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°C±1°C. Allowing at least two hours for recovery, Ss were observed in batches (N=10) in plexiglass cells (2x5x9 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:

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: $\theta_1=\theta_2=\theta_3$ 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 ($\chi^2=6.50$, 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.

identify a large number of protein fractions in larval haemolymph. A re-examination of the situation in lgl therefore would seem appropriate.

Two alleles lgl and lglB have been studied. Both mutants were maintained as balanced lethals over the SM5 chromosome. Haemolymph samples from non-lethal larvae were collected at about 5 days post-hatching when the larvae leave the food medium prior to pupation. Development in lethal larvae is delayed and haemolymph samples were taken therefore from at least 6 days post-hatching. The technique of acrylamide gel disc electrophoresis was used. The procedure follows Davis (1964) except that the spacer and sample gels were omitted and 50μl of sample applied directly to the top of each gel. Gels were stained in 0.5% amido black in 7% acetic acid.

No differences could be detected in the haemolymph proteins from lgl/lgl and lgl/SM5 third instar larvae, and lglB/SM5 larvae also gave a normal protein pattern. However, the electrophoretic separation of haemolymph samples from lglB/lglB larvae revealed clear differences in protein content; fraction 7 was entirely missing and the amount of stainable material in fractions 12 and 15 considerably elevated (Fig. 1).

FIGURE 1. Densitometer tracings of electropherograms obtained from haemolymph samples of third instar lglB/lglB larvae (A, B) and lglB/SM5 larvae (C, D). The protein fractions are numbered from the running front to the origin (0).

Fractions 12 and 15 show considerable quantitative variation between strains so the inheritance of the elevated quantities in lglB homozygotes was not examined further. The possibility that the absence of fraction 7 in lethal larvae depends on another gene locus on the lglB second chromosome was tested by outcrossing the lglB/SM5 strain to the Edinburgh wild type. Non-SM5 F1 progeny was mated to expose the lglB chromosome to recombination and haemolymph samples were taken from the resulting third instar larvae. In a total of 104 lglB/ lglB larvae examined, no recombinants were obtained.