Higgins, C.O. and B. Hochman. University of Tennessee, Knoxville, Tennessee. Free amino acids and the genetic control of transfer RNA in Drosophila.

Minute (M) mutations have been detected at some 55 different loci distributed over the four chromosome pairs of Drosophila melanogaster. Regardless of the mutation's location, or whether the M^+ gene has been delated or simply altered to an M allele, the phenotypic effects of the genetic changes are essentially similar; i.e., homozygotes (M/M) die as embryos or larvae and heterozygotes (M^+/M) have delayed development, short, thin bristles and reduced viability and fertility.

One hypothesis (advanced by Atwood and Ritossa) for the Minute syndrome is that the various M^+ genes each specify a particular transfer RNA and heterozygotes (with only one dose of a normal gene) suffer from a reduced protein synthesizing capacity while M/M flies die due to inability to utilize adequately the amino acid corresponding to the species of t RNA they are unable to encode. (See Ritossa et al., 1966.)

There is at least one M^+ locus on the fourth chromosome in D. melanogaster. As a test of the above hypothesis, we measured the free amino acids in samples of 150 adults carrying different doses of chromosome 4 and of the M^+ gene(s) therein situated. Our idea was simply that an otherwise diploid fly with one fourth chromosome (haplo-4) might exhibit a higher concentration of at least one amino acid than normal (diplo-4) individuals. Actually, the tests involved eleven different genotypes as follows: triplo-4, c(4)RM (attached-4), Oregon-R and Canton-S (two wild-type diploid strains), $M(4)^{57g}$/Df(4)G, $M(4)^{63a}/ci^D$, Df(4)M/ey^D, Df(4)M/Df(4)G and two haplo-4 types (Oregon-R and Canton-S). ($M(4)^{57g}$ is a presumed point mutation, $M(4)^{63a}$ is a small deletion and Df(4)M a large deficiency for the M^+ and neighboring loci, Df(4)G is a deficiency for the distal 10-15 percent of the chromosome, ey^D is a small duplication and ci^D is a dominant visible.)

Samples were prepared both from flies fed on a standard medium and those fed only a 1% sucrose solution for 48 hr prior to the extraction of amino acids. The effect of differences in age of adults (0-24 hr versus 72-96 hr) on amino acid content was also examined. A Beckman-Spinco amino acid analyzer, located at the University of Tennessee Medical Research Center and made available through the courtesy of Dr. D. Dupourque, was utilized.

Fifteen amino acids were found in measurable amounts in all samples tested and traces of threonine were also detected. Technical difficulties prevented the resolution of asparagine, cysteine, glutamine and tryptophan. The concentration of proline was consistently highest followed by serine, glutamic acid and arginine in order of abundance varying from sample to sample.

On the basis of t-tests it was concluded that no important differences existed between the samples of fed and 48 hr-"starved" flies and the two sets of data could be considered experimental replicates. To test the eleven genotypes for possible significant differences in amino acid content, Duncan's multiple range tests were performed. It was found that the concentration of proline was significantly different between triplo-4 and attached-4 samples and that the concentration of alanine in Oregon-R differed significantly from that in triplo-4, attached-4, $M(4)^{63a}/ci^D$ and Df(4)M/ey^D. These differences, however, were not correlated with the number of doses of chromosome 4 or the M^+ locus.

While apparent differences in amino acid content were found between one and three day old adults, the absence of replicates precluded statistical analysis.

These results confirm what others (Chen, 1962 and Fahrig, 1970) have reported for free amino acids in Drosophila and other insects but they do not support the Atwood-Ritossa hypothesis. On the other hand, no outright rejection of the hypothesis is warranted for the following reasons: (1) The M^+ locus on 4 might specify a t RNA for one of the four amino acids not measured in these experiments; (2) The small number of replicates in this study (dictated by insufficient funds) unfortunately engendered a high within-group error. Significant differences in amino acid content, correlated with doses of M^+, may exist but statistical limitations prevent their exposure; (3) It is possible that an amino acid in high concentration as a result of t RNA inadequacy may be altered or metabolized so as to render it undetectable by the methods here employed.

Additional studies, in which all of the common acids are measured and a greater number of replicates of the crucial genotypes (haplo-4, diplo-4 and triplo-4) are tested, are required before conclusive evidence, either confirming or rejecting the hypothesis, can be obtained. Results from such experiments could then be compared to proposed nucleic acid hybridizations of 4s RNA (t RNA) with polytene chromosome DNA (Steffensen and Wimber, 1971) to determine the correctness of the postulated M^+ - t RNA relationship.

Literature cited: Chen, P.S. 1962 In Amino Acid Pools, pp. 115-135, Elsevier, Amsterdam; Fahrig, R. 1970 DIS 45:62; Ritossa, F.M., K.C. Atwood and S. Spiegelman 1966 Genetics 54: 663-676; Steffensen, D.M. and D.E. Wimber 1971 Genetics 69:163-178.

Premlatha, N. and M. Sanjeeva Rao Osmania University, Hyderabad, India. Induction of mutations by thioridazine hydrochloride in Drosophila melanogaster.

The pioneering work of Auerbach and Robson (1942) on the chemical induction of mutations by mustard gas in D. melanogaster followed by an extensive study on chemical induction of mutations led us to understand how the genes act. Various chemicals have been tried for their genetic damage in various organisms. However, the studies on the mutagenic potential of tranquilizers has been scanty. The review available was only on their toxic and physiological effects. With a view to find out whether these tranquilizers commonly used would also produce genetic damage, experiments were undertaken to assess the damage, if any, of thioridazine hydrochloride, a chemical which is one of the most important ingredients in tranquilizers.

Oregon-K strain of D. melanogaster flies were allowed to feed on a normal medium containing 10 mgs of thioridazine hydrochloride for every 100 cc of food and 20 mgs. of the chemical for every 100 cc of food. The males developed on these media were crossed to y sc^{S1} In-49 sc^{8}; bw;st virgin females, to screen the incidence of sex-linked recessive lethals and translocations. A brood pattern of 3 days interval was used and six broods were studied. Each male was allowed to mate with 3 virgin females. The virgin F_1 females were mated individually with y sc^{S1} In-49 sc^{8} males, while the F_1 males were mated individually with bw;st virgin females to score for sex-linked recessive lethals and translocations respectively. The results are presented in Table 1.

Table 1.

Sex-linked recessive lethals.

Brood	Control			thioridazine hydrochloride 10mg/100cc food			thioridazine hydrochloride 20mg/100cc food		
	T	l	%	T	l	%	T	l	%
A Brood	1336	3	0.22	477	6	1.34	362	4	1.10
B Brood	1716	8	0.47	424	6	1.40	364	1	0.27
C Brood	1894	3	0.24	322	5	1.52	243	3	1.23
D Brood	1599	7	0.43	123	4	3.25	257	7	2.72
E Brood	1015	0	-	283	10	3.60	301	12	3.98
F Brood	1073	3	0.27	179	4	2.23	125	4	3.20
Translocations									
				T	t	%	T	t	%
A Brood	1516	0	-	388	0	-	357	0	-
B Brood	1496	0	-	411	2	0.48	486	4	0.82
C Brood	1668	0	-	276	1	0.39	152	4	2.62
D Brood	1539	0	-	173	4	2.30	244	4	1.63
E Brood	1321	0	-	229	6	2.62	264	3	1.52
F Brood	1367	0	-	295	8	2.71	300	4	1.33

T = total number of X chromosomes or F_1 sons scored
l = lethals recorded
t = translocations recorded

The chi square test has been done to compare the following groups: 1) control versus thioridazine hydrochloride, 10mg/100cc food; 2) control versus thioridazine hydrochloride,