Genet. Res., Camb. (1984), **43**, *pp.* 27–33
With 2 plates and 1 text-figure
Printed in Great Britain

A new *t*-complex embryonic lethal (tcl^0) has arisen in the T^{hp} chromosome of the mouse and is allelic to t^0

BY EDWIN R. SANCHEZ AND CRAIG HAMMERBERG*

Department of Human Genetics, Box 015, University of Michigan Medical School, Ann Arbor, Michigan 48109

(*Received 10 October 1983*)

SUMMARY

Complement testing of a T^{hp} line revealed a failure of survival of T^{hp}/t^0 embryos. The genetic factor responsible for this lethality maps between Brachyury (*T*) and tufted (*tf*) on the murine seventeenth chromosome. This lethal factor permits recombination between *T* and *tf* and does not affect the transmission of its seventeenth chromosome. Its effect upon embryonic development is similar to that of the t^0 haplotype. It would appear to represent a point mutation of a single gene, *t-complex lethal zero* (tcl^0).

1. INTRODUCTION

Within the *t*-complex of the mouse there exists a series of recessive lethal mutations (l^t) which affect the embryo at different stages of its development. These recessive lethals are all associated with an array of genetic abnormalities such as transmission ratio distortion of the *t* chromosome by the male and crossing-over suppression with the *t*-complex and beyond (Erickson *et al.* 1980). A dominant point mutation Brachyury (*T*), which results in embryonic lethality in the homozygous state, and a viable short-tail animal when heterozygous, is present on the centromeric end of the *t*-complex. Recent work has placed the recessive lethal mutations at several locations within the *t*-complex (Lyon *et al.* 1979; Artzt *et al.* 1982). All of the l^t mutations have been isolated from wild mice or derived from wild mice by recombinational events (Bennett *et al.* 1976). The only mutations that have been reported that arose spontaneously or were induced in laboratory mice are allelic to *T* (Bennett, 1975).

One such allele of *T* is Hairpin-tail, T^{hp}, which arose in the AKR/J strain (Johnson, 1975). T^{hp} appears to be a deletion of at least 3 cM of chromosome 17 (Bennett, 1975). Its lethal effect in the homozygous state (T^{hp}/T^{hp}) occurs between the morulae and blastocyst stages (Babiarz *et al.* 1982). T^{hp} does not seem to affect chiasma frequency or recombination on chromosome 17 (Johnson, 1974). T^{hp} transmitted by the male results in decreased numbers of T^{hp} offspring. However, this is proposed to be the result of death at birth of T^{hp} offspring rather than transmission ratio distortion during fertilization (Johnson, 1974).

* Present address: College of Veterinary Medicine, Virginia Polytechnic Institute and University, Blacksburg, VA 24060.

In a T^{hp} line which we received from Dr M. F. Lyon we have discovered a lethal effect that lies outside the deleted region of T^{hp}. This lethal belongs to the t^0 complementation group (l^0) and maps to the left of the hair-mutation, *tufted*, *tf*. It has no effect upon male transmission ratios or recombination within the centromeric end of chromosome 17. The l^0 factor would appear to have arisen spontaneously and may represent a point mutation of a single locus within the *t*-complex. This gene can be appropriately named *t complex lethal zero* (tcl^0).

Table 1. *Complementation of* T^{hp} *to various* t*-haplotypes**

t-Haplotypes	Number of males tested	Offspring			
		$+/t^x$	$T/+$	T^{hp}/t^x	Total
t^{w5}	1	7	6	2	15
t^{w18}	2	12	4	6	22
t^{12}	1	5	4	4	13
t^0	2	30	23	1	54
t^6	3	25	17	0	42

* All crosses were T/t^x (♀) × T^{hp} (♂).

Table 2. *Recombination* (R) *frequency between* T^{hp} *and* tf *in the presence of the* t^0*-like lethal*

Matings		Number of animals tested	Parental chromosomes		Recombinant chromosomes		Total	%R
♂	♀		Normal-tail, tufted	Short-tail, non-tufted	Normal-tail, non-tufted	Short-tail, tufted		
$\frac{T^{hp}l^0+}{++tf}$	$\frac{++tf}{++tf}$	9	143	102	7	3	255	3·9
$\frac{++tf}{++tf}$	$\frac{T^{hp}l^0+}{++tf}$	7	35	1	4	0	40	10·0

2. MATERIALS AND METHODS

(*i*) *Mice*

The T^{hp} stock was received from Dr M. F. Lyon (MRC Radiobiology Unit, Harwell, England) and maintained by crosses to normal-tail tufted ($+tf/+tf$) littermates. The *t*-haplotypes: t^{w5}, t^{w18}, t^{12}, t^0, t^6 as well as T^{hp} were bred in the mouse colony of Dr R. P. Erickson of the Department of Human Genetics at the University of Michigan Medical School.

(*ii*) *Embryos*

Embryos of known gestational age were obtained by detection of vaginal plugs in naturally ovulated females. The mid-point of the dark period preceding the day the plugs were found was taken to be time zero. Embryos were fixed with Bouin's solution *in utero*. Serial sections (6 μm) of paraffin-embedded embryos were examined under a light microscope after staining with haematoxylin and eosin.

3. RESULTS

(*i*) *Genetic studies*

T^{hp} has the ability to complement t^{w5}, t^{w18} and t^{12} (Table 1). However, unlike previous reports (Lyon *et al.* 1979*a*; Babiarz *et al.* 1982), T/t^6 or T/t^0, with one exception, failed to produce tailless (T^{hp}/t^x) offspring upon mating with T^{hp}. If a t^0-like lethal mutation (l^0) arose in the T^{hp} line, the one exceptional T^{hp}/t^0 offspring could represent a recombinant T^{hp} which had lost l^0.

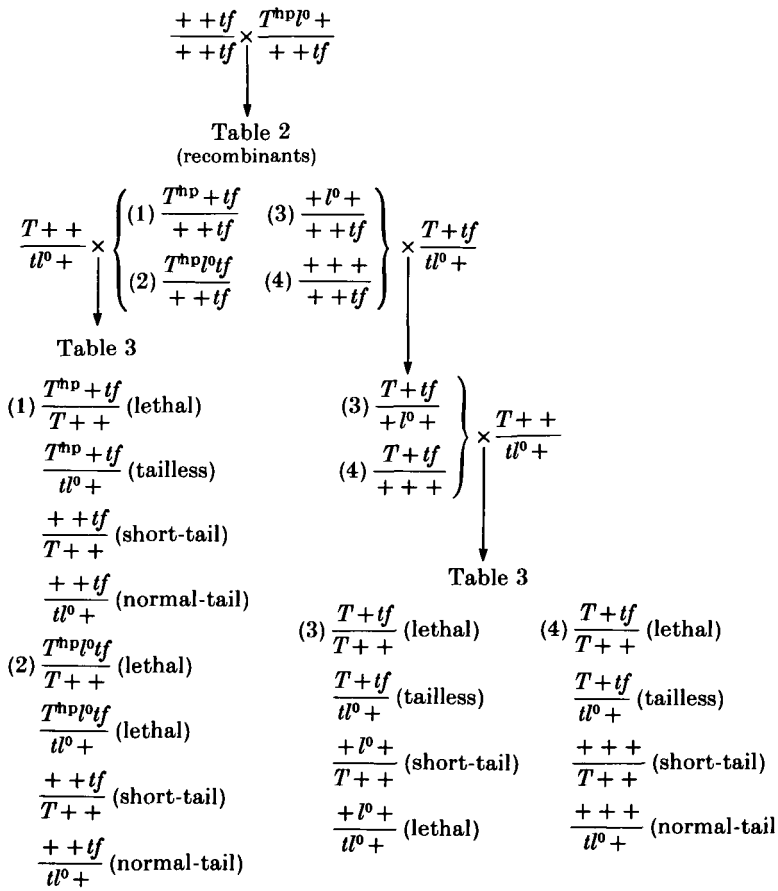


Fig. 1. Mating scheme for the isolation of a t^0-like lethal from the T^{hp} haplotype.

To test for a lethal factor which is separate from the T^{hp} deletion, a three-point cross between T^{hp}, *tf* and l^0 was designed (Fig. 1). Recombination in such a cross is permitted between T^{hp} and *tf* (Table 2). Several of the resulting recombinants were then tested for the presence of the l^0 (Fig. 1) by mating to T/t^0 (Table 3). Since tailless offspring from crosses involving a short-tail tufted recombinant (left-hand side of Fig. 1) are produced, the l^0 factor was lost when recombination occurred between T^{hp} and *tf*.

The normal-tail, nontufted recombinants (right-hand side of Fig. 1) were first crossed to Ttf/t^6 to place the recombinant chromosome in repulsion to *Ttf*. Three

https://doi.org/10.1017/S0016672300025684 Published online by Cambridge University Press

such recombinants were then mated to T/t^0 or T/t^6 (Table 3). Two of them yielded no normal-tail ($+l^0+/tl^0+$) offspring or a very reduced number. One of the three did produce normal-tail offspring in amounts suggesting that is lost the l^0 factor upon recombination between T^{hp} and *tf*. These results would place the l^0 factor to the left of *tf*, separable from T^{hp}. Of the five putative normal-tail recombinants from the cross $+l^0+/T+tf \times T/t^0$ or T/t^6, three were tested for the presence if *tf*, and all carried *tf*, indicating that a recombinational event did occur, with the l^0 factor being lost as a result. Given five normal-tail recombinants out of 130 offspring (Table 3, lines 1 and 2), this gives a recombinational frequency of 3·8%.

Table 3. *Testing of recombinant chromosomes*

Recombinant chromosome	Female	Number males tested	Offspring: Short-tail	Zero tail	Normal-tail	Total
$+l^0+/T+tf$	$\times T/t^0$ or T/t^6	2	47	50	5	102
$+l^0+/T+tf$	$\times T/t^0$ or T/t^6	1	8	20	0	28
$+++/T+tf$	$\times T/t^0$ or T/t^6	2	32	22	36	90
$T^{hp}+tf/++tf$	$\times T/t^0$	1	10	5	7	22

Table 4. *Transmission ratios of* T^{hp} *and recombinants*

Matings ♂	Matings ♀	Number tested	Offspring: Normal-tail	Short-tail	Zero tail	Total	% Transmission*	χ_1^2
$\frac{T^{hp}l^0+}{++tf}$	$\times\frac{++tf}{++tf}$	9	150	105	0	255	41	7·9
$\frac{t^{h2}+tf}{T^{hp}l^0+}$	$\times\frac{+++}{+++}$	3	48	144	0	192	25	48·0
$\frac{+l^0+\dagger}{T+tf}$	$\times\frac{T}{t^0}$ or $\frac{T}{t^6}$	3	—	55	70	125	44	1·8
$\frac{+l^0+\dagger}{T+tf}$	$\times\frac{++tf}{++tf}$	1	10	10	0	20	50	—
$\frac{+++\dagger}{T+tf}$	$\times\frac{T}{t^0}$ or $\frac{T}{t^6}$	2	—	32	22	54	59	1·9

* Transmission ratios indicated are for the top male chromosome of each mating.
† The indicated chromosomes are recombinants.

The l^0 factor *cis* to T^{hp} allows recombination between T^{hp} and *tf* (Table 2). Another property of wild-derived *t* haplotypes is their effect upon male transmission ratios of the *t* chromosome. T^{hp} and the l^0 factor in *cis* show a deficiency of short-tail (T^{hp}) offspring (Table 4). However, earlier studies have demonstrated this deficiency to be due to post-natal death of T^{hp} heterozygotes (Johnson, 1974). The l^0 factor by itself has no effect upon transmission ratios (Table 4). In addition, $T^{hp}-l^0$ in *trans* to t^{h2} does not increase the transmission of t^{h2} (Table 4).

https://doi.org/10.1017/S0016672300025684 Published online by Cambridge University Press

(*ii*) *Embryonic studies*

The lack of zero-tail offspring from $T/t^0 \times T^{hp}/+$ matings suggested that T^{hp}/t^0 embryos were dying *in utero*. Some $5\frac{1}{2}$-day post-coitum (p.c.) embryos from this cross were examined histologically, since t^0/t^0 embryos are routinely identified at the early egg-cylinder stage (Gluecksohn-Schoenheimer, 1940). Twenty-six per cent of the embryos were classified as abnormal (Table 5). The typical early

Table 5. In utero *histology of* $5\frac{1}{2}$ *day p.c. embryos*

Matings ♀	Matings ♂	Number of litters	Number normal	Number abnormal	Total embryos
$\frac{+\,+\,+}{tl^0+}$	$\times \frac{T^{hp}l^0+}{+\,+\,+}$	2	14	5	19
$\frac{+l^0+}{+\,+tf}$	$\times \frac{+l^0+}{+\,+tf}$	2	14	6	20
$\frac{+l^0+}{+\,+tf}$	$\times \frac{+\,+\,+}{tl^0+}$	3	10	10	20

egg-cylinder embryo (Plate 1*a*) is comprised of two ectodermal masses, embryonic and extra-embryonic, a layer of endoderm, a small but rapidly expanding ectoplacental cone mass and a thin layer of mural trophectoderm. Both primary and secondary giant cells can be seen invading the uterine stroma. In contrast, the putative T^{hp}/t^0 embryos at this stage (Plate 1*b*) showed few or no giant cells. The layer of endoderm overlying embryonic ectoderm is rough, due primarily to the rounded shape of the individual cells. By comparison, normal endoderm cells are cuboidal and yield a smooth-surfaced layer. Although the abnormals had both embryonic and extra-embryonic ectoderm, these masses are much reduced in size. Few or no ectoplacental cone cells could be seen.

When the lethal region (l^0) is separated from T^{hp} it continues to act as a t^0-like lethal when made homozygous ($+l^0+/+l^0+$) (Table 5). Embryos from the cross $+l^0+/+\,+tf \times +l^0+/+\,+tf$ were examined histologically. Thirty per cent of these $5\frac{1}{2}$-day p.c. embryos were abnormal. These abnormals had the same aberrant morphologies as T^{hp}/t^0 embryos at this stage (Plate 2*a*). Moreover, $+l^0+/tl^0+$ embryos, obtained from $+l^0+/+\,+tf \times +\,+\,+/tl^0+$ matings, were virtually identical to $T^{hp}l^0+/tl^0+$ and $+l^0+/+l^0+$ embryos of the same gestational age (Plate 2*b*). The expected frequencies of abnormal embryos reported in Table 5 depend on the transmission ratio distortion exhibited by the males of each genotype. The $T^{hp}l^0+/+\,+\,+$ and $+l^0+/+\,+tf$ males are normal transmitters. The $+\,+\,+/tl^0+$ males, however, donate the tl^0+ chromosome to 98% of their offspring. This is a result of an interaction between the t^0 chromosome and the wild-type chromosome 17 which, in this case, is the Robertsonian translocation marker Rb7 (personal observation; data not reported).

https://doi.org/10.1017/S0016672300025684 Published online by Cambridge University Press

4. DISCUSSION

The T^{hp} line in our laboratory carries with it a lethal factor that does not complement t^0-like haplotypes. Embryonically, the l^0 factor causes lethality in much the same way as does t^0. The typical t^0/t^0 $5\frac{1}{4}$ day p.c. embryo contains little or no ectoplacental cone, smaller than normal ectodermal masses, an irregular endoderm and a few giant cells (Sanchez *et al.*, manuscript submitted). Death of the embryo occurs at $6\frac{1}{2}$–7-days p.c. due primarily to failure of ectoplacental cone formation. The putative $T^{hp}l^0/lt^0$, l^0/l^0 and l^0/t^0 $5\frac{1}{2}$-day embryos demonstrate this same pattern of abnormalities.

This l^0 factor can be separated by recombination from T^{hp} and maps 3·8 cM to the right of Brachy. The occurrence of the l^0 factor does not represent an extension of the T^{hp} deletion, as the lethal of t^{w18} which lies to the left of l^0 (Lyon *et al.* 1979) is not expressed when combined with T^{hp}. This l^0 factor would appear to have arisen spontaneously in the T^{hp} stock. The ancestors of our T^{hp} line were never crossed to t^0 or t^6 (M. F. Lyon, personal communication). However, Lyon *et al.* (1979) did report an absence of tailless offspring (T^{hp}/t^{h20}) in a cross involving a T^{hp} male, suggesting that the l^0 factor is segregating in the T^{hp} population. In addition, no abnormality in transmission ratio distortion or crossing-over suppression is seen which would be suggestive of the presence of a *t* haplotype. In this way it resembles the t^6-recombinant, t^{h18}, which only possesses the lethal portion of the t^6 haplotype (Lyon & Mason, 1977). However, our l^0 factor differs from t^{h18} in one important respect – it does not affect the low transmission of t^{h2}, whereas t^{h18} in combination with t^{h2} gives normal Mendelian transmission (Lyon & Mason, 1977). These observations indicate that our T^{hp} line acquired its l^0 factor through a mutational event or a very precise recombinational event that picked up only the l^0 factor. In either case, this lethal effect appears to be the result of a single gene, *t-complex lethal zero* (tcl^0), and may represent the first opportunity to study *t* embryonic lethality in the complete absence of other regions and loci normally associated with *t*-haplotypes. In addition, the existence of this single developmental gene in the absence of other *t* regions suggests that developmental recessive lethals within the *t*-complex are the result of changes in the structural genes themselves rather than the effect of abnormal *t*-chromatin (Lyon *et al.* 1979*b*).

These experiments were supported by NIH Grant HD 11738. The authors wish to thank Dr Robert P. Erickson for his scientific encouragement, Dr Michael Rosenberg for critical reading of the manuscript, and all mouse room employees past and present, especially Cynthia Abel and Liz Rush.

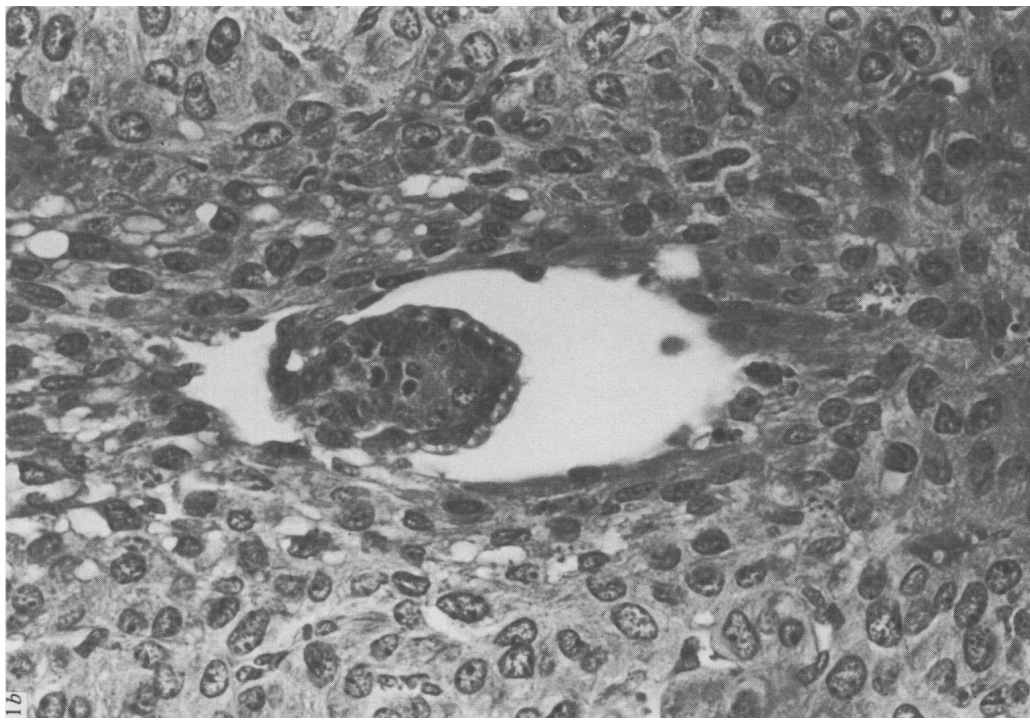
REFERENCES

Artzt, K., McCormick, P. & Bennett, D. (1982). Gene mapping within the *T/t*-complex of the mouse. I. *t*-lethal genes are nonallelic. *Cell* **28**, 463–470.

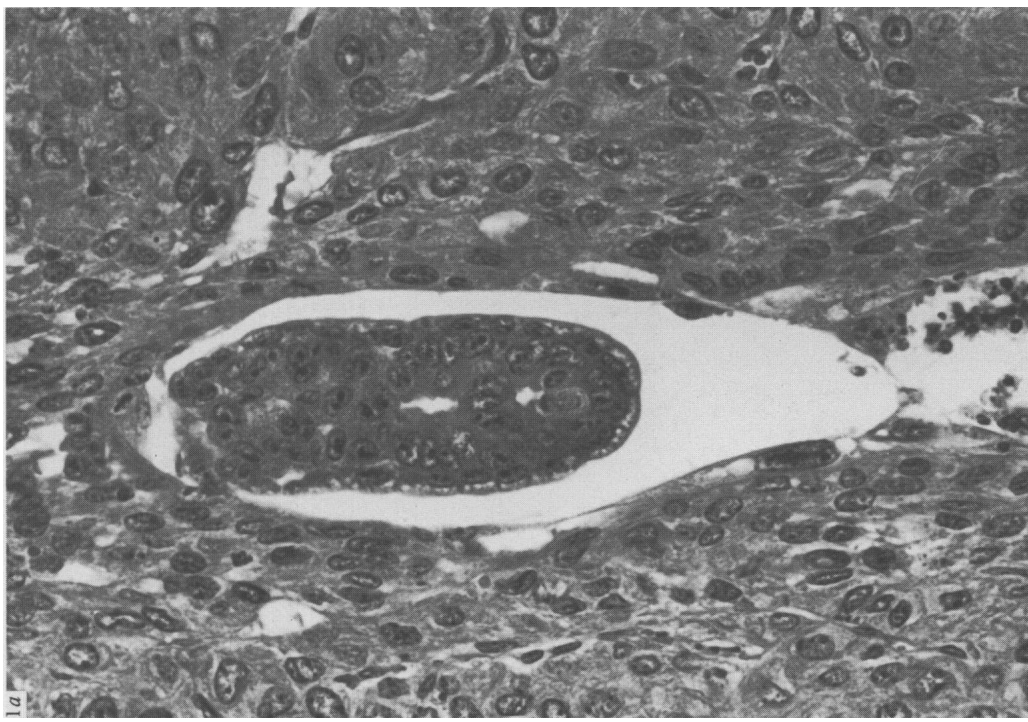
Babiarz, B., Garrisi, G. J. & Bennett, D. (1982). Genetic analysis of the t^{w73} haplotype of the mouse using deletion mutations; evidence for a parasitic lethal mutation. *Genetical Research* **39**, 111–120.

Bennett, D. (1975). The *t*-locus of the mouse. *Cell* **6**, 441–445.

Bennett, D., Dunn, L. C. & Artzt, K. (1976). Genetic change in mutations at the *T/t* locus in the mouse. *Genetics* **83**, 361–372.



1*b*

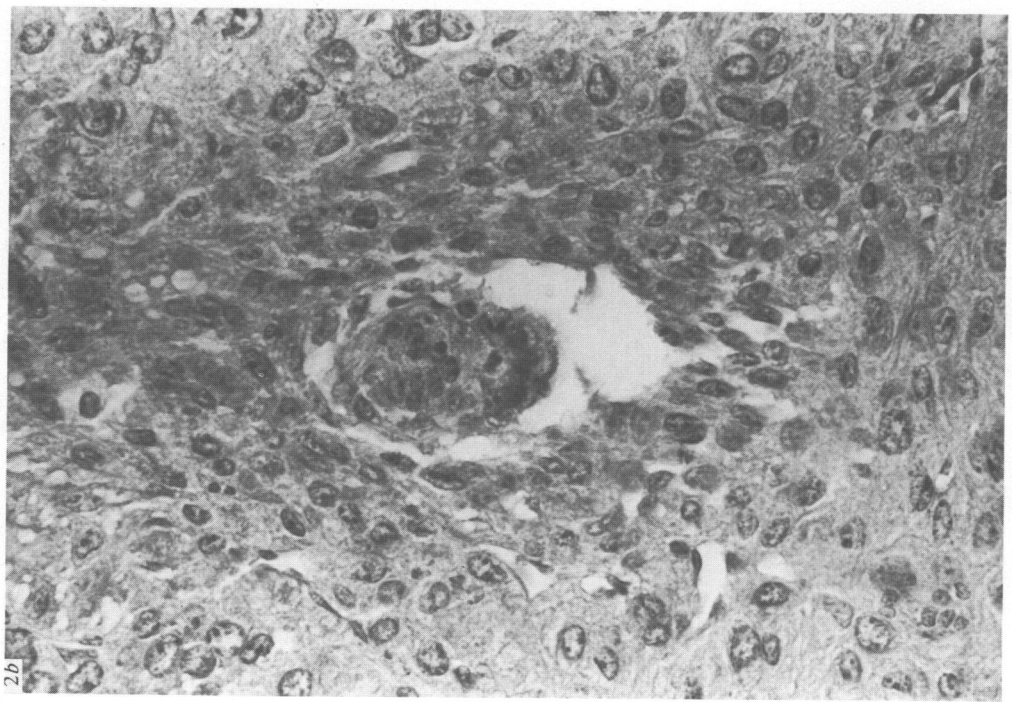


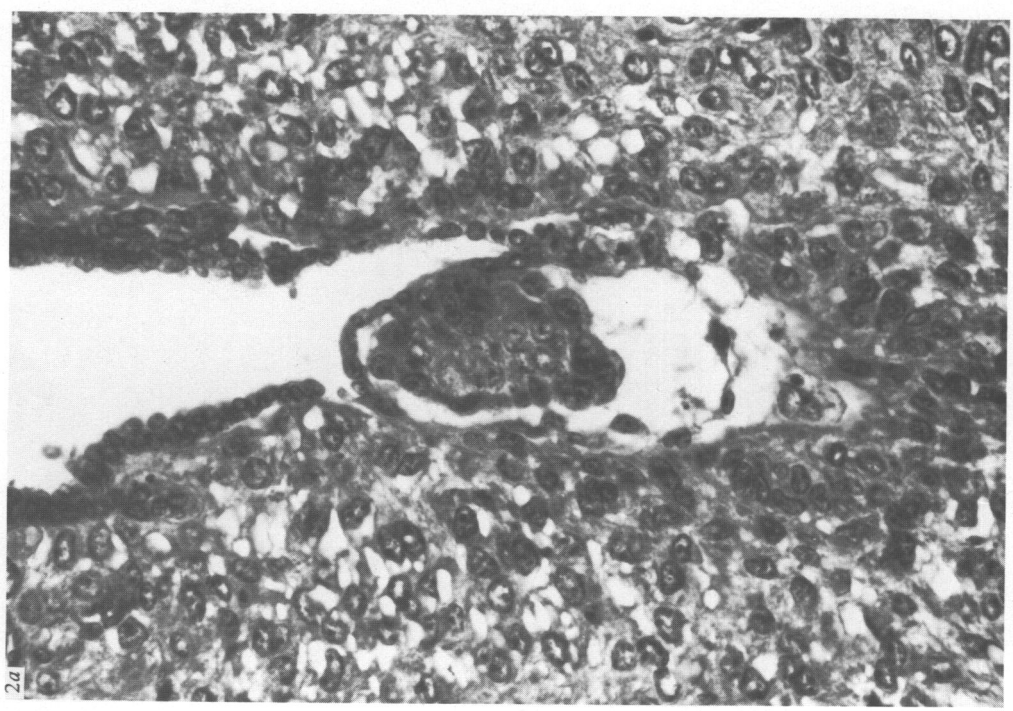
1*a*

E. R. SANCHEZ AND C. HAMMERBERG

(*Facing p.* 32)

https://doi.org/10.1017/S0016672300025684 Published online by Cambridge University Press





E. R. SANCHEZ AND C. HAMMERBERG

https://doi.org/10.1017/S0016672300025684 Published online by Cambridge University Press

ERICKSON, R. P., HAMMERBERG, C. & SANCHEZ, E. (1980). *t*-mutants in the mouse and alterations in early development. In *Current Research Trends in Prenatal Craniofacial Development* (ed. Pratt and Christiansen). Amsterdam: Elsevier North-Holland.

GLUECKSOHN-SCHOENHEIMER, S. (1940). The effect of an early lethal (t^0) in the house mouse. *Genetics* **25**, 391–400.

JOHNSON, D. R. (1974). Hairpin-tail: a case of post-reductional gene action in the mouse egg. *Genetics* **76**, 795–805.

LYON, M. F. & MASON, I. (1977). Information on the nature of *t*-haplotypes from the interaction of mutant haplotypes in male fertility and segregation ratio. *Genetical Research* **29**, 255–266.

LYON, M. F., JARVIS, S. E., SAYERS, I. & JOHNSON, D. R. (1979*a*). Complementation reactions of a lethal mouse *t*-haplotype believed to include a deletion. *Genetical Research* **33**, 153–161.

LYON, M. F., EVANS, E. P., JARVIS, S. E. & SAYERS, I. (1979*b*). t-haplotypes of the mouse may involve a change in intercalary DNA. *Nature* **279**, 38–42.

EXPLANATION OF PLATES

PLATE 1

Light micrographs (× 375) of longitudinal sections from litters segregating for $T^{hp}l^0+$ and tl^0+. (*a*) A normal early egg-cylinder embryo at day 5·5. (*b*) A putative $T^{hp}l^0+/tl^0+$ embryo at day 5·5.

PLATE 2

Light micrographs (× 375) of longitudinal sections from litters segregating for $+l^0+$ and tl^0+. (*a*) A putative $+l^0+/+l^0+$ embryo at day 5·5. (*b*) A putative $+l^0+/tl^0+$ embryo at day 5·5.