Table 1

	Min. with CO_2					Min. with cold					Min. with ether					Controls
No. surviving	1/2	1	2	4	8	1/2	1	2	4	8	1/2	1	2	4	8	--
after 24 hours	19	19	19	10	4	20	18	19	20	20	19	20	18	1	0	29
" 48 "	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 (χ^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°. 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 CO_2-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_o:$\theta_1=\theta_2=\theta_3$ against the alternative that the proportions are not equal, we reject H_o (χ^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 (χ_r^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 CO_2 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

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 lgl^B 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 lgl^B/SM5 larvae also gave a normal protein pattern. However, the electrophoretic separation of haemolymph samples from lgl^B/lgl^B 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).

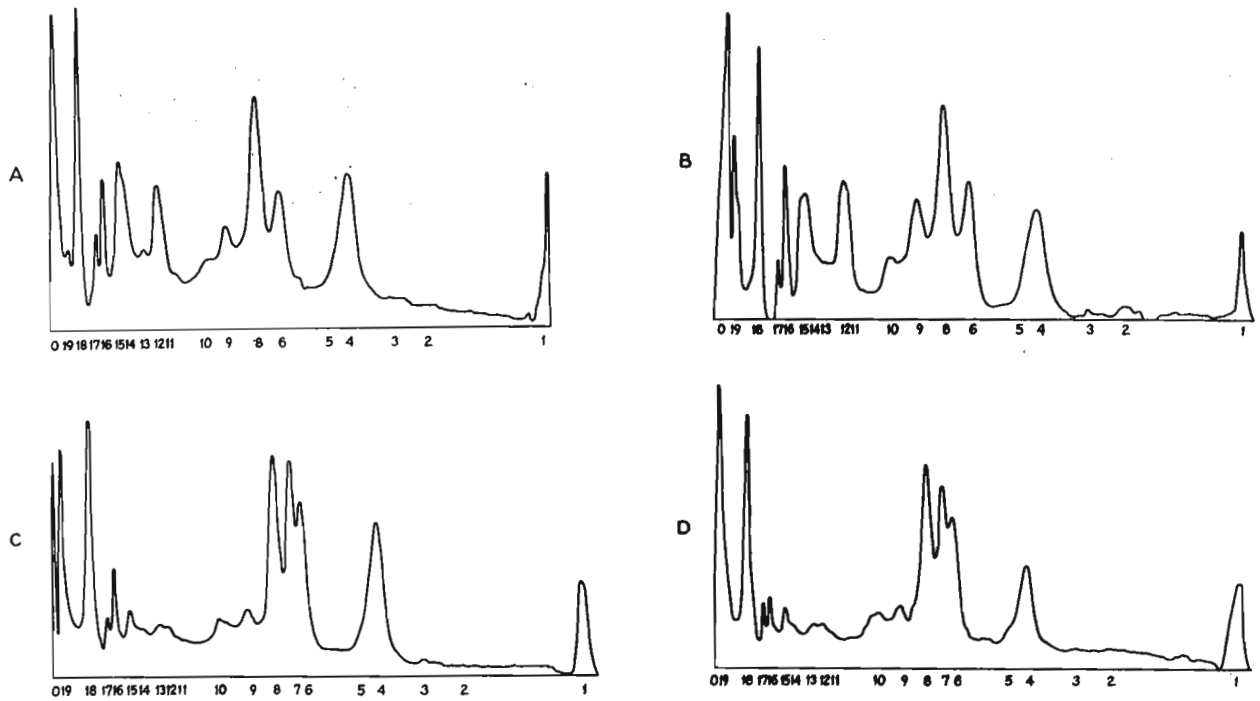

FIGURE 1. Densitometer tracings of electropherograms obtained from haemolymph samples of third instar lgl^B/lgl^B larvae (A,B) and lgl^B/SM5 larvae (C,D). The protein fractions are numbered from the running front to the origin (0).

Fractions 12 and 15 show considerable quantitative variation between strains so the inheritance of the elevated quantities in lgl^B homozygotes was not examined further. The possibility that the absence of fraction 7 in lethal larvae depends on another gene locus on the lgl^B second chromosome was tested by outcrossing the lgl^B/SM5 strain to the Edinburgh wild type. Non-SM5 F_1 progeny was mated to expose the lgl^B chromosome to recombination and haemolymph samples were taken from the resulting third instar larvae. In a total of 104 lgl^B/lgl^B larvae examined, no recombinants were obtained.

References: Davis, B.J., 1964, Ann. N.Y. Acad. Sci. 121: 404; Faulhaber, I., 1959, Z. Induktive Abstammungs-Vererbungslehre 90: 299.