GENETIC AND DEVELOPMENTAL STUDY OF A COMPLEX LOCUS
IN THE HOUSE MOUSE

Progress Report

for period August 1, 1975 - July 31, 1976

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Abstract of Progress Report

We have maintained and studied seven dominant T-like mutations, eleven recessive lethal t-alleles in six different complementation groups, two semilethal t-mutations, and fifteen viable t-variants. Seven of the latter are newly arisen and have been characterized during this year. Six other, non T/t locus, markers are also maintained. Interest has centered on the following three points. (1) Further analysis of the four different types of dominant T-mutations that we have available shows that two of these, and possibly a third, seem to have chromosome abnormalities associated with them. The etiology of the maternal effect seen in T^{HP} has also been studied; no mechanism has yet been found, although trisomy 17 has been excluded. (2) Eleven new alleles derived by recombination in lethal t-mutations have been fully or substantially analyzed; all are homozygous viables, male-fertile, and most have low transmission ratios. (3) Attempts at fine structure mapping of the T-tf region in the presence of two other mutations (t³⁸ and Low) have produced anomalous results that suggest that the presence of t³⁸ and Low in the trans position may interfere with crossing over in the t³⁸-Low interval.

PROGRESS REPORT

Principal Investigator:

Dorothea Bennett, Ph. D. 10% of time (no change anticipated).
Contract requirements have been complied with.

1) Stock Maintenance and Supply

I. The following mutations at the T/t locus are maintained:

<u>Complementation Group</u>	<u>Mutations</u>
T	T, T ¹ Or, T ² Or, T ³ Or, T ⁶ Or, T ^{Hp} , T ^{Or1}
t ⁰	t ⁰ , t ¹
t ⁹	tw ¹⁸
t ¹²	t ¹² , tw ³²
tw ¹	tw ¹ , tw ¹² , tw ⁷¹
tw ⁵	tw ⁵ , tw ⁷⁵
tw ⁷³	tw ⁷³
t ^{semilethal}	tw ² , tw ⁸
t ^{viable}	t ³⁸ , t ⁴⁴ , t ⁴⁵ , t ⁴⁶ , t ⁴⁷ , t ⁴⁸ , t ⁴⁹ , t ⁵⁰ tw ⁸² , tw ⁸³ , tw ⁸⁴ , tw ⁸⁵ , tw ⁸⁶ , tw ⁸⁷ , tw ⁸⁸ .

II. The following chromosome 17 markers or mutations are maintained: Ki, tf, qk, T¹⁹⁰, T¹³⁸.

III. Because of the ectromelia epidemic in late 1974 our colony was quarantined until early 1976. We have now begun to supply mice to other investigators again, and have supplied the following laboratories:

Dr. J. Larry
Department of Biology
University of Alabama
University, Alabama

Terry Magnuson
Sloan Kettering Institute
Rye, New York

Dr. Davor Solter
The Wistar Institute
Philadelphia, Pennsylvania

Dr. L. Flaherty
Research Division
Department of Health
Albany, New York

Dr. H. McDevitt
Department of Medicine
Stanford University Medical School
Stanford, California

Dr. Kurt Stern
Mt. Sinai Hospital
New York, New York

Dr. M. Verghese
Department of Microbiology and Immunology
Duke University
Durham, North Carolina

2) Genetic and Developmental Analysis

I. Dominant T-mutations.

We have available four classes of dominant T-mutations, each of which is in some respect different from any other, as indicated in the Table below.

<u>Allele</u>	<u>Time of Homozygous Lethality</u>	<u>Pseudo- dominance of quaking</u>	<u>Maternal Effect</u>
T	10 1/2 days	No	No
T ^{Or}	7 - 9 days	No	No
T ^{Orl}	before 9 days	Yes	No
T ^{Hp}	before 9 days	Yes	Yes

This table summarizes information published in Genetical Research (Bennett, D., L. C. Dunn, M. Spiegelman, K. Artzt, J. Cookingham, and E. Schermerhorn (1975) 26: 95-108). ~~(Six reprints are attached).~~

New work done on these mutations during the past year has yielded the following results:

Time of death and lethal syndrome of TOrl and THp homozygotes:

These homozygotes appear to die shortly after implantation, showing severe defects of ectoplacental cone development.

Chromosomal Defects in TOr, TOrl, and THp:

Each of these mutations has effects which are compatible with their being small deletions; that is, TOr mutations produce no specific sperm T-antigen detectable by allo-immunization, suggesting that no gene product exists. TOrl and THp, on the other hand, although they do specify a sperm antigen detectable by our conventional anti-T antisera, show evidence of deficiency in another way; namely, by eliciting pseudodominance of the marker qk 3 units to the right of the T/t locus. This evidence thus suggests that all three may be deletions, but that TOrl and THp cover different regions than TOr. In collaboration with Dr. J. Biedler we are examining chromosome 17 in these stocks by Giemsa banding methods. The evidence is that THp shows alteration of the first band adjacent to the centromere such that there appears to be a diffusion of the same band into the adjacent interband area, and sometimes contact of the band with the centromeric heterochromatin. TOrl shows the same effect but in lesser degree.

The Etiology of "Maternal Effect" in THp Females:

As already reported, the THp mutation is transmitted to viable offspring only through sperm. THp-bearing eggs from THp/+ females, even though fertilized by normal sperm, produce embryos that die late in pregnancy. We have begun the examination of litters of embryos segregating for these lethal heterozygotes and find that the lethal embryos survive frequently to at least 17 days of gestation, showing only diffuse and minor abnormalities such as edema, polydactyly, and some neural tube defects. Histological studies are in progress. This is the only such clear cut effect known in mammals, and the reason for it is not at all obvious. One possibility that we examined was that the THp-carrying oocytes in such females might undergo non-disjunction and thus, on fertilization, yield embryos trisomic for chromosome 17, a situation that might be compatible with their relatively late death. However, no evidence for aneuploidy was found in such dying embryos.

3) Complete or partial genetic analysis has been carried out for eleven newly arisen t-alleles in our colony. All of these have arisen from a pre-existing lethal or semilethal mutation by abnormal recombinational events, and all are known to produce viable homozygotes. The mutations that we have analyzed in the past year, and their characteristics, are given in the Table below:

<u>Allele</u>	<u>Parent Allele</u>	(1) <u>Relative Viability t/t</u>	(2) <u>Transmission Ratio</u>	(3) <u>Male t/t Fertility</u>
t44	t0	88%	31%	6.8
t45	t0	69%	23%	i. d.
t46	t0	i. d.	i. d.	9.6
t47	t0	i. d.	17%	6.9
t48	t0	76%	37%	7.3
t49	t1	i. d.	24%	i. d.
t50	t12	i. d.	i. d.	i. d.
tw85	tw2	100%	48%	5.6
tw86	tw12	42%	57%	i. d.
tw87	tw ³²	i. d.	38%	i. d.
tw88	tw8	i. d.	64%	i. d.

Preliminary tests are underway for an additional eight newly arisen variants.

(1)

Relative viability of t/t compared to T/t in progeny from T/t x T/t matings. Expectation of t/t calculated on basis of average transmission ratio. All percentages based on at least 50 offspring. i. d. =insufficient data.

(2)

Transmission ratio of t; determined by progeny tests of T/t males by ++ females. = $\frac{\# t/+}{\# \text{ total offspring}}$

Percentages based on at least 50 offspring. i. d. = insufficient data.

(3)

Litter size average in at least four litters.

4) Fine Structure Mapping of the T/t Region:

The reason for the skewed distribution of recombinants from

$$\begin{array}{ccccc} + & \text{Low} & \text{tf} & & \\ \hline t^{38} & + & + & & \end{array}$$
 females has still not been solved. As reported in the

last two progress reports these females show the normal amount of over-all crossing over between t^{38} and tf , but progeny tests of the recombinants in terms of transmission ratio show that virtually all of them had to be considered to be recombinant between Low and tf, and almost none between t^{38} and Low, the region which by standard mapping is four times as long as the Low-tf interval. We have now tested a model which proposed that the t^{38} mutation consists of two separable factors, one controlling transmission ratio which was far to the right of the conventionally mapped T-locus. On this model the original females would be:

$$\begin{array}{ccc} (1) & & (2) \\ t^{38} & t^{38} + \text{Low} & +tf \end{array}$$

ered to be the transmission distorting unit of t^{38} , then virtually all the $+T - +tf$ recombinants would be expected to carry a factor lowering transmission ratio, either $t^{38} (2)$ or Low. We tested this by putting the recombinant chromosome over $tw^{12} tf$, the expectation (from previous experience) being that $\frac{tw^{12}}{\text{Low} +} tf$ males would be fertile and have

normal transmission ratios, whereas $\frac{tw^{12}}{(2)} tf$ would be semisterile and

show disturbed transmission ratios. These tests have now been done, and all males tested have been fertile with normal ratios, suggesting that this model is not correct, and that there is indeed a maldistribution of recombination in this particular kind of heterozygote.

Publications:

Dorothea Bennett, L. C. Dunn, Martha Spiegelman, Karen Artzt, Janice Cookingham and Elizabeth Schermerhorn. (1975) Observations on a set of radiation-induced dominant T-like mutations in the mouse. *Genetical Research* 26: 95-108. (Six reprints enclosed).

* Karen Artzt, and Dorothea Bennett. (1975) Analogies between embryonic (T/t) antigens and adult major histocompatibility (H-2) antigens. *Nature* 256: 545-547. (Six reprints enclosed).

* Hammerberg, C., Klein, J., Artzt, K., and Bennett, D. Histocompatibility-2 System in Wild Mice. II. H-2 Haplotypes of t-bearing mice. *Transplantation* (in press).

* Bennett, D., Dunn, L. C., and Artzt, K. Genetic change in mutations at the T/t-locus. *Genetics* (in press).

* Spiegelman, M., Artzt, K., and Bennett, D. Embryological study of a T/t locus mutation (tw⁷³) affecting trophoctoderm development. *J. Embryol. exp. Morph.* (in press).

*Preprints were submitted as Document Numbers C00-2497-03, C00-2497-04, C00-2497-05, C00-2497-06 respectively.

*Reprints +
preprints removed*