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Curtailed, a new dominant T-allele in the house mouse

By A. G. SEARLE

M.R.C. Radiobiological Research Unit, Harwell, Berkshire

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1. INTRODUCTION

The *T*-region in linkage group IX of the mouse is one of particular interest, because of the unusual properties (summarized by Lyon & Meredith, 1964*a*) possessed by the extensive series of recessive *t*-alleles and the major effects which dominant and recessive alleles have on the development of axial organization. Two dominant alleles have been described so far: *T* itself, otherwise known as brachyury or short-tail (*cf.* Grüneberg, 1963) and the Harwell allele T^h (Lyon, 1959). Hertwig's allele T^{hg} appears to be a reoccurrence of *T* (Kuminek, 1959, 1960). Curtailed, the new allele to be described here, shows a number of abnormalities not previously described in members of the *T* series and thus throws additional light on the functions of this complex region.

2. ORIGIN AND APPEARANCE

A single female with a very short caudal filament instead of a normal tail turned up in a mixed litter of 19 derived from a cross between two females of a multiple recessive stock and a male given 200 r. acute X-irradiation when a 17½-day foetus (Carter, *et al.*, 1960). The male produced no other offspring of this phenotype out of a total progeny of 102. The abnormal female was mated to an unrelated hybrid male and produced 46 (19♀, 27♂) offspring with the same mutant phenotype out of a total of 108. The deviation from a 1:1 ratio is not significant. Thus the mutant gene was regarded as a fully penetrant autosomal dominant and given the name curtailed, being assigned the symbol T^c when tests (described later) proved it to be allelic with *T*.

A typical curtailed mouse has no normal tail but a short boneless caudal filament. About 5% of curtailed mice have a tail, not more than 20% normal length, and ending in a filament. 3% lack the filament, but this may be accidental, as some with caudal filaments lost them later. The following abnormalities were also seen very occasionally in curtailed young: (i) small blood-blisters just dorsal to the caudal filament associated with low grade spina bifida, (ii) paralysis of the hind-limbs, (iii) atresia ani with reduced genital papilla.

3. GENETICS

Outcrosses gave 370 curtailed: 446 normal among offspring classified shortly after birth. This differs significantly from a 1:1 ratio ($\chi_1^2 = 7{\cdot}08$) and suggests

that there is some selective elimination of newborn curtailed. 32·2% of curtailed mice died between classification at birth and weaning, which is much higher than the usual figure in this laboratory of about 10%, especially as large litters were reduced in number by killing off some non-curtailed mice. Intercrosses gave 302 curtailed:159 normal progeny, a close approach to the 2:1 ratio expected with homozygous lethality *in utero*. Thus there was much less evidence for neonatal elimination of curtailed progeny than on outcrossing, but post-natal mortality was very similar (33·4%).

Table 1. *Linkage tests between* T^c *and* tf: *results of backcross matings*

Heterozygous parent	Phenotypes of progeny					Recombination	
	T^c+	$T^c tf$	++	$+tf$	Total	%	Standard error
$T^c+/+tf$ ♀	96	16	11	159	282	9·57	1·75
$T^c tf/++$ ♀	8	32	82	17	139	17·99	3·26
$T^c+/+tf$ ♂	142	9	19	152	322	8·70	1·57
$T^c tf/++$ ♂	2	49	81	4	136	4·41	1·76

Curtailed was tested for linkage with the following genes: c^{ch}, *d*, *se*, *s*, *a*, *Ra*, *Ca*, *Re*, *wa*-2, *b*, *tf*, *v*, Mi^{wh}, *wa*-1, *je*, *fz*, *ln*, *f*, *W* and *ru*. Recombination values did not differ significantly from 50% except with tufted *tf* on linkage group IX (Table 1), as would be expected if curtailed was a *T*-allele. Mice were classified for tufted at the age of about 1 month; there was 42·1% mortality of curtailed mice between classification at birth and at this age but only 11·9% mortality of non-curtailed mice. This differential mortality does not seem to have affected linkage estimates, for the figures arrived at by considering only non-curtailed mice are very similar to the overall percentages of recombination. The recombination frequencies in the two sexes are similar when T^c and *tf* are on different chromosomes, but significantly different when they are on the same chromosome. The difference between coupling and repulsion recombination values is significant in females but not in males. Combining all the data gives an estimated recombination frequency of 9·8%.

Other tests made to gain information on the suspected *T*-allelism were as follows:

(i) A curtailed heterozygote was mated to a t^{h7}/t^{h3} mouse and produced 29 curtailed-like, 32 short-tailed with caudal filament (the tail varying from 10–50% of normal length but mostly around 20%) and 55 normal progeny. This approximate 1:1:2 ratio is in agreement with *T*-allelism, since t^{h7} elongates the tails of brachyury mice so that T/t^{h7} mice have normal tails (Lyon & Meredith, 1964*b*), while T/t^{h3} mice are tailless. The presumed T^c/t^{h3} mice were apparently more severely affected than $T^c/+$, since over 80% died before the age of 14 days, while about 30% showed dorsal red scars or blood-blisters at birth, some of these having paralysed hind-limbs as well. Thus it seems probable there was a high incidence of spina bifida occulta.

(ii) Curtailed heterozygotes were mated to $T/+$ mice and females dissected on the ninth to tenth day of gestation. Characteristically abnormal embryos of the T/T type were found.

(iii) Dissections of pregnant females following $T^c/+$ intercrosses showed that between days 9 and 11 there were 29 dead or live malformed embryos among 95 implantations (31%). These presumptive curtailed homozygotes were similar to lethal T/T embryos but showed more extreme abnormalities, which are described in detail later.

4. ABNORMALITIES OF HETEROZYGOTES

Alizarin transparencies were prepared of 45 curtailed mice and 54 normal sibs for detailed comparison of skeletal abnormalities, supplemented by papain preparations of individual bones and methylene blue transparencies of 13–16-day foetuses.

The following abnormalities were noted in curtailed heterozygotes:

(i) *Absence of the odontoid process of the axis*

The axis had no odontoid process in any of ten papain preparations (Fig. 1), it was also absent in the cartilaginous skeleton (Fig. 2). It was, however, undoubtedly present in $T/+$, $T^h/+$ and tailless T/t^6 mice. The axis acquires the same smooth

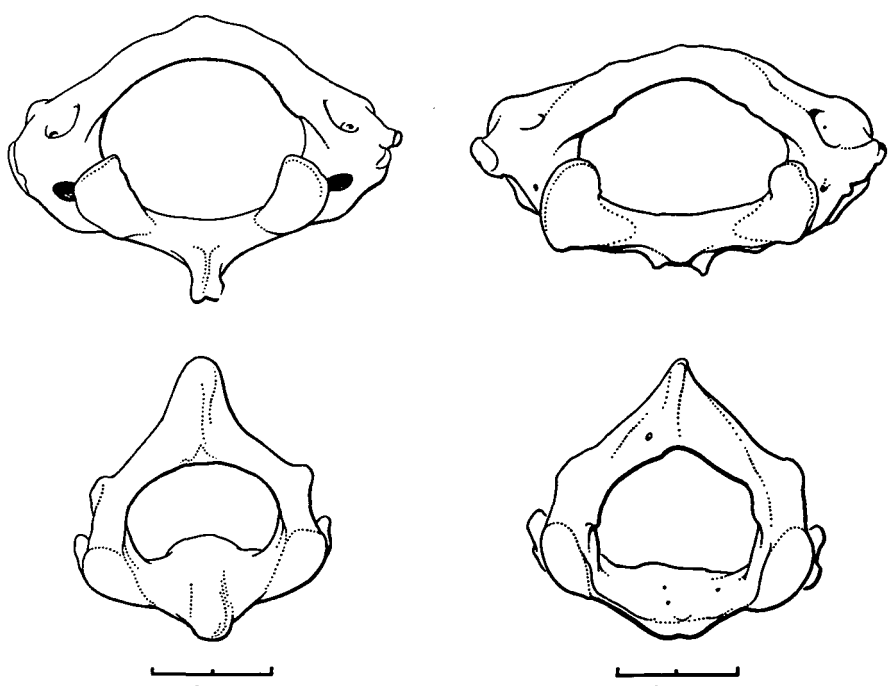

Fig. 1. Atlas and axis of normal (left) and curtailed mice, the latter showing absence of the axis odontoid process and changes in the ventral arch of the atlas. Camera lucida drawings.

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horseshoe-shaped articulation with the atlas as is found in mice heterozygous for Danforth's short-tail (Grüneberg, 1953).

(ii) *Other atlas-axis anomalies*

Six curtailed mice showed a curious anomaly of the axis or of the atlas and axis, in which the neural arch was partially double, as shown in Fig. 3. This was associated with dyssymphysis of the neural arches concerned. In one curtailed mouse the atlas right neural arch was completely fused with the axis.

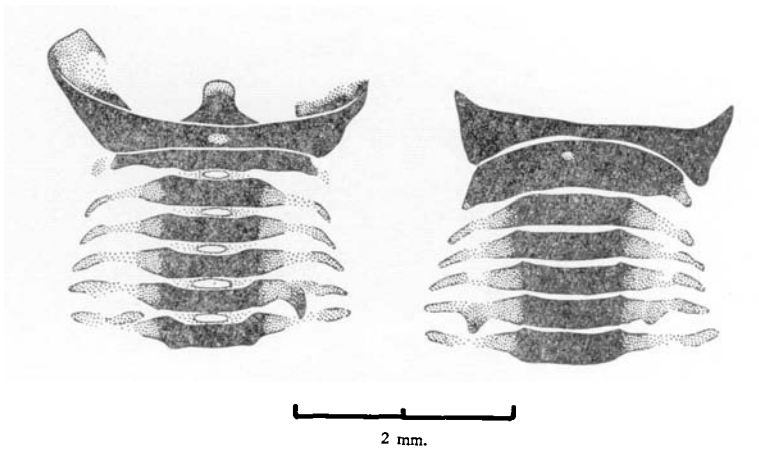

Fig. 2. Ventral views of cervical vertebrae of normal (left) and curtailed 16-day foetal mice, showing absence of the odontoid process of the axis and of the nuclei pulposi in curtailed. Camera lucida drawings of methylene-blue transparencies.

(iii) *Vertebral and rib fusions*

Vertebrae in all regions tended to become fused (Table 2), although in the cervical region the fusions were all between the 2nd and 3rd vertebrae, apart from the atlas-axis fusion mentioned above (ii). Associated with the fusions of thoracic vertebrae were rib fusions which tended to be quite extensive (Fig. 3). They could already be seen in the cartilaginous skeleton of a 14-day $T^c/+$ foetus. Other rib abnormalities were occasionally seen, three curtailed mice having 7th cervical vertebrae with ribs on one or both sides which articulated with 1st thoracic ribs; two also lacked the sternal element of the 1st thoracic rib, the costal part of which articulated with the 2nd thoracic instead.

(iv) *Increased width of centra*

The centra of $T^c/+$ cervical vertebrae are significantly wider than normal, as judged by measurements of 6th cervicals from alizarin preparations of five pairs of litter-mates. Mean widths were:

T^c	+	Difference	t	P
15·5 mm.	12·3 mm.	3·2 mm.	6·39	$<0·01$

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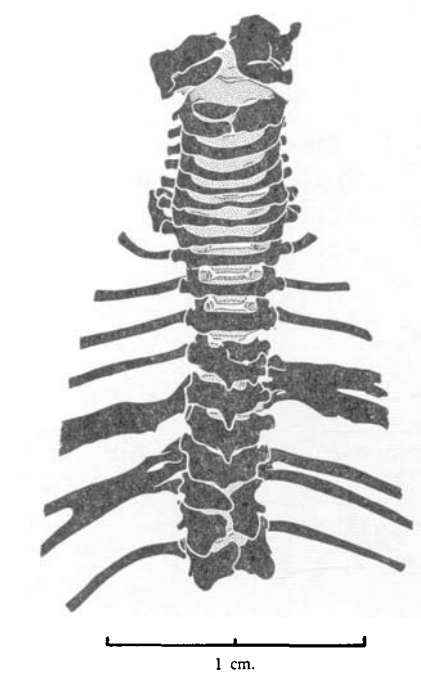

Fig. 3. Dorsal view of cervical and upper thoracic vertebrae of adult curtailed mouse, showing neural arch abnormalities of atlas, axis and some thoracic vertebrae as well as rib fusions. Camera lucida drawing of alizarin transparency.

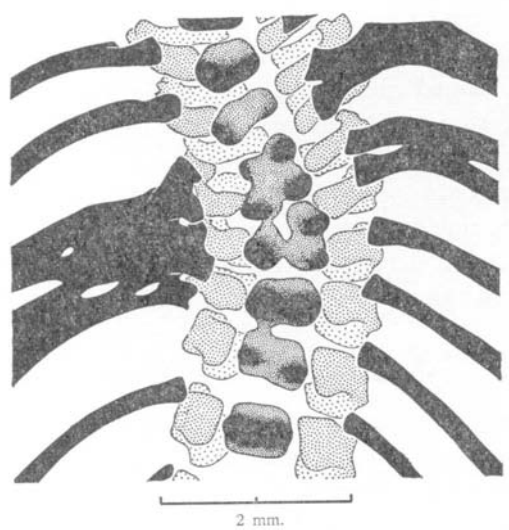

Fig. 4. Ventral view of middle thoracic vertebrae of 2-day curtailed mouse showing irregular ossification of centra and rib fusions. Camera lucida drawing of alizarin transparency.

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Measurements of widths of 1st lumber vertebral centra showed no significant differences between $T^c/+$ and normal mice.

Alizarin preparations of 2-day-old curtailed and normal mice showed that the T^c axis tended to ossify from twin centres, as did some thoracic centra, which also showed abnormal distortion and fusion (Fig. 4) associated with rib fusions.

(v) *Absence of nucleus pulposus*

Methylene-blue transparencies of foetal and 3-week-old curtailed mice showed that they lacked the intervertebral nuclei pulposi, apart from some slight traces of them in the lower thoracic and lumbar regions at 3 weeks. As Fig. 2 shows, the difference from the normal condition was very marked.

Table 2. *Incidence of vertebral and rib fusions in curtailed and normal mice*

		Number of vertebral fusions			
Phenotype	Total examined	Cervical	Thoracic	Lumbar	Number of rib fusions
Curtailed	45	6 (13·3%)	5 (11·1%)	3 (6·7%)	10 (22·2%)
Normal	54	1 (1·9%)	0	0	0

(vi) *Decreased number of presacral vertebrae*

Table 3 shows that curtailed mice had on the average 0·2 presacral vertebrae less than normal. The difference was significant on a 2×2 comparison of number of sides with 26 or with less than 26 presacral transverse processes in the two groups, χ_1^2 being 5·64, $P = 0{\cdot}02$. Numbers of lumbar and of thoracic vertebrae were both reduced.

Table 3. *Numbers of presacral vertebrae (left/right)*

	26/26	26/25	25/26	25/25	25/24	24/24	Total	Mean
Curtailed								
♂♂	9	1	1	14	0	0	25	25·40
♀♀	11	1	2	4	1	1	20	25·55
Total	20	2	3	18	1	1	45	25·47
Normal								
♂♂	18	2	3	8	0	0	31	25·66
♀♀	15	1	0	7	0	0	23	25·67
Total	33	3	3	15	0	0	54	25·67

(vii) *Sacro-caudal abnormalities*

The total number of sacral + post-sacral vertebrae varied from 1–8. The most caudal vertebrae were always distorted and reduced in size, with asymmetrical fusions between them.

5. ABNORMALITIES OF HOMOZYGOTES

Dissections of $T^c/+$ females on the tenth to eleventh day of gestation, after mating with $T^c/+$ males, revealed a distinctive type of abnormal embryo (Fig. 5). This resembled lethal T/T embryos (also shown in Fig. 5) in lacking (i) the posterior part of the body, including hind-limb buds and tail, (ii) the normal allantoic outgrowth, (iii) externally visible somites. However, these presumptive T^c/T^c embryos showed more extreme abnormalities than T/T ones in that (i) posterior reduction

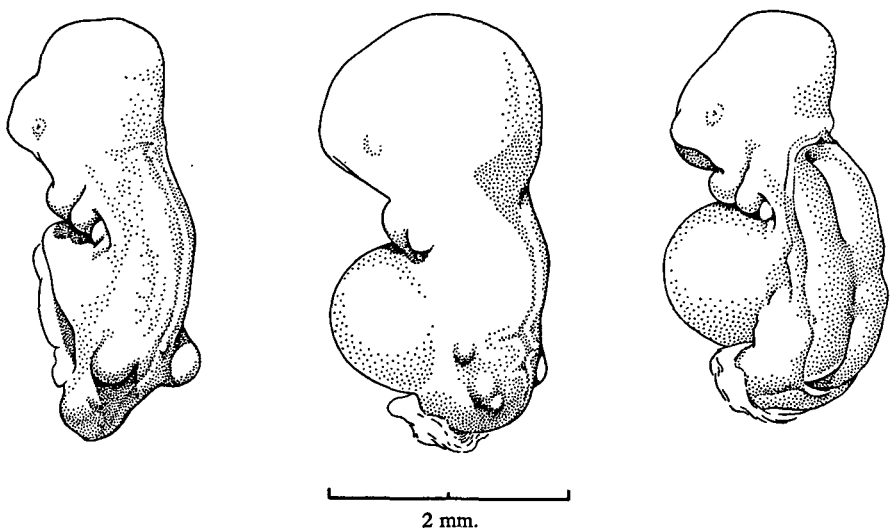

Fig. 5. 10–11-day T/T (left), T/T^c (centre) and T^c/T^c (right) embryos. Note that T^c/T^c is more severely affected, while T/T^c shows an intermediate phenotype in some respects. Camera lucida drawings.

of the body was even greater, with loss of the fore-limb buds too, (ii) the neural folds remained open in the trunk region, (iii) there were kinks in the spinal cord on each side of the open neural folds, (iv) the pericardium was distended.

$T^c/+$ mice were crossed to $T/+$ or $T^h/+$ mice and similar dissections made. No differences were found between the presumptive T^c/T^h phenotype and T^c/T^c, suggesting that T^h is completely recessive to T^c. T/T^c embryos were similar to T/T but seemed more variable, tending to show some characteristics of T^c/T^c. Thus there was occasional partial non-closure of the trunk neural folds and waviness of the spinal cord. Fore-limb buds were present but rather small and irregular in shape, sometimes being split up into several small outgrowths (Fig. 5).

6. DISCUSSION

Several of the pleiotropic effects of the T^c-allele resemble those of T. Thus T heterozygotes may also lack the tail altogether on occasion, have fusions between vertebrae, have fewer presacral vertebrae on the average, and very occasionally

show some more extreme abnormality, such as spina bifida, paralysis of the hind-limbs or atresia ani. But curtailed mice clearly differ from brachyury ones in that the tail always shows extreme abbreviation or absence, while on some genetic backgrounds the tail of a $T+$ mouse may be indistinguishable from normal. Moreover, the absence of the axis odontoid process in curtailed mice may well be an absolute difference between effects of the two alleles, since this abnormality has never been reported in brachyury mice although it has been found in all curtailed mice examined from this point of view. The clear-cut differences between lethal T/T and T^c/T^c embryos serve to confirm that we are dealing with different alleles. Judging from the phenotypes of compounds, the order of dominance of the three T-alleles now known is T, T^c, T^h.

Comparing the three alleles, T, T^c and T^h, we find the peculiar situation that while T^h is less extreme than T^c in heterozygous form, since it has essentially the same phenotype as $T/+$, yet it is decidely more extreme in homozygous form, since T^h/T^h embryos are already highly abnormal at 8 days' gestation, consisting only of a small cone of tissue in an embryo sac of half normal size (Lyon, 1959). It is also of interest that the recessive allele t^{h7}, which suppresses the heterozygous expression of both T and T^h (Lyon & Meredith, 1964; Lyon, personal communication), only partially suppresses T^c+ expression, since a tail is present but is much shortened. Another point to be considered concerns linkage relationships with tufted. The values for recombination between T and *tf* given by Lyon (1956) and derived from coupling backcrosses ($7{\cdot}7 \pm 1{\cdot}8\%$ in females and $9{\cdot}2 \pm 2{\cdot}4 \pm$ in males) do not differ significantly from the coupling backcross figures given in Table 1 of this paper. But Lyon (1959) found some evidence for a lower recombination value ($4{\cdot}9 \pm 2{\cdot}4\%$ between T^h and *tf*, although the difference from the T-*tf* and T^c-*tf* figures does not reach a significant level. Thus, while the relationships between T and T^c seem of the normal allelic type, T^h behaves more like a length of abnormal chromosome (as discussed by Lyon, 1959) which includes the T locus but has additional recessive properties. Recessive *t*-alleles seem to consist of different lengths of abnormal region (Lyon & Meredith, 1964) and several of them die at about the same stage as T^h when homozygous (Bennett, 1964).

Curtailed heterozygotes show interesting resemblances to Danforth's short-tail mice (*Sd*/+) in the absence of the odontoid process of the axis and of the nuclei pulposi (Theiler, 1951; Grüneberg, 1953, 1958). Nuclei pulposi are also affected in pintail (*Pt*/+) mice, being much reduced in size (Berry, 1960). Both *Sd* and T^c develop the same novel type of horseshoe-shaped articulation between atlas and axis as a result of lack of the odontoid process, and both heterozygotes also have wider than normal cervical centra. Grüneberg (1958) traced all the defects of *Sd*/+, and the much more severe defects of *Sd*/*Sd*, back to an abnormality of the notochord leading to its early disintegration. He considered that this in turn might stem from a more general disturbance of the primitive streak. Studies of $T/+$ and T/T embryos also implicate the notochord and the primitive streak in the disturbed developmental pathway (cf. Grüneberg, 1963). The nature of the T^c defects leads to the same conclusion and suggests that in this allele there is an

earlier disintegration of the notochord than in T, leading to even greater losses and defects of posterior structures in homozygotes as well as to the loss of the odontoid process, absence of nuclei pulposi and other defects of the axial skeleton.

The fusions between ribs found in some curtailed heterozygotes links T^c also to rib fusions *Rf* (Mackensen & Stevens, 1960; Theiler & Stevens, 1960). *Rf*/*Rf* mice usually die *in utero* with extensive rib and vertebral fusions; they also lack somites and frequently show exencephaly. Heterozygotes usually have fused ribs and may also show a number of vertebral abnormalities, including fusions between neural arches and between centra as well as ossifications from bilateral centres. These similar skeletal defects in T^c and *Rf* heterozygotes all suggest relatively slight irregularities in somite differentiation, while in both homozygotes the normal development of somites is very seriously affected. The appearance of these somite-derived defects in T^c but not in T heterozygotes is presumably connected with the greater severity of effect in the T^c homozygote. It provides additional evidence for the belief that the primary effect of both alleles is on the notochord-mesoderm system (Bennett, 1964). The coexistence of abnormalities of both notochordal and paraxial mesodermal derivatives in T^c heterozygotes as well as homozygotes suggests that the primary defect may well reside in the progenitor of both these structures, namely, the primitive streak.

SUMMARY

Curtailed (T^c) is a new T-allele which leads to complete or near-complete absence of the tail in heterozygotes, apart from a small caudal filament. Other heterozygous defects include absence of the axis odontoid process and of the nuclei pulposi, a tendency to have rib and vertebral fusions and a slight decrease in the average number of presacral vertebrae. T^c is lethal when homozygous, causing similar but more extreme defects than T, with absence of all limb-buds and non-closure of the neural folds. A comparison of T^c with other mutants suggests that the anomalies are mainly the result of more severe effects on the notochord-mesoderm system than in T. Differences between the genetic behaviour of T, T^c and T^h are discussed.

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