Drosophila melanogaster*.

Faulhaber (1959) demonstrated a reduction in the haemolymph protein content of larvae homozygous for the *lgl* mutant. However, the paper electrophoresis technique employed by Faulhaber allowed the clear separation of only two protein fractions. With the introduction of acrylamide gel as a supporting medium for electrophoresis, it is now possible to