Sega, G.A. and W.R. Lee. Department of Zoology, Louisiana State University, Baton Rouge. A vacuum injection technique for obtaining uniform dosages in D. melanogaster.

A new method to administer quantitatively chemical mutagens to D. melanogaster by vacuum injection has been developed at our laboratory. Previous micro-injection techniques have been criticized by Carlson and Oster (Genetics 47:561) because individual flies vary as to the amount of injected material that is retained. Preliminary tests in our lab in which 0.2µl. of ^{14}C-ethyl methanesulfonate (EMS) was injected per fly confirmed this variability. In several experiments we obtained a coefficient of variation (C.V.) of the retained radionuclide that averaged near 60%. (The coefficient of variation is the percentage ratio of the sample standard deviation to the sample mean: $s/\bar{x} \cdot 100\%$)

The feeding method of E.B. Lewis and F. Bacher (DIS 43:193) was also used and we obtained in this case a C.V. of 16%. However, a considerable amount of mutagen was necessary to carry out a feeding experiment. A method of treatment was therefore devised that gives a comparable C.V. while using only 10µl. of mutagenic solution.

In our vacuum injection method, a C.V. of 10% was obtained using ^{14}C-EMS. The procedure was to place 10 nonetherized adult males in a 25 ml. serum vial and then lower the absolute pressure to between 40 and 50 mm of Hg. Freshly etherized flies were killed by the vacuum treatment. Increasing the number of flies per vial above 10 gave a lower received dose per fly. Ten µl. of water containing ^{14}C-EMS was then taken up into a syringe needle attached to a 1 ml. syringe with the syringe plunger withdrawn to take in 0.1 ml. of air. The syringe needle containing the 10µl. was then inserted through the rubber stopper of the serum vial, and atmospheric pressure forced the mutagen into the vial as an aerosol. Absolute pressure rose only slightly. After flies were left in the vials from one to two hours the vacuum was broken by inserting a large hypodermic needle through the rubber stopper. It was thought that the sudden increase in air pressure caused the mutagen that had diffused into the trachea to be forced into the tissues of the fly, although this point is still open to question.

Increasing the concentration of ^{14}C-EMS by a factor of 10 resulted in a ten-fold increase in the amount of retained radionuclide. The incorporation of the radionuclide was found to increase with time in the vacuum; however, leaving the flies in the vials for longer than two hours caused death in some cases. Genetic tests have shown this method to be effective in producing mutations. A test of 323 chromosomes from males treated with 10µl. of 0.12 M non-labeled EMS for one hour gave 42% sex-linked recessive lethals in the F_2.

Hunt, Virginia. Developmental Biology Center, Case Western Reserve University, Cleveland, Ohio. A qualitatively minimal amino acid diet for D. melanogaster.

Using a modification of Geer's amino acid diet (DIS 40:96) as a basic test medium, we have developed a medium which is completely defined and quantitatively minimal. Three amino acids can be omitted from Geer's original formulation and RNA replaced by inosine and uridine, without adversely affecting either development time or survival. The omission of either glycine or tyrosine causes no significant change in development time or survival, while the omission of cystine causes a significant decrease in development time and a significant increase in survival

	mg.		mg.		mg.		mg.
L-Arginine·HCl	80	L-Threonine	200	Thiamine	0.20	$MgSO_4 \cdot 7H_2O$	24.60
L-Glutamic acid	840	L-Tryptophan	50	Nicotinic acid	1.20	$NaHCO_3$	100.00
L-Histidine·HCl	100	L-Valine	280	Riboflavin	1.00	KH_2PO_4	71.90
L-Isoleucine	300	Agar ("Bacto")	1500	Ca pantothenate	1.60	K_2HPO_4	373.60
L-Leucine	200	Sucrose	1000	Pyridoxin	0.25	Water to 100 ml.	
L-Lysine.HCl	190	Inosine	80	Biotin	0.03		
L-Methionine	80	Uridine	70	Folic acid	1.00		
L-Phenylalanine	130	Cholesterol	30	Choline chloride	8.00		

Glutamic acid was found to be non-essential for survival but essential for normal development time. The minimal medium follows: ($FeSO_4$, $CaCl_2$, $MnSO_4.H_2O$ were omitted as they were found to be unnecessary by Sang (J. Exptl. Biol. 33: 45-72).