

## Observations on a set of radiation-induced dominant *T*-like mutations in the mouse\*

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### SUMMARY

Genetic analysis of seven dominant short tailed mutations independently induced by radiation of male mice showed that six were allelic to *T* (*Brachyury*) but not identical to it. Homozygotes for each mutant die at least 2 days earlier than *T/T* homozygotes; two that were studied histologically are indistinguishable from one another. The development of these abnormal embryos is arrested by seven days of gestation, when cells of embryonic ectoderm cease proliferation and become pycnotic. Endoderm and extra-embryonic ectoderm do not seem to be primarily affected, and survive and grow for at least 2 days more. Serological studies of one of these mutations suggest that it is a deletion. A review is presented of these and other *T*-like mutations that have been described; from this it appears that five different categories of *T*-like mutants are discernible.

### 1. INTRODUCTION

The *T*-locus in the mouse is a chromosome region whose genetic complexity is still incompletely understood. The first mutation identified at this locus was a dominant gene, *Brachyury* (*T*) (Dobrovolskaia-Zavadskaia, 1927); *T/+* animals are short-tailed, and *T/T* embryos die in mid-gestation with precisely definable abnormalities (Chesley, 1935). *T* also has the remarkable property of interacting with recessive mutants that act as genetic alleles to each other and to *T*; *T/t* animals have a specific abnormal phenotype, taillessness.

A variety of recessive alleles has been identified and studied, and their peculiar genetic properties have led to considering them as pseudo-alleles, perhaps chromosomal changes rather than point mutations, that together identify a complex genetic locus (Dunn, Bennett & Beasley, 1962; Lyon & Meredith, 1964*a, b*). On the other hand, the dominant mutation *T* initially appeared to possess none of the

\* Most of the data reported here were complete before the death of L. C. Dunn, but he shares no responsibility for the defects in the manuscript which his co-authors regretfully produced without his help.

peculiar genetic properties of the recessive genes at this locus, and was thought of as an ordinary point mutation. However, evidence has been accumulating that not all  $T$ -mutants are alike (Lyon, 1959; Searle, 1966; Johnson, 1974; Moutier, 1973) and, further, that radiation induced mutations to  $T$ -like alleles may be unusually frequent (Batchelor, Phillips & Searle, 1966; Searle, personal communication; Lyon, personal communication). We report here additional evidence on these points; six out of a total of seven short-tailed mutations induced by radiation that we have studied are allelic to  $T$ , but not identical to it. This suggests either that there is hypermutability to  $T$ -alleles, or that changes that behave as  $T$ -mutants can occur over an extensive chromosome region.

## 2. OBSERVATIONS

### (i) Genetic data

In the course of studies on radiation induced mutations at the Oak Ridge National Laboratory, Dr Paul B. Selby recovered seven transmissible dominant short-tailed mutations of independent origin from a total of 186 000 offspring whose male parents were irradiated early in life (1973*a, b*, and personal communication). He kindly made these available to us for testing for similarity to our known  $T$  mutant.

Table 1. *Crosses of short-tailed ( $T^{Or}$ ) males to  $t^{38}/t^{38}$  females*

Allele designation*	Progeny obtained by $t^{38}/t^{38}$ females		
	Tailless	Normal-tailed	Short-tailed
Or-1	34	29	0
Or-2	34	38	0
Or-3	18	17	0
Or-4	4	8	0
Or-5	14	20	0
Or-6	63	73	0
PBS-51	0	17	17

\* The Or designations correspond, in numerical order, to P. B. Selby's mutations produced at the Oak Ridge National Laboratory originally referred to as PBS-4, PBS-27, PBS-35, PBS-40, PBS-42 and PBS-50 (Selby, personal communication). For clarity we have given the symbols  $T^{1Or}$ ,  $T^{2Or}$ , ..., etc., to mutations similar to  $T$ .

Initial genetic tests were done by crossing male heterozygotes from each of these lines to females homozygous for the viable  $t$ -allele  $t^{38}$ . The expectation is that males carrying an allele with interactive properties similar to  $T$  will produce litters that contain tailless ( $T^{Or}/t$ ) and normal-tailed ( $+/t$ ) offspring only, but no short tailed progeny. Table 1 shows that six of the seven short-tailed mutations behaved like  $T$  in this respect. These have been designated  $T^{1Or}$ ,  $T^{2Or}$ , etc. One mutation was not like  $T$ ; PBS-51 heterozygotes produced 17 short-tailed and 17 normal offspring but no tailless offspring in these tests.

Crosses of  $T^{Or}/+ \times T/+$  animals produced 111 normal-tailed and 225 short-tailed offspring; the 1:2 ratio is evidence that the  $T^{Or}/T$  genotype is lethal. Four of the  $T^{Or}$  mutants were tested for linkage with the marker *tufted* (*tf*) which is approximately 8 centimorgans from *T*. Table 2 shows that the  $T^{Or}$  mutations tested were also linked to *tf*. Sufficient data for statistical treatment are available only for  $T^{1Or}$ ; in this case the recombination percentage is 7.5% which is not significantly different from values reported for the *T*-*tf* distance (Dunn & Bennett, 1967). Thus, genetic tests unequivocally establish that six of these seven radiation induced short-tailed mutations closely resemble *T*.

Table 2. Tests of linkage between  $T^{Or}$  and *tf*

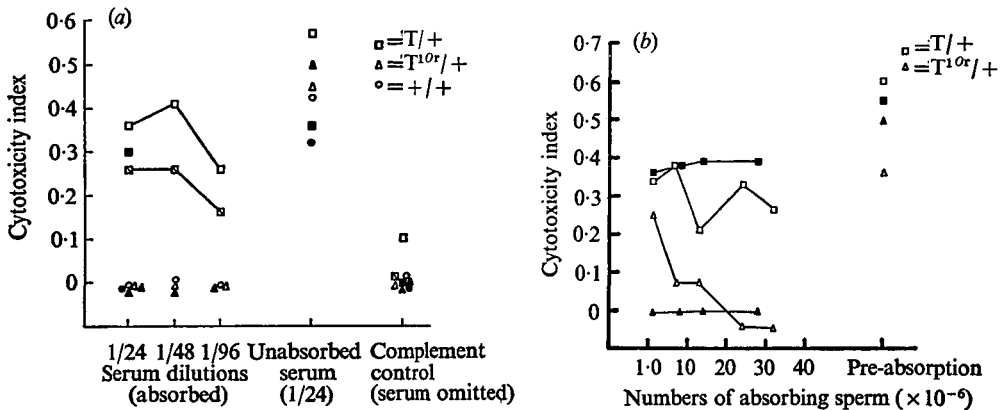
Cross	Allele	Phenotype of offspring*			
		Parental		Recombinant	
		Brachy non-tufted	Normal tailed tufted	Brachy tufted	Normal-tailed non-tufted
$\begin{matrix} \text{♂} & \text{♀} \\ T^{Or}/+ & +tf \\ +tf & +tf \end{matrix} \times$	$T^{1Or}$	112	15	2	4
	$T^{2Or}$	54	8	0	0
	$T^{3Or}$	66	10	1	0
	$T^{6Or}$	40	10	1	0
$\begin{matrix} T^{Or}/+ & T^{Or}/+ \\ +tf & +tf \end{matrix} \times$	$T^{1Or}$	423	27	32	9
	$T^{2Or}$	16	0	1	0
	$T^{3Or}$	18	2	2	1
	$T^{6Or}$	41	4	2	0

\* Offspring from litters culled at birth to remove most normal-tailed animals, and scored at 28 days for tufted phenotype.

(ii) Serological data

One of these newly detected *T*-mutants,  $T^{1Or}$ , has been examined for the presence of the sperm antigen known to be specified by *T*. Antiserum raised against sperm from  $T/+$  males was rendered specific for *T*-antigen by standard absorption procedures using sperm from wild-type males to remove non-specific sperm autoantibody (Bennett *et al.* 1972). This anti-*T* serum was assayed in complement-dependent cytotoxicity tests for activity against sperm from  $T^{1Or}$  males. In three such tests, shown in Text-fig. 1(a),  $T^{1Or}$  sperm did not react with anti-*T* antiserum. Furthermore, in two additional tests, absorptions of specific anti-*T* antiserum with  $T^{Or}/+$  sperm did not remove any activity against  $T/+$  sperm (Text-fig. 1(b)). Both of these experiments indicate that if  $T^{1Or}$  specifies an antigen on sperm, it is not the same and not cross-reacting with the antigen determined by *T*. Two different attempts have been made to generate a specific antiserum against a component on sperm specified by  $T^{1Or}$ . In both cases wild type male mice were immunized according to a standard method with sperm from  $T^{1Or}/+$  males (Bennett *et al.* 1972). At the end of the course of immunization, antiserum from both groups had high titres of sperm-specific antibody, but had no

residual activity for  $T^{Or}/+$  sperm after absorption with wild-type sperm to remove sperm autoantibody. In conjunction with the first two experiments these findings suggest strongly that  $T^{1Or}$  does not specify a serologically detectable antigen on sperm, and thus imply that it may be a deletion.



Text-fig. 1. (a) Cytotoxicity tests of specific anti- $T$  antiserum tested against sperm from  $T/+$  (□, positive control)  $+/+$  (○, negative control) and  $T^{1Or}/+$  (Δ) mice. Specific anti- $T$  serum is negative on both  $+/+$  and  $T^{1Or}/+$  sperm, although sperm of all genotypes react with unabsorbed serum. Different symbols (e.g. □, ■) represent independent tests on sperm from different males of the same genotype.

(b) Cytotoxicity tests of specific anti- $T$  antiserum tested against sperm from  $T/+$  (□) and  $T^{1Or}/+$  (Δ) mice after quantitative absorption with increasing numbers of sperm from  $T^{1Or}/+$  males. Absorption with  $T^{1Or}/+$  sperm does not remove activity against  $T/+$  sperm.

### (iii) Embryological data

#### (a) Gross observations

Crosses were made between  $T/+$  animals and  $T^{Or}$  heterozygotes representing four independent mutations. We examined embryos between 8 and 10 days by removing them from their decidual capsules; during this period  $T/T$  homozygotes can easily be recognized in the dissecting microscope because they show absent or abnormally small and diffuse somites, failure of posterior body development, enlarged pericardial sac, and abnormally positioned forelimbs. Table 3 presents evidence that each of the  $T^{Or}$  mutations studied produces  $T^{Or}/T$  embryos that are morphologically indistinguishable from  $T/T$  embryos.

However, when *inter se* crosses were made within stocks of the same four mutations and litters examined at comparable stages, no embryos showing the syndrome typical of  $T/T$  homozygotes were seen. Rather, as Table 4 shows, about 25% of embryos were already dead and resorbing by 8 days.

It seems clear that in the cases tested, the phenotype of  $T^{Or}/T$  appears identical to that of  $T/T$  homozygotes, but that each class of  $T^{Or}/T^{Or}$  embryos dies considerably earlier, usually being resorbed by 8 days.

(b) *Histological observations*

Embryos were obtained from matings between *T<sup>1Or</sup>/+* animals, and between *T<sup>1Or</sup>/+* and *T<sup>3Or</sup>/+* mice at 6, 7 and 8 days post-fertilization; they were fixed intact within their capsules in Bouin's fluid for 12–24 h, embedded in paraffin, sectioned at 6–10  $\mu$ m and stained with haematoxylin and eosin. Age of embryos

Table 3. *Classification of embryos obtained from matings of T/ +  $\times$  T<sup>Or</sup>/ +*

Cross	No. of decidual capsules examined	No. of normal embryos	No. of typical <i>T/T</i> homozygotes	No. dead or resorbed
<i>T/ + <math>\times</math> T<sup>1Or</sup>/ +</i>	64	45	10	9
<i>T/ + <math>\times</math> T<sup>2Or</sup>/ +</i>	25	16	9	0
<i>T/ + <math>\times</math> T<sup>3Or</sup>/ +</i>	19	13	3	3
<i>T/ + <math>\times</math> T<sup>6Or</sup>/ +</i>	47	34	8	5
Totals	155	108	30	17

Table 4. *Classification of embryos obtained from inter se matings of T<sup>Or</sup>/ +  $\times$  T<sup>Or</sup>/ +*

Cross	No. of decidual capsules examined	No. of normal embryos	No. of typical <i>T/T</i> homozygotes	No. dead or resorbed
<i>T<sup>1Or</sup>/ + <math>\times</math> T<sup>1Or</sup>/ +</i>	35	24	0	11
<i>T<sup>2Or</sup>/ + <math>\times</math> T<sup>2Or</sup>/ +</i>	7	6	0	1
<i>T<sup>3Or</sup>/ + <math>\times</math> T<sup>3Or</sup>/ +</i>	15	12	0	3
<i>T<sup>6Or</sup>/ + <math>\times</math> T<sup>6Or</sup>/ +</i>	17	12	0	5
Totals	74	54	0	20

was predicted by copulation plug and more precisely determined by comparing histological sections of normal embryos to Sobotta's drawings (1911). Table 5 summarizes the histological observations. No differences were noted between embryos homozygous for *T<sup>1Or</sup>/T<sup>1Or</sup>* and *T<sup>1Or</sup>/T<sup>3Or</sup>*. Because mouse embryos vary greatly in degree of development with respect to their age as determined by the vaginal plug method, the data in Table 5 are presented according to stage of development, rather than chronological age. (See footnote to Table 5 for description of developmental stages of normal embryos.)

*Group A: 6½–7 days.* Although a few abnormal embryos, putative *T<sup>Or</sup>*-homozygotes, are detected early in this period, a morphologically abnormal class is first regularly observed in embryos approaching 7 days.

At this time, the normal embryo is an advanced egg-cylinder stage with ectoderm completely separated into embryonic and extraembryonic portions; in both regions the ectoderm is composed of high columnar cells. The extraembryonic ectoderm is apposed to a cuboidal or columnar layer of proximal endoderm that appears absorptive or secretory; over the embryonic ectoderm the proximal

endoderm forms a flattened layer of single cells. Development of the yolk sac and growth of the ectoplacental cone are well underway. All cell layers display mitotic figures, with the embryonic ectoderm appearing especially proliferative at this time (Fig. 2).\*

Littermates classed as  $T^{Or}$ -homozygotes are recognized by a syndrome of defects restricted specifically to cells of the embryo proper. The most prominent defect is pycnosis in the ectodermal cells, which usually contain large, dense cytoplasmic granules. These embryonic ectoderm cells rarely form an orderly columnar layer but instead are crowded together in a disorganized fashion. The overlying endoderm is not a flattened single cell layer; nuclei are very close together and the cells

Table 5. *Classification of embryos obtained from matings of  $T^{1Or}/+ \times T^{1Or}/+$  and  $T^{1Or}/+ \times T^{3Or}/+$*

Stage of development*	No. of litters	Total embryos	Normal embryos	Embryos classified as $T^{Or}/T^{Or}$	Resorbed	Other†
<i><math>T^{1Or}/+ \times T^{1Or}/+</math></i>						
A	8	74	56	14	1	2, 1 <sup>+</sup>
B	2	16	14	2	0	0
C	3	33	26	5	1	1
Totals	13	123	96	21	2	4
<i><math>T^{1Or}/+ \times T^{3Or}/+</math></i>						
A	3	26	16	7	2	1
B	1	13	10	3	0	0
C	1	14	10	3	1	0
Totals	5	53	36	13	3	1
Combined totals	18	176	132	34	5	5

\* A: 6½–7 days; normal embryos are at elongating egg-cylinder stages and early primitive streak formation. B: 7–7½ days; normal embryos complete migration of mesoderm and approach completion of amnion and chorion. C: 7½–8 days; normal embryos complete amnion and chorion formation, show headfolds and 1–4 somites.

† Abnormal dying embryo not typical of  $T^{Or}/T^{Or}$ . + ? early signs of typical  $T^{Or}/T^{Or}$ .

are cuboidal with vacuolated apical cytoplasm. In contrast to these obvious abnormalities of the embryonic portion of the egg cylinder, the extraembryonic ectoderm and endoderm, ectoplacental cone, and yolk sac appear quite regular in form and these cell layers have normal relationships to one another (Fig. 3).

It is clear, moreover, that the abnormal embryo is smaller than the normal embryo of the same age; this applies to the yolk-sac cavity, the ectoplacental cone, the extraembryonic portion of the egg cylinder, as well as the embryo proper. With increasing age the size differences between normal and mutant embryos become more obvious. In embryos prior to the primitive streak stage, the size of the mutant egg cylinder is three-fourths to one-half that of the normal embryo;

\* Figs. 2 and 3 are shown on Plate 1, Figs. 4–8 on Plate 2, Figs. 9 and 10 on Plate 3 and Figs. 11–15 on Plate 4.

by the time the normal embryo reaches the primitive streak stage, the mutant embryo arrests as a diminutive egg cylinder and is less than one-fourth the size of its normal counterpart.

Older normal embryos of this group have begun the proliferation of mesoderm from the primitive streak and are noticeably larger than at the preceding stage. In none of the litter-mates that show the abnormal syndrome has a primitive streak zone or mesoderm been recognized.

Corresponding to the retarded development of the mutant is the observation that fewer mitotic figures are seen in every embryonic tissue of the abnormal embryo than in the normal embryo (Table 6). The depression of cell division is most evident in the embryonic ectoderm where mitotic figures occur at less than one-fourth the number seen in the same tissue of the normal embryo. Even more striking is the fact that the great majority of division figures in the embryonic ectoderm of the mutant are unquestionably abnormal in appearance (Figs. 4, 5). These cells usually display one or more large dense bodies, possibly clumped chromatin, associated with the mitotic chromosomes or the spindle apparatus (Fig. 6). Although the other tissues show a similar reduction in division figures, the mitotic chromosomes and spindle apparatus in these cells appear to be normal and comparable to mitotic figures seen in the same tissues in normal embryos (Figs. 7, 8).

*Group B: 7–7½ days.* Mesoderm has penetrated into almost all regions in normal embryos of this group. Embryonic ectoderm remains a thick layer of columnar cells. Endoderm is a flat layer in most of the embryo but some cells are modified into a columnar epithelium to form the archenteron roof. Notochord formation may also be started by the end of this period. The ectoplacental cone consists of a large compact mass of cells and giant cells are present among the decidual cells. The amnion and chorion are developing and an extraembryonic coelom is obvious (Fig. 9).

The homozygotes are considerably retarded in development, and show little further development of the embryo *per se*; their size is only slightly increased but more significant, morphogenesis appears to be blocked. The embryo is an abnormal egg-cylinder with irregular, pycnotic ectoderm cells and crowded vacuolated endoderm cells which sometimes are detached from the underlying ectoderm. There is no evidence of mesoderm formation, or of amnion or chorion. The ectoplacental cone and yolk sac have continued to expand; these tissues are smaller than those in the normal counterpart but they have grown proportionately more than has the mutant embryo proper. Thus, the extraembryonic structures are capable of proliferation and some development in spite of the arrested development of the embryo (Fig. 10).

Mitotic figures are abundant in all tissues of the normal embryos; they are virtually absent in the arrested embryonic tissues of the mutant, although extra-embryonic tissues continue to show a small number of cells that appear to be dividing normally. The obvious size difference between the normal and mutant embryos reflects the lack of cell proliferation in the mutant.

*Group C: 7½–8 days.* Normal embryos of this group progress through

Table 6. *Mitotic counts in embryonic tissues*

Specimen	Kind of section	Mitotic figures					
		Embryonic ectoderm	Embryonic endoderm	Extraemb. ectoderm	Extraemb. endoderm	Ectopl. cone	Distal endoderm
Normals							
305-1	Long.	39	18	17	24	34	16
305-3	Trans.	42	6	28	16	31	6
309-8	Trans.	59	13	39	38	39	18
310-1	Trans.	71	26	35	13	48	24
310-6	Trans.	64	12	41	28	18	8
311-3	Trans.	15	1	22	8	16	8
311-4	Long.	66	7	21	5	11	4
311-5	Trans.	31	7	9	11	12	5
312-2	Long.	17	12	16	9	25	11
312-10	Trans.	49	14	25	14	18	2
315-2	Long.	18	5	1	1	10	6
315-3	Long.	17	16	9	7	11	3
316-1	Long.	15	9	8	7	19	4
316-10	Long.	43	13	18	16	18	5
Mean		39	11	21	14	22	9

Total of 14 specimens from 7 litters.

## Mutants

305-2	Long.	6	3	5	6	3	7
309-3	Trans.	4	5	0	0	3	1
309-4	Trans.	2	0	1	0	0	7
309-7	Trans.	4	2	9	8	10	15
309-9	Trans.	2	4	9	8	0	3
310-3	Trans.	9	4	14	8	7	9
311-2	Long.	6	2	4	0	2	2
312-5	Long.	15	5	9	8	9	12
313-4	Trans.	16	9	23	13	2	14
314-2	Trans.	4	2	4	0	9	4
315-5	Long.	3	4	5	1	0	5
315-9	Long.	9	6	4	0	1	3
316-3	Long.	3	8	2	2	6	2
316-9	Long.	1	7	7	4	4	4
Mean		6	4	7	4	4	6

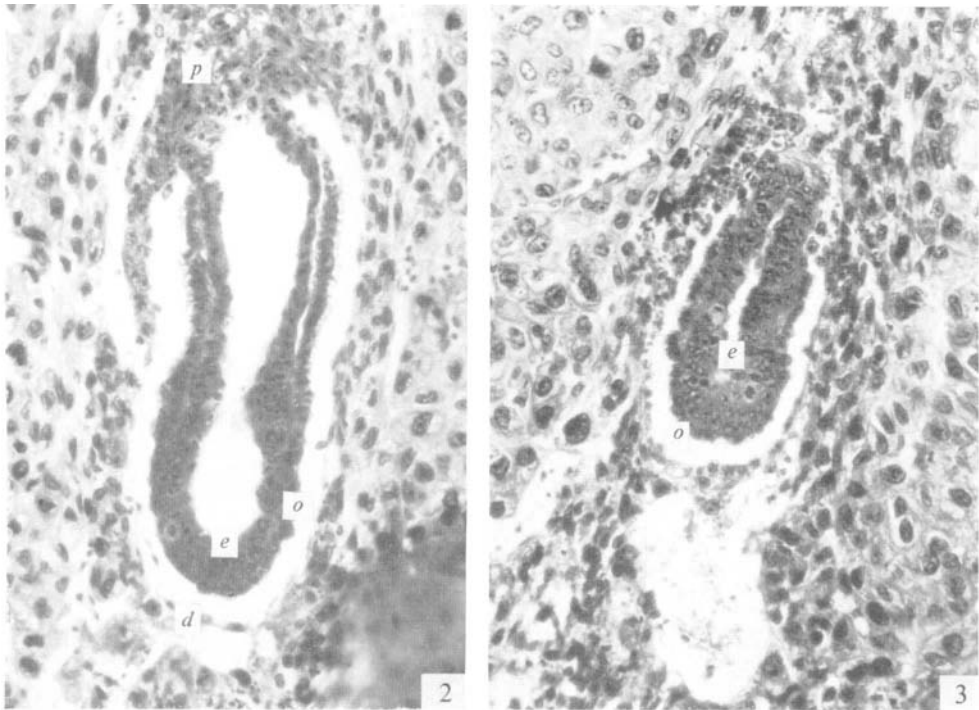
Total of 14 specimens from 9 litters.

All embryos examined were at mid- to late egg-cylinder stages (stage A); their genotypes were distributed approximately equally among  $T^{10r}/T^{10r}$ ,  $T^{30r}/T^{30r}$  and  $T^{10r}/T^{30r}$ . All mitotic counts were made at a magnification of  $400\times$ .

organization of the longitudinal axis; head folds and notochord form, somites begin to appear, neural tube closure proceeds, the amnion and chorion are complete, and an allantois develops. The yolk sac and ectoplacental cone are large and many giant cells are present (Fig. 11).

Arrested embryos of the type previously seen are no longer observed. Instead, a prominent yolk-sac cavity with a definite ectoplacental cone and yolk-sac epithelium with Reichert's membrane, but containing no embryo, is present. Giant

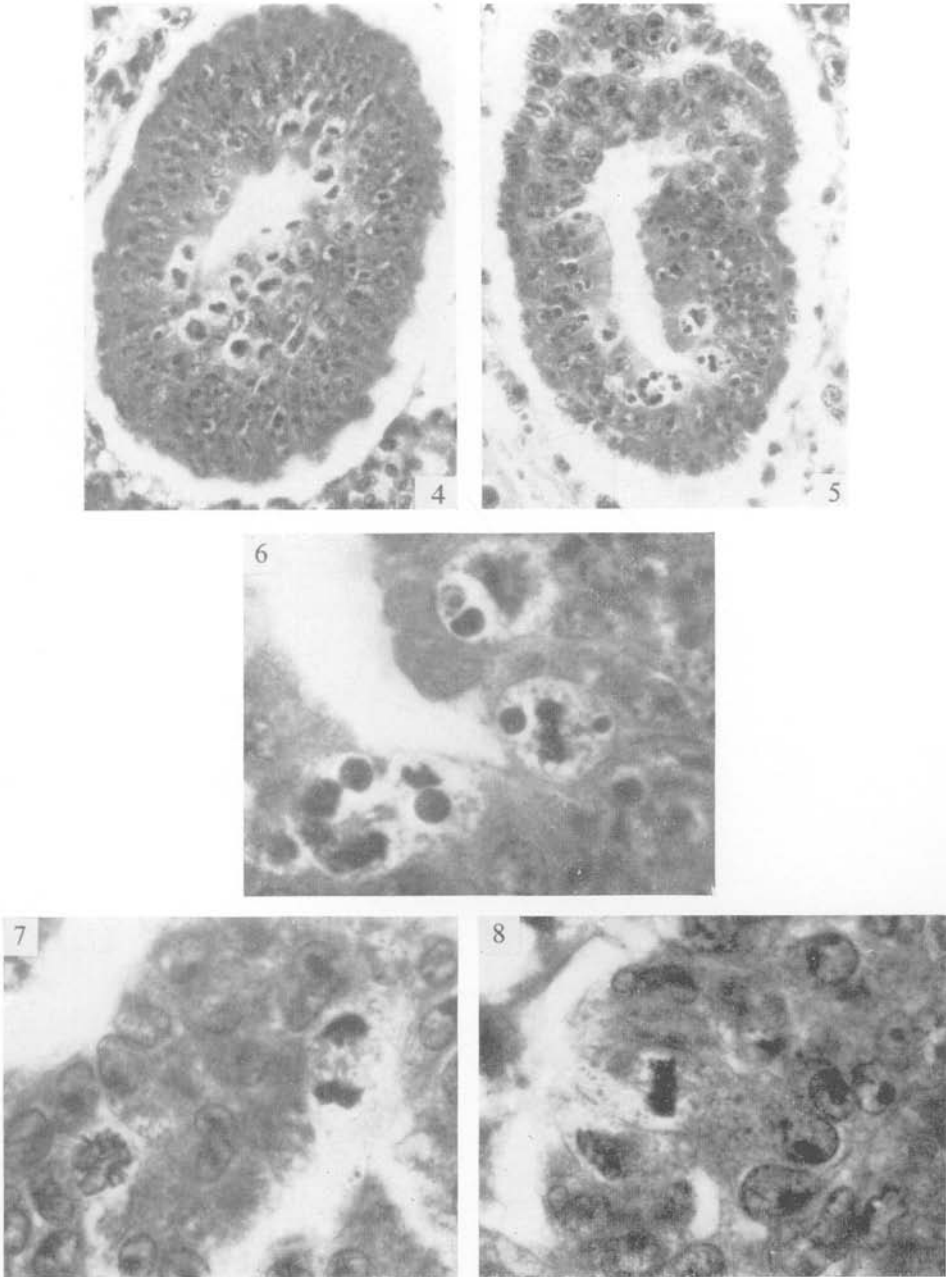




Figs. 2-3. Group A.

Fig. 2. Longitudinal section through normal egg cylinder. *e*, Embryonic ectoderm; *o*, embryonic endoderm; *p*, ectoplacental cone; *d*, distal endoderm.  $\times 200$ .

Fig. 3. Longitudinal section through mutant egg cylinder. Embryonic ectoderm, *e*, is pycnotic; embryonic endoderm, *o*, is crowded and vacuolated.  $\times 200$ .



Figs. 4-8. Group A.

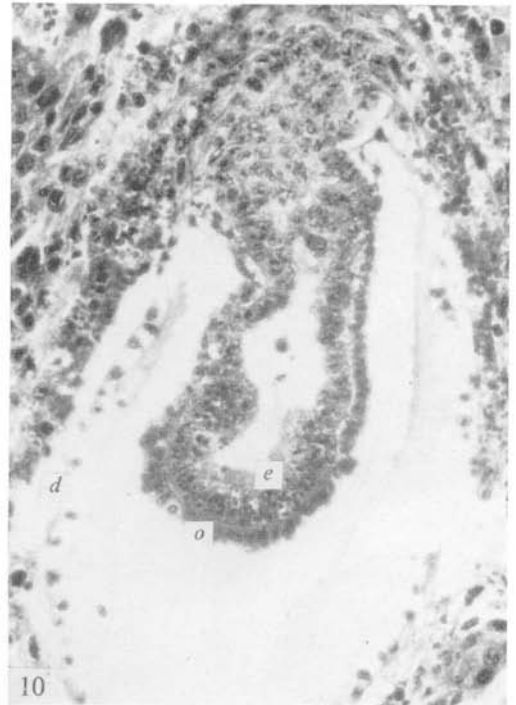
Fig. 4. Oblique section through normal embryo showing relationship of embryonic ectoderm and endoderm and numerous mitotic figures in ectoderm.  $\times 330$ .

Fig. 5. Oblique section through mutant embryo showing pycnotic ectoderm and vacuolated endoderm. Mitotic figures in ectoderm are very abnormal.  $\times 330$ .

Fig. 6. Abnormal mitotic figures in embryonic ectoderm of mutant. Large, round, dense bodies are often located near poles of spindle.  $\times 1200$ .

Fig. 7. Mitotic figures in extraembryonic ectoderm of normal embryo.  $\times 1200$ .

Fig. 8. Mitotic figure in ectoplacental cone of mutant appears as regular as those seen in normal embryos (Fig. 7).  $\times 1200$ .

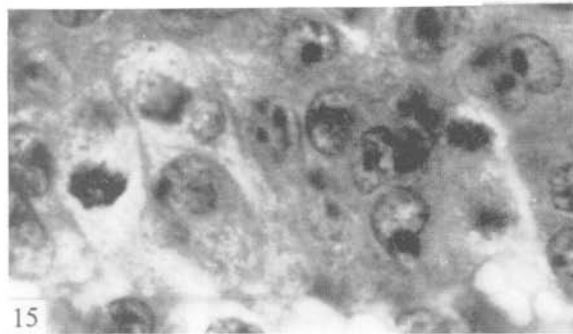
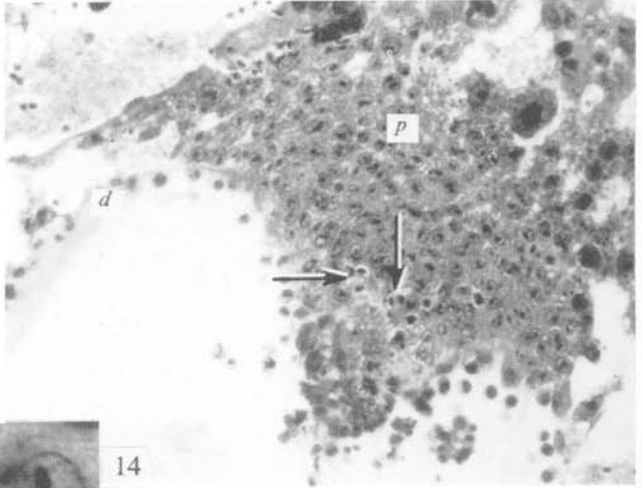
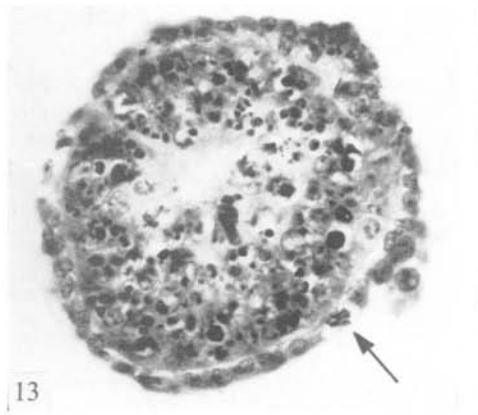
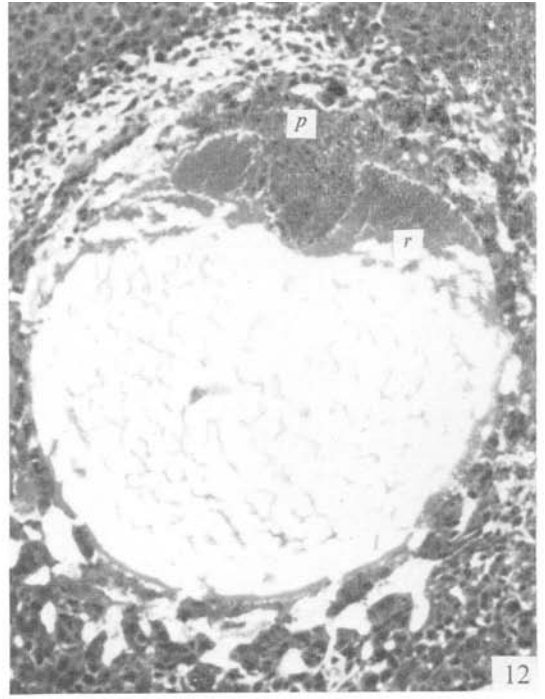


Figs. 9 and 10. Group B.

Fig. 9. Longitudinal section of normal primitive streak stage. *E*, Embryo; *s*, primitive streak; *a*, amnion; *c*, chorion; *p*, ectoplacental cone; *d*, distal endoderm.  $\times 200$ .

Fig. 10. Longitudinal section of mutant arrested as an egg cylinder. *e*, Embryonic ectoderm; *o*, embryonic endoderm; *d*, distal endoderm.  $\times 200$ .

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Figs. 11-15. Group C. For legend see facing page

cells are also seen and the maternal circulation in the decidua resembles that in capsules of normal embryos; many maternal red blood cells are present in the yolk sac cavity (Fig. 12). The ectoplacental cone protrudes into the yolk-sac cavity and is covered with a small cap of pycnotic and dying cells probably representing the remnant of the embryo, which consists of a core of crowded, extremely pycnotic and abnormal ectoderm cells covered by a layer of cuboidal endoderm cells that still show occasional mitotic figures (Fig. 13).

The cells of the ectoplacental cone appear normal and regular in form as do the cells of the other persisting extraembryonic tissues, and many cells of the ectoplacental cone are in mitosis (Figs. 14, 15).

(c) *Summary of histological observations in the T<sup>Or</sup> mutant*

Initially, cell division is specifically aberrant or blocked in the embryonic ectoderm cells of the egg cylinder. Embryonic endoderm is indirectly impaired; cells appear to continue to proliferate for a time, but do not have an adequate cellular substrate on which to spread because differentiation of embryonic ectoderm is already impeded. Other tissues have deficient numbers of dividing cells but do not appear otherwise abnormal.

By the primitive streak stage, the embryo proper is clearly arrested, having undergone little or no proliferation or morphogenesis beyond a two-layered egg cylinder. Notwithstanding developmental retardation, cells of extraembryonic structures are normal in morphology.

Even after the embryo is dead, at 8 days, the ectoplacental cone and yolk sac remain and expand. The tissues involved in implantation are capable of fulfilling a developmental program independent of the embryo at least up to the head fold and early somite stage.

### 3. DISCUSSION

(i) *Development of T<sup>Or</sup>/T<sup>Or</sup> embryos*

Gross embryological observations on four independently derived *T<sup>Or</sup>* homozygotes, and detailed histological study of two of them suggest that they are phenotypically indistinguishable. Like all of the other lethal mutations at the *T*-locus, *T<sup>Or</sup>*-homozygotes are arrested at a specific time during development and with a specific group of cells primarily affected (Bennett, 1964). In many of these

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Fig. 11. Longitudinal section through normal head fold stage. *h*, Head fold; *b*, allantoic bud; *a*, amnion; *c*, chorion; *p*, ectoplacental cone.  $\times 80$ .

Fig. 12. Longitudinal section through yolk sac cavity of mutant. No embryo is present although both the yolk sac and the ectoplacental cone, *p*, are quite large. Many maternal red blood cells, *r*, are often accumulated in the yolk sac cavity.  $\times 80$ .

Fig. 13. A mutant embryo remnant arrested as a pycnotic egg cylinder. Ectoderm is extremely pycnotic but endoderm still shows an occasional normal mitotic figure, *arrow*.  $\times 330$ .

Fig. 14. Ectoplacental cone, *p*, of mutant is large with many normal mitotic figures, *arrows*. *d*, Distal endoderm.  $\times 200$ .

Fig. 15. Mitotic figures in ectoplacental cone of mutant appear normal.  $\times 1200$ .

mutant embryos a cascade of organizational abnormalities seems to centre around initial defects in the ectoderm.

In the present study of  $T^{Or}$ -mutants, abnormal mitotic figures, as well as a decided decrease in numbers of cell divisions, are detected in the embryonic ectoderm of egg cylinders at day six of development. Large clumps of chromatin are often associated with the mitotic apparatus; presumably, mitosis is blocked, pycnotic cells accumulate, and further growth ceases. It cannot be determined if these aberrant mitoses result from other initial cellular disorders affecting the nucleus or machinery of cell division.

Cells of the other tissues are not pycnotic and, in fact, appear to undergo normal mitosis although also at lower numbers. Nevertheless, some irregularities are observed; the crowding of cells of the embryonic endoderm is probably due to the physical inability of the non-proliferative ectoderm to afford an adequate surface upon which the overlying endoderm can grow and spread. Furthermore, appropriate interactions between ectoderm and endoderm are no doubt impaired.

Interestingly, although there is a general depression of cell division in all tissues, the extraembryonic structures continue to develop and appear relatively normal. Fewer divisions in themselves do not appear to impede development, and the extraembryonic structures seem to have little or no dependence upon the normal development of the embryo *per se*. These tissues, therefore, continue to proliferate and differentiate normally even after the embryo proper is frankly moribund.

We know from studying 7-day embryos that chorion and mesoderm do not develop in the mutants; the ectoplacental cone, which looks fairly normal at 8 days of development, subsequently becomes necrotic. This may mean that placenta formation cannot proceed without the participation of chorion and mesodermal elements.

Of the  $T$ -locus mutants that have been studied in some histological detail,  $t^{w5}/t^{w5}$  embryos most closely resemble  $T^{Or}/T^{Or}$  embryos; homozygotes for  $t^{w5}$  are also arrested at the egg cylinder stage with abnormalities in cells of the embryonic ectoderm (Bennett & Dunn, 1958).

Homozygotes for  $T^{Or}$  genes differ in some respects, however, from the  $t^{w5}$ -homozygotes. The latter mutants are more variable in lethal phenotype and time of death. Some die by day 8 as degenerating egg cylinders, while some develop extraembryonic structures including yolk sac, chorion, and allantois, and may live into the 10th day. The  $T^{Or}$ -homozygotes bear a striking resemblance to the first class of  $t^{w5}$ -mutants; mesodermal derivatives never appear in either of these types. Nevertheless, crosses between  $T^{Or}/+$  and  $+/t^{w5}$  animals produce the expected number of viable tailless  $T^{Or}/t^{w5}$  compounds (Bennett, unpublished).

#### (ii) *Genetics and relationships to other T-mutations*

The surprising fact that six out of seven radiation-induced dominant mutations affecting the tail were  $T$ -like alleles suggests that there must be some sort of hypermutability with respect to genetic changes that produce the phenotype associated with  $T$ . This surmise is amply supported by existing data. A number of

spontaneous mutations to *T*-mutants have been described. The available information about spontaneous mutations securely identified as *T* mutants, in addition to the *T* first described by Dobrovolskaia-Zavadskaia (1927), are as follows:

(1) A mutation found at the Jackson Laboratory in 1944; this gene interacted with  $t^0$  and  $t^1$  to produce a tailless phenotype, and when heterozygotes were crossed with known *T*/+ animals abnormal embryos comparable to *T*/*T* homozygotes were found. Homozygotes for the new mutation were not examined (Gluecksohn-Waelsch, personal communication).

(2) A *T* mutation described by Carter & Phillips (1950); this mutation was examined genetically (Dunn, unpublished) and embryologically (Gluecksohn-Waelsch, personal communication) as described in (1) above, with the same results.

(3)  $T^h$  described by Lyon (1959); this mutation arose in a control series of mutagenesis experiments. *T*/ $T^h$  embryos are lethal and probably similar to *T*/*T* homozygotes.  $T^h$ / $T^h$  homozygotes, however, die about 2 days earlier than *T*/*T* embryos, but histological observations were not made. Nevertheless, the time of death appears to be comparable to that in  $T^{Or}$ / $T^{Or}$  embryos.

(4)  $T^{hg}$  described by Kuminek (1960);  $T^{hg}$ / $T^{hg}$  homozygotes were histologically indistinguishable from *T*/*T* embryos.

(5) A *T* mutation described by Dunn *et al.* (1962); this gene behaved genetically like *T*, and had essentially the same degree of linkage to *tf*. Heterozygotes crossed to known *T*/+ animals produced abnormal embryos of typical *T*/*T* morphology. Homozygotes for this mutation were not examined.

(6)  $T^J$  described by Hummel (1963);  $T^J$ /*T* and  $T^J$ / $T^J$  embryos are indistinguishable (Bennett, unpublished).  $T^J$  and *T* also produce cell surface components on spermatozoa which are antigenically indistinguishable (Bennett *et al.* 1972).

(7)  $T^{2J}$  discovered by D. Bailey in C57 BL/6 stock (personal communication).

(8)  $T^{Or1}$  described by Moutier (1973*a*); this allele produces taillessness in *T*/*t* genotypes, and  $T^{Or1}$ /*T* embryos are indistinguishable from *T*/*T* (Bennett & Spiegelman, unpublished). It has also the most unusual property of leading to pseudodominance of *quaking*, a mutation about three map units from *T* (Moutier, 1973*b*; Bennett, unpublished). The phenomenon of pseudodominance does not extend to the locus of *tf*, about 4 units distal to *qk* (Bennett, unpublished).

(9)  $T^{Hp}$ ; this allele appears unique since the phenotype of heterozygotes depends on whether their  $T^{Hp}$  gene is maternal or paternal in origin. If the mutation is transmitted through the egg, heterozygous embryos are grossly abnormal and die before birth; if the source of the mutant gene is sperm, heterozygotes are fully viable with the short-tailed phenotype typical of *T*/+ (Johnson, 1974).  $T^{Hp}$ / $T^{Hp}$  homozygotes have not been identified.  $T^{Hp}$ /*T* embryos are lethal and indistinguishable from *T*/*T* (Bennett & Spiegelman, unpublished).  $T^{Hp}$  also causes pseudodominance of *qk* (Bennett, unpublished).

In addition to these apparently spontaneous occurrences numerous radiation induced mutations to *T*-like mutants have been described.

The first of these was *Curtailed* ( $T^c$ ). The phenotype of  $T^c$ /+ animals is more

extreme than that of  $T/+$ , with the tail being very much reduced or absent.  $T^c/T^c$  embryos are correspondingly more abnormal than are  $T/T$  embryos; the posterior body is still more abbreviated, neural folds usually fail to close, and anterior limb buds are absent.  $T^c/T$  embryos are of intermediate phenotype; the neural folds often are partially open, and the anterior limb-bud, although present, is frequently split into several outgrowths.  $T^c/T^h$  embryos, on the other hand, are indistinguishable from  $T^c/T^c$  (Searle, 1966).

Batchelor *et al.* (1966, 1967) and Searle (personal communication) report that of 39 dominant visible mutations induced by either neutron or gamma radiation to spermatogonia, 13 were confirmed as  $T$ -allele mutations. Of the three that were studied embryologically, one produced homozygotes resembling  $T/T$  embryos, while homozygotes of the others died at earlier stages.

Another set of radiation mutagenesis experiments done by Lyon and Morris produced several more recurrences of  $T$ -like mutations. These results have never been published in detail, but again mutations to  $T$ -like alleles were unusually frequent, and several that were embryologically studied varied in the time of death of the homozygote, some being like  $T$  and some like  $T^h$  (Lyon, personal communication).

The  $T$ -alleles so far described can be classified into at least five different types on the basis of criteria which include homozygous lethal phenotype, interaction with one another, and ancillary genetic effects. Thus each of the type-alleles  $T$ ,  $T^h$ ,  $T^c$ ,  $T^{Hp}$  and  $T^{Or1}$  seems to be different from any of the others. The new  $T^{Or}$  described in this paper cannot be discriminated from  $T^h$  on the basis of information available at present. One of the  $T^{Or}$  mutants appears, on the basis of serological evidence, to be a deletion, and this is compatible with its radiation-induced origin. On the other hand, two spontaneous mutations,  $T^{Hp}$  and  $T^{Or1}$ , are also most simply interpreted as being deletions, since they impart pseudodominance to  $qk$  at a locus three units distant.  $T^{1Or}$  does not interact with  $qk$  in this way, however, and therefore must not cover an identical chromosome region.

It should be noted that in spite of these differences the  $T$ -mutations have not been shown to complement one another. In fact, compounds of  $T$  have been made with each of the other  $T$ -alleles, and in all cases but one ( $T^c$ ) the phenotype of  $T/T^x$  embryos is identical to  $T/T$  embryos. This sets the dominant mutations as a group apart from recessive mutations at this locus, where as a rule complementation occurs between alleles with different embryonic effects.

Further genetic, embryological and serological studies of these various independent occurrences of  $T$ -like mutations should produce valuable information about the complex locus where they exist. At present it is evident only that some  $T$ -like mutations have bizarre genetic effects that make it unlikely that they are simple point mutations, and that mutation to  $T$ -like alleles is unusually frequent. This apparent hypermutability is shared by several other loci at which dominant mutations are known, notably  $W$ ,  $Sl$ ,  $Ta$  and  $Mo$ . Although  $T$ -like mutations are especially numerous after fission neutron irradiation, where such mutations are more numerous than any other dominant visible, the spontaneous mutation



frequency of *W*-locus mutations appears to be decidedly higher than those at any other single locus, including *T* (Searle, 1974). The reason for this discrepancy is not clear.

Although the genetic basis for hypermutability is unknown, it may imply the existence of a long region with many repetitive genes, each of which is necessary for normal development. On the other hand it may suggest that a single gene produces an essential product in which no mutational alteration is tolerated. Whatever the reason for the hypermutability to *T*-mutants, this situation contrasts strongly with the situation in respect to the recessive alleles at this locus, where there have been no recorded instances of mutations, either spontaneous or induced, of a wild-type gene to a *t*-allele. Thus the series of dominant mutations at the *T*-locus promises to exhibit a genetic and developmental complexity comparable to the already well-known complexity of the recessive alleles.

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