A STUDY OF A GENETIC ABNORMALITY IN THE DENTITION OF THE MOUSE

by

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General Introduction

The general form of the dentition of the house mouse is remarkably constant, but variation does occur. A wide survey by Grüneberg (1965) included examples both of major effects on the teeth, which are known to be caused by single mutant genes, and of minor differences between inbred strains. Two of the mutant genes already studied, the mimics tabby, Ta, and crinkled, cr, (Grüneberg, 1965 and 1966a) together with two other similar mimics, Ta^c, an allele of tabby (Mouse News Letter 35), and downless, dl (Mouse News Letter 23 and 34), have been the main object of this study. In addition to a consideration of these four mutants, an investigation was made to determine the nature of the genetic control of a minor morphological variant, a supernumerary cusp on the lower first molar. The work on the supernumerary cusp will be described first.

A. THE GENETIC CONTROL OF A MINOR MORPHOLOGICAL VARIANT, A SUPERNUMERARY CUSP ON THE LOWER FIRST MOLAR.

1. Introduction

Teeth are the most durable parts of the body and accordingly constitute a large part of the fossil material available for the study of phylogeny. In living animals they are usually reasonably accessible, and, if required, permanent preparations can be made with little difficulty. Thus modern and fossil populations can be included in a single study. It is therefore of interest to know something of the genetics of tooth morphology, and this can only be done by making use of the variation that exists in living populations.

Variation in the form of human teeth is known to occur both between and within populations (Dahlberg, 1945; Lasker, 1950; Lasker and Lee, 1957; Kraus, 1957). A number of attempts at analysis of the nature and degree of genetic control of this variation have been made (Mandeville, 1949; Kraus, 1951; Ludwig, 1957; Lundström, 1963; Hanihara, 1963; Garn, 1966). Studies of interspecific and intraspecific variation of tooth morphology in other mammals have included gibbons (Frisch, 1963), mice of the genus Peromyscus (Hooper, 1957), and the microtene rodents (Guthrie, 1965). No genetic analyses were made.

Minor dental variation in the house mouse has been considered in a number of investigations. These have, however, been primarily concerned with the control of tooth size rather than shape. Absence and size of third molars have been shown to be under genetic control (Grüneberg, 1951), but also dependent to a considerable extent on maternal physiology (Grüneberg, 1951; Searle, 1954b; Deol and Truslove, 1957). The heritability and components of variation of tooth size have been calculated for all three mandibular molars by Bader (1965a and b) and Tenczar and Bader (1966).

Variation in form of the molars is largely composed of small differences of relative size and position of the normal complement of cusps, but may also include a difference of cusp number. At least two supernumerary cusps are known to occur on the upper first molar (Grüneberg, 1965).

The normal crown of the lower first molar is composed of seven cusps. Numbered from anterior to posterior there are three buccal, B1, B2, and B3; three lingual, L1, L2, and L3; and a single central posterior cusp, 4. A supernumerary cusp, S, between B1 and L1 was found to occur with high frequency among animals of the Tuck No. 1 strain (Fig. 1).

Affected Tuck animals were crossed to four inbred strains. There was a striking difference between the F_1 of one of the crosses, where the character behaved as almost completely dominant, and those of the other three, where it behaved as almost completely recessive. Further crosses were made to distinguish between a single gene with variable penetrance and multifactorial inheritance.

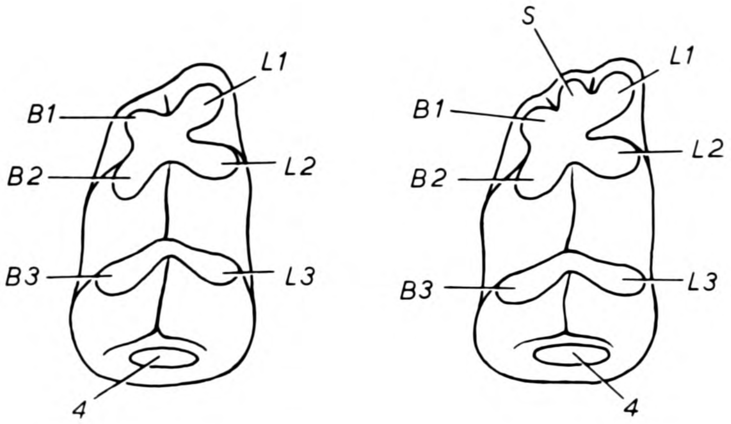
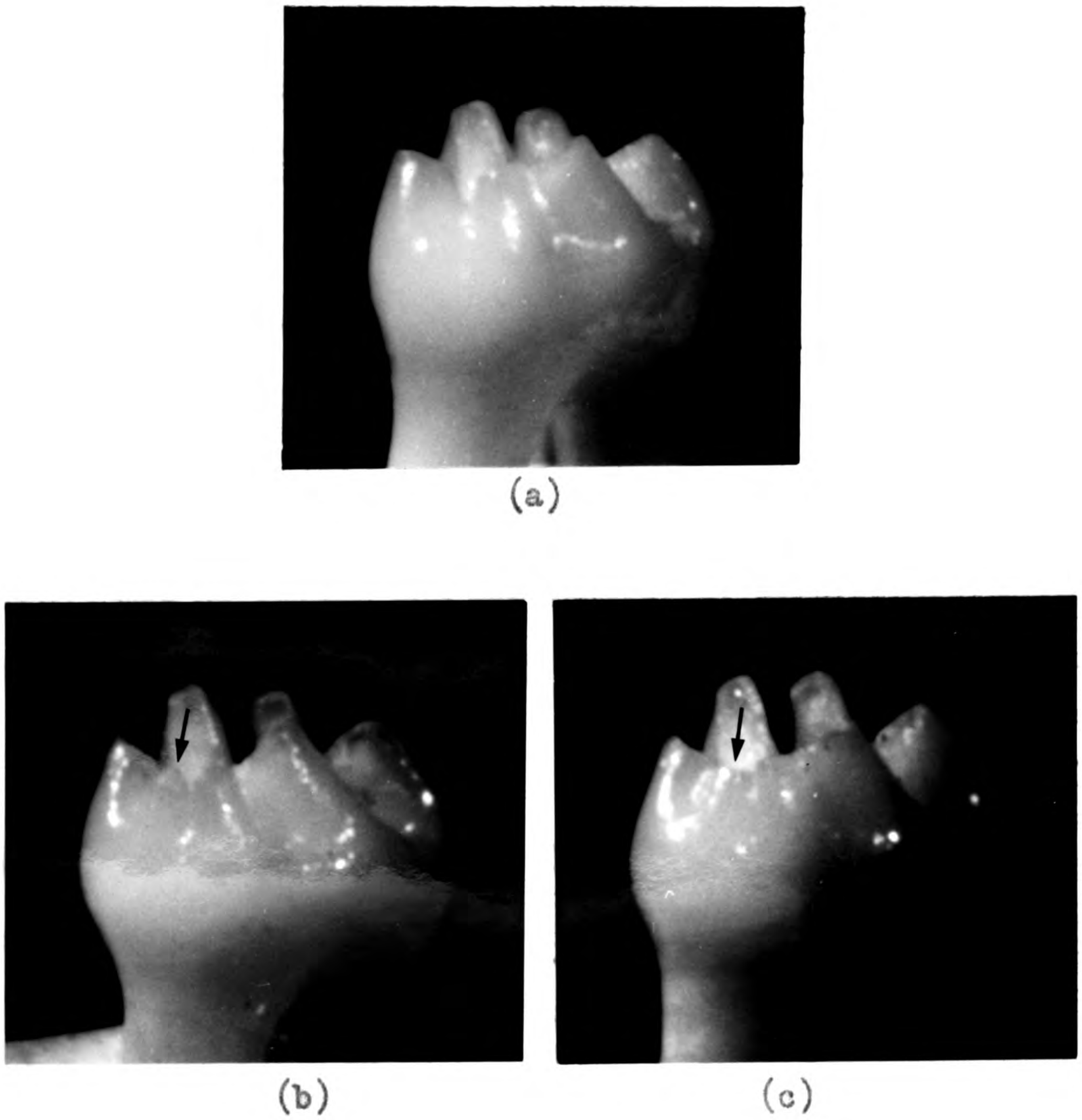


Fig. 1. Diagrams of occlusal surfaces of normal (left) and Tuck (right) lower left first molars.


(a)

(b)

(c)

Fig. 2. Lower left first molars (X31). (a) Normal (b) Small supernumerary cusp (c) Large supernumerary cusp

2. Material

The Tuck No. 1 strain has been maintained by random mating within a closed colony for twenty years. Tuck animals are therefore not highly inbred but can be regarded as being genetically similar. Three matings were obtained from Tuck's Mousery, Rayleigh, Essex, and the offspring of these matings were subsequently mated at random to provide material for the experiment. Eighty out of 89 Tuck animals showed the supernumerary cusp, some on one side and others on both. At its largest the cusp was comparable in size and form with its neighbours, but more usually it consisted of a projection of variable size, either from the groove between B1 and L1, or from the anterobuccal surface of L1. In very many cases the impression was given of different degrees of division of L1 into two daughter cusps. In a few cases the cusp was restricted to the base of a widened groove between B1 and L1. Normal and Tuck teeth are shown in Fig. 2.

Phenotypic variation does not necessarily imply genetic heterogeneity (Searle, 1954a), but to reduce the possibility of significant genetic variation in the material, only the most severely affected Tuck animals were used for mating.

The four inbred strains, A, C57, JU, and CBA, have been maintained by brother x sister matings at the Institute of Animal Genetics, Edinburgh, for between 40 and 50 generations. Animals of the same strain can therefore be regarded as being genetically identical. Lower first molars of a number of inbred mice were examined. Six out of 87 C57 animals showed unilateral slight but definite grooving of the anterobuccal surface of L1 towards its tip, similar to that seen in the most mildly affected Tuck animals, (Fig. 2b). The remaining C57 animals, and all those of the other three strains, were entirely normal in this respect. It should be mentioned that B1 and L1 of C57 lower first molars are always much less well separated than they are in the other three strains. This minimal separation of B1 and L1 in C57 mice has already been noted by Grüneberg (1965). Cusps B1 and L1 of the few unaffected Tuck teeth were well separated.

3. Method

(i) Examination

Animals required for mating were examined under anaesthesia produced by an intraperitoneal injection of Nembutal (0.1 ml. of a 0.9% solution per 10G. body wt.).

Examination required illumination of the teeth which are some way back from the small opening of the oral cavity. This was accomplished by using a dissecting microscope with a modified light source. The modification consisted of a glass slide, held at 45° just below the objective lens, at which a horizontal light beam was directed. The slide reflected light down along the optical axis of the lens and at the same time allowed an image of sufficient intensity to pass back through it unreflected and to be observed through the microscope. A pair of adapted tweezers served as a mouth prop , and a small funnel-shaped instrument was constructed to act as a retractor for the tongue and lips through which the observer could see. The apparatus is shown in Fig. 3.

Animals not required for mating were sacrificed and dissected prior to examination.

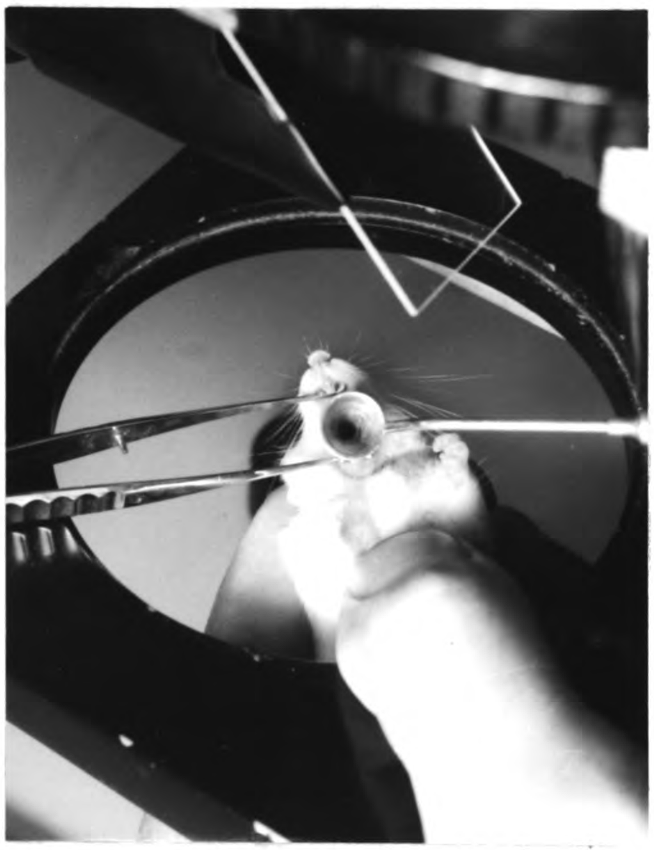


Fig. 3. Examination of mice under anaesthesia.

Tuck animals and the progeny of the crosses were examined between weaning at three weeks and mating at six weeks of age. Some of the inbred mice were a little older, but not enough to allow wear to affect scoring.

(ii) Scoring

In the main analysis of the behaviour of the cusp it was treated as an all-or-none character, but, as it varied in size, some attempt was made to score the degree to which animals were affected. Animals were scored 0 for no cusp, 1 for a small cusp, and 2 for a large cusp. Each side was scored separately so that the maximum possible total score was 4. The results of the crosses are expressed as histograms based on this method of scoring.

(iii) Crosses

The method was similar to that used by Bloom and Falconer (1964) to demonstrate a recessive gene conferring resistance to pulmonary tumours.

Affected Tuck animals were crossed to the four inbred strains, A, C57, JU and CBA. The F_1 results are shown in Fig. 7. In view of the relative dominance of the character on the C57 background and recessiveness of it on the other three the following backcrosses were made. F_1 animals of the C57 cross were backcrossed to C57, and then a second backcross was made to C57. F_1 animals of the JU cross, taken as being representative of the recessive situation, were backcrossed to affected Tuck animals, and then a second backcross was made to Tuck.

Because of its variable expressivity and incomplete penetrance in the Tuck strain the cusp was regarded as a threshold character. When dealing with such characters meaningful conclusions can only be drawn if an underlying scale of continuous variation is assumed to exist. Such a scale would be a measure of some attribute immediately related to the development of the cusp, and all individuals below the threshold value would be normal and all those above it affected. The more the value exceeded the threshold the more severely would the individual be affected. On this basis the theory behind the system of crosses is illustrated in Figs. 4 and 5.

In the single gene case (Fig. 4) the two strains differ in respect of the gene A and one is all affected and one all non-affected. F_1 individuals are all identical heterozygotes with some above and some below the threshold. In the real case the F_1s were not intermediate but high in the C57 cross and low in the others. Backcrossing the F_1 produces two distributions, one identical to the F_1 and one identical to the parental strain to which it was crossed. The proportion of affected individuals amongst the first backcross progeny is therefore intermediate between the F_1 and the parental strain to which it was crossed.

In the case of the multifactorial inheritance (Fig. 5) F_1 individuals are all identical heterozygotes with some above and some below the threshold, just as in the single gene case. But unlike the single gene case backcrossing produces a single distribution of greatly increased variance, composed of individuals of 2^n different genotypes, where n is the number of loci which are different between the two parental strains. The progeny of the F_1 back-

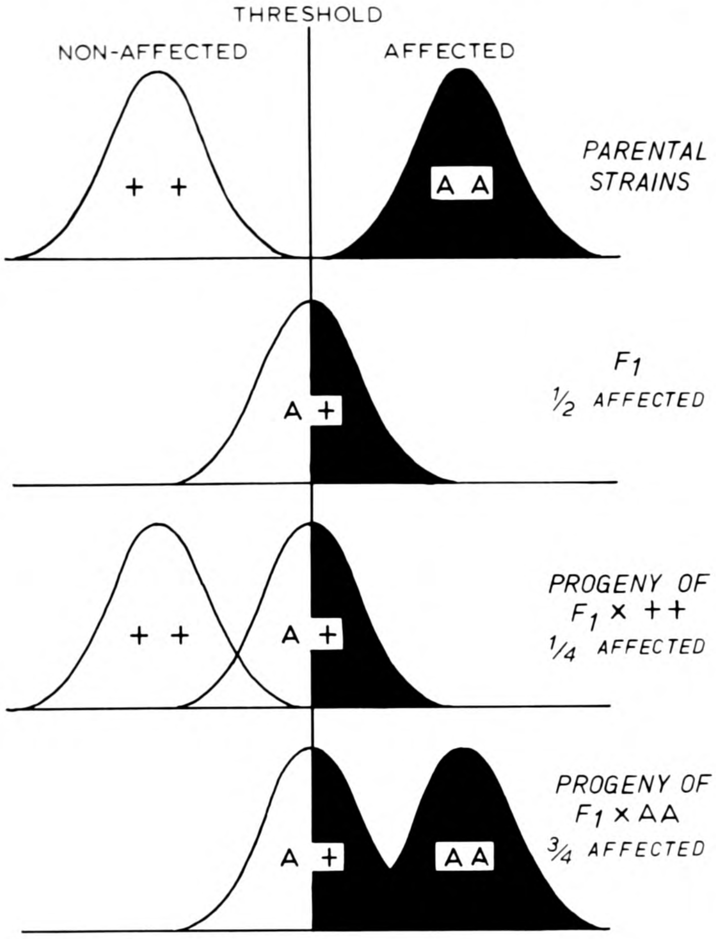

THE BEHAVIOUR OF A SINGLE PARTIALLY DOMINANT GENE

Fig. 4.

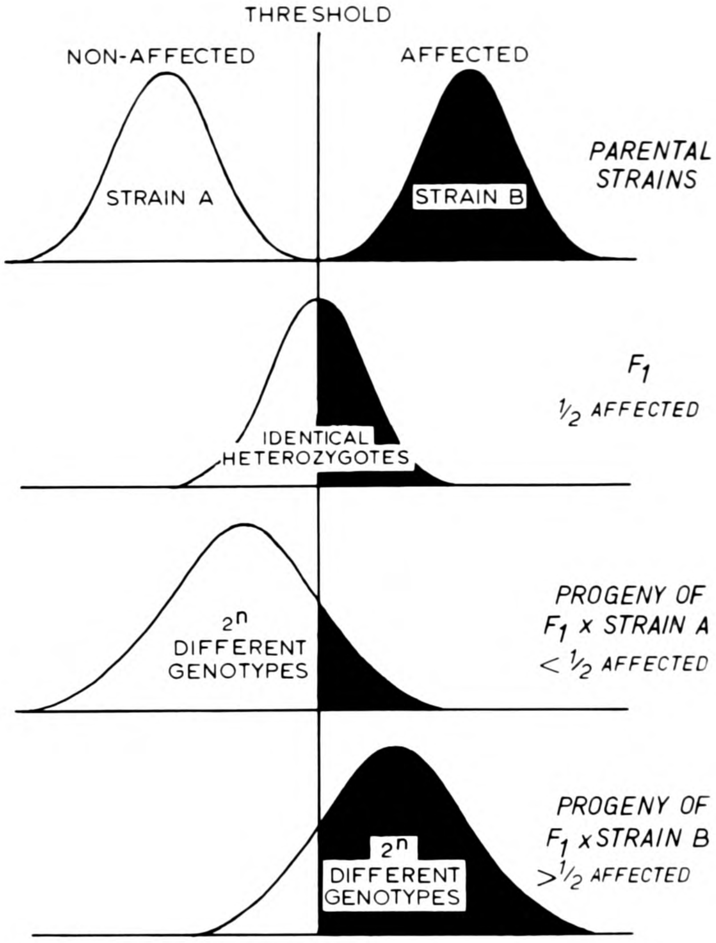

MULTIFACTORIAL INHERITANCE

Fig. 5.

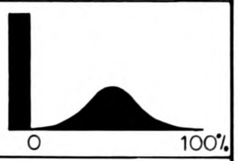
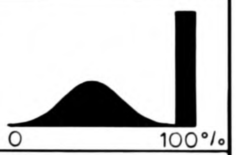
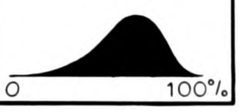
		1st BACKCROSS PROGENY	2nd BACKCROSS TO	TYPES of MATING	PROP^N. AFFECTED INDIVIDUALS	DISTRIBUTION of FAMILY SCORES
SINGLE GENE	DOMINANT	½ + + ½ A +	+ +	+ + × + + A + × + +	0% 50%	
	RECESSIVE	½ a a ½ a +	a a	a a × a a a + × a a	100% 50%	
MULTIFACTORIAL		2^n DIFFERENT GENOTYPES	NON-AFFECTED PARENT	MAXIMUM NUMBER OF 2^n	WIDE VARIATION BETWEEN FAMILIES	
			AFFECTED PARENT			

Fig. 6.

(For explanation see text)

crossed to the unaffected parent form a distribution whose mean is shifted down from the threshold, and the progeny of the F_1 backcrossed to the affected parent form a distribution whose mean is shifted up from the threshold. The outcome, on an all-or-none basis, is just as in the single gene case. The proportion of affected individuals amongst the first backcross progeny is intermediate between the F_1 and the parental strain to which it was crossed. The two alternatives, single gene and multifactorial inheritance, are therefore indistinguishable at this stage; unless there is a method of scoring the degree to which animals are affected which is sufficiently accurate to indicate the shapes of the distributions of first backcross progeny.

The critical test to distinguish between single gene and multifactorial inheritance is made by a second backcross, where first backcross progeny are taken at random to be used as partners in mating to the original parental stocks. The genotypes of first backcross progeny are then reflected in the families they produce, and the families can be scored on the basis of the proportion of affected individuals they contain.

The behaviour of the two alternative situations in a second backcross is illustrated in Fig. 6. A single gene is expected to produce a bimodal distribution of second backcross family scores, as there are two distinct genotypes of first backcross progeny which are used as parents. Multifactorial inheritance is expected to produce a unimodal distribution, as there are many different genotypes amongst the first backcross progeny.

(iv) Matings made

The matings made are shown below:

Cross	♀ Parent	♂ Parent	Code	Number of Matings	Number of Progeny
Tuck x A	Tuck	A	TA	4	87
Tuck x C57	Tuck	C57	TCa	4	63
	C57	Tuck	TCb	8	68
Tuck x JU	JU	Tuck	TJa	3	57
	Tuck	JU	TJb	1	15
Tuck x CBA	CBA	Tuck	TCBA	4	36
BC_1 to C57	$C57F_1$	C57	TCC_1a	5	47
	C57	$C57F_1$	TCC_1b	5	32
BC_1 to Tuck	JUF_1	Tuck	TJT_1a	5	56
	Tuck	JUF_1	TJT_1b	4	41
BC_2 to C57	$C57BC_1$	C57	TCC_2a	11	193
	C57	$C57BC_1$	TCC_2b	13	266
BC_2 to Tuck	Tuck BC_1	Tuck	TJT_2a	13	253
	Tuck	Tuck BC_1	TJT_2b	12	228

4. Results

The incidence of affected individuals amongst the parental strains and the progeny of the crosses, according to the scoring method described, is shown in Figs. 7 and 8.

Comparison of the F_1 groups shows that the character was almost completely dominant on the C57 background and almost completely recessive on the other three. As expected, the first backcross, both to C57 and to Tuck, produced progeny amongst which the proportion of affected individuals was

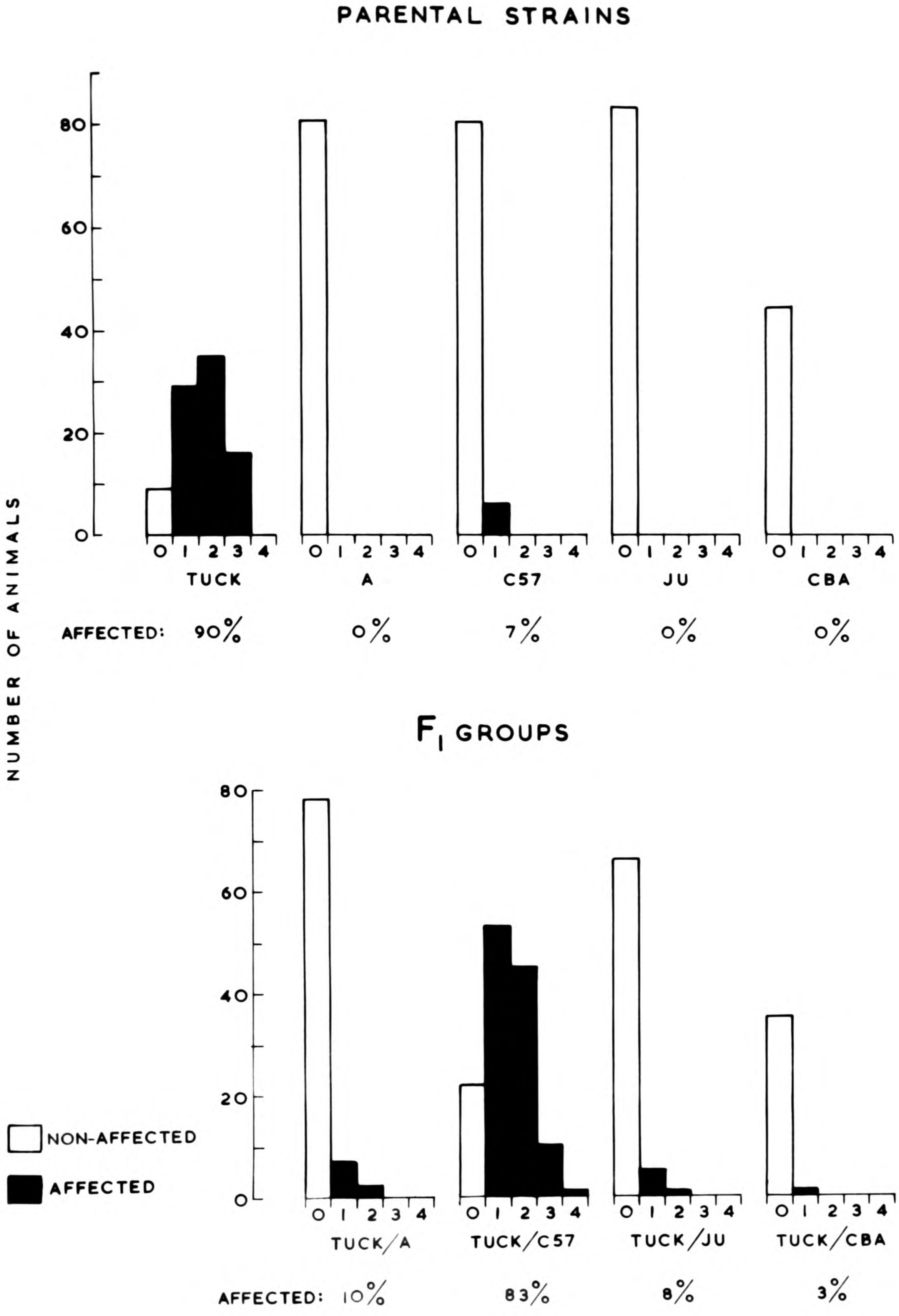


Fig. 7. The incidence of affected animals in five parental and four F_1 groups according to the scoring method described in the text.

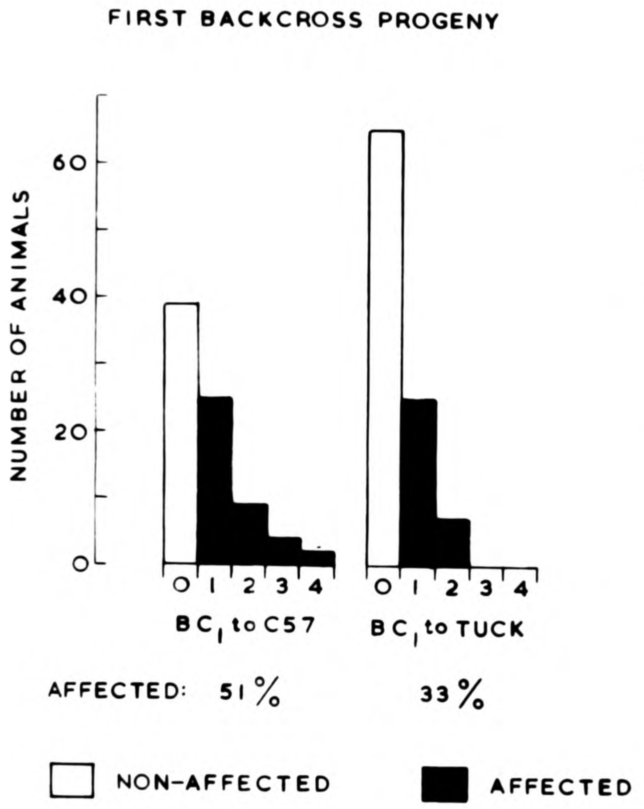
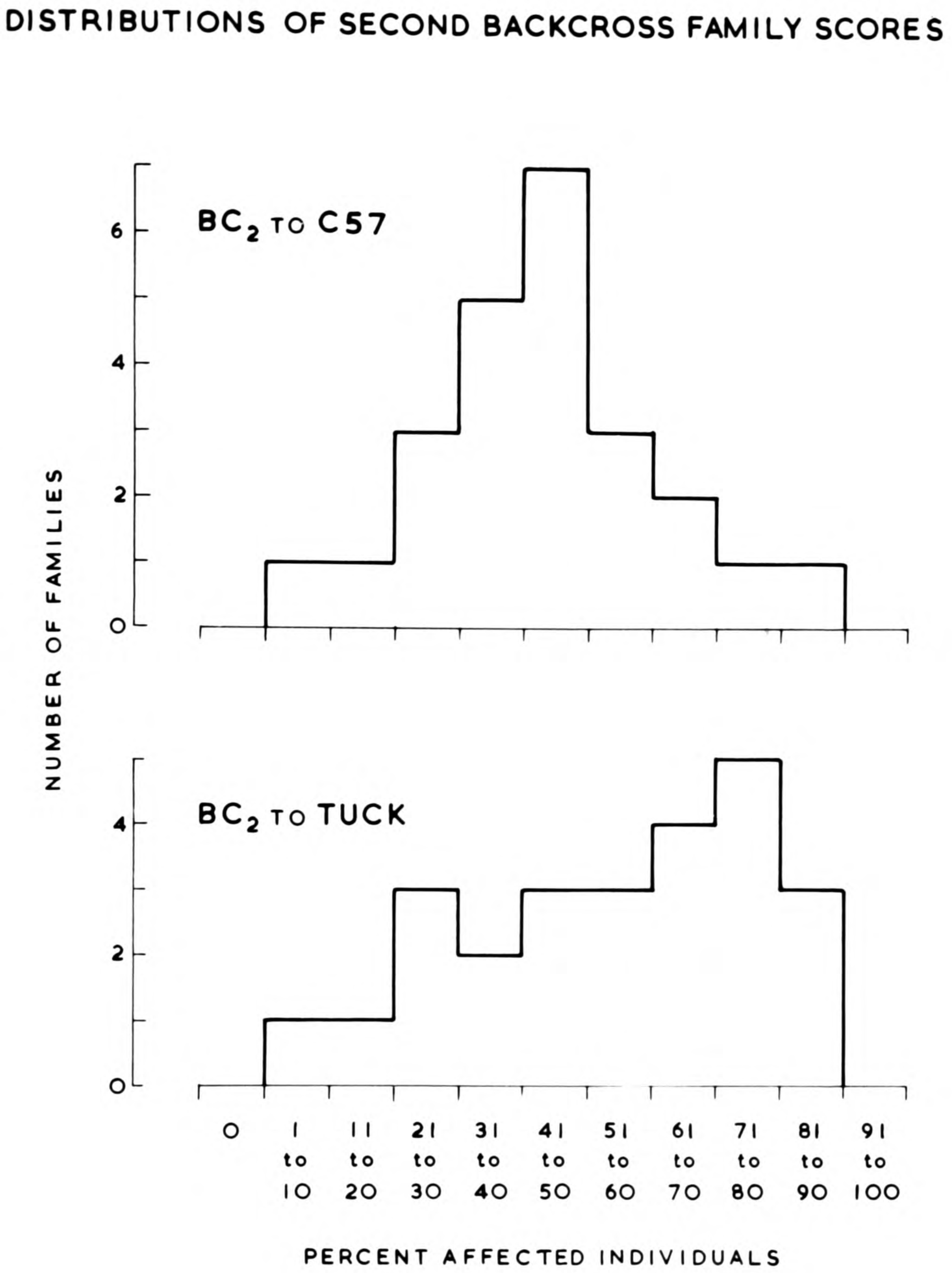


Fig. 8. The incidence of affected animals amongst first and second backcross progeny.



Family size	12	13	14	15	16	17	18	19	20	21	22	23	24	25
No. of C57 BC$_2$ families	1	0	2	0	1	5	1	3	2	3	2	1	2	1
No. of Tuck BC$_2$ families	0	0	0	0	4	5	4	1	3	0	5	2	0	1

Fig. 9. Distributions of second backcross family scores.

intermediate between the F_1 and the parental strain to which it was crossed. The scoring method was not fine enough to indicate the shapes of the distributions. The results at this stage are therefore equally compatible with single gene and multifactorial inheritance.

The results of the second backcross treated in the same way are similarly compatible with both forms of inheritance. The proportions of affected individuals in both cases were intermediate between that of each first backcross group and the parental strain to which it was crossed. If, however, the second backcross results are plotted as distributions of family scores the presence of a major gene should be detectable as a bimodality.

Fig. 9 shows the distributions of second backcross family scores. There was no definite trend towards bimodality in either cross, which indicates that there is no single gene responsible for the presence of the cusp. The genetic control of this supernumerary cusp is therefore an example of multifactorial inheritance.

5. Discussion

The most striking feature of the results was the variable expression of the character on different genetic backgrounds. This enabled two parallel sets of crosses to be carried out, one of which (the C57 set) would have been able to demonstrate a dominant gene, and the other of which (the JU set) would have been able to demonstrate a recessive gene. There was no evidence for the presence of a single major gene.

The proportions of affected individuals in each of the crosses conformed with what would be expected of an underlying scale of continuous variation. The group distributions of the two sets of crosses moved in opposite directions with successive backcrossing. Further evidence for such an underlying scale was obtained by examining the distributions of animals between classes within each affected group. This will now be described.

If an underlying scale of continuous variation does exist, then the greater the proportion of animals affected in any particular group the more severely will they be affected on average. This relationship was tested. Animals were scored in five classes; 0, 1, 2, 3, and 4. Zero must represent the threshold, so, if all classes above zero are to be of equal size, 1, 2, 3, and 4 must be the upper limits of each class. The midpoints of the four classes are then 0.5, 1.5, 2.5 and 3.5. It was from these midpoints that the mean score of affected animals in each group (MSA) was calculated (Moroney, 1962).

Given the proportion of affected individuals in any one group, and assuming that the group is normally distributed, two values can be read from tables. These are, x, the distance of the threshold from the group mean, and a, the distance of the mean of affected individuals from the group mean. They are both in terms of σ , the standard deviation of the group (Falconer, 1965). Thus if σ is known, purely theoretical positions of the means of affected individuals, relative to the constant threshold (that is a-x), can be calculated for distributions which have different positions on the underlying scale.

Strict comparison between groups can only be made if they all have the same σ. Accordingly, an estimate of σ for each group in terms of a constant, the threshold interval, was made (Falconer, 1964). For this purpose animals were divided into three classes; those scored as 0, those scored as 1, and those scored as 2 and above. The values for σ were then in terms of the interval between the 0-1 and 1-2 thresholds. Table 1 shows the particulars of nine groups considered. Fig. 10 shows the relationship between the proportion of individuals affected and the mean score of affected individuals for six of these groups. The line indicates the theoretical expectation for $\bar{\sigma} = 0.96$, the mean of the six groups plotted. The A, JU, and CBA F_1 groups were omitted from the plot and from the calculation of the mean as these had respectively only 2, 1, and 0 animals in the upper class. A σ for the CBA F_1 group could therefore not be calculated, and σ and MSA values for the other groups were unlikely to have been reliable.

Of the six points plotted two fall above the line, the Tuck strain and the C57 F_1 groups, expected to have relatively low variances. The remaining four groups fall below the line. The two furthest away are the first backcross groups, expected to have relatively high variances, and the two closest to the line are the second backcross groups, expected to have variances intermediate between parental or F_1 and first backcross values. Both the general trend of the points and their relationship to each other are therefore reasonably consistent with what would be expected of underlying continuous variation.

Table 1. (For explanation see text)

Group Code	Numbers scored in each category			% Affected	MSA	σ
	0	1	2 and above			
Tuck	9	29	51	90	1.34	0.90
TA	78	7	2	10	-	-
TC a&b	22	53	56	83	1.12	0.88
TJ a&b	66	5	1	8	-	-
TCBA	35	1	0	3	-	-
TCC_1 a&b	39	25	15	51	1.08	1.17
TJT_1 a&b	65	25	7	33	0.72	0.97
TCC_2 a&b	258	158	43	44	0.77	0.84
TJT_2 a&b	227	167	87	53	0.86	1.01

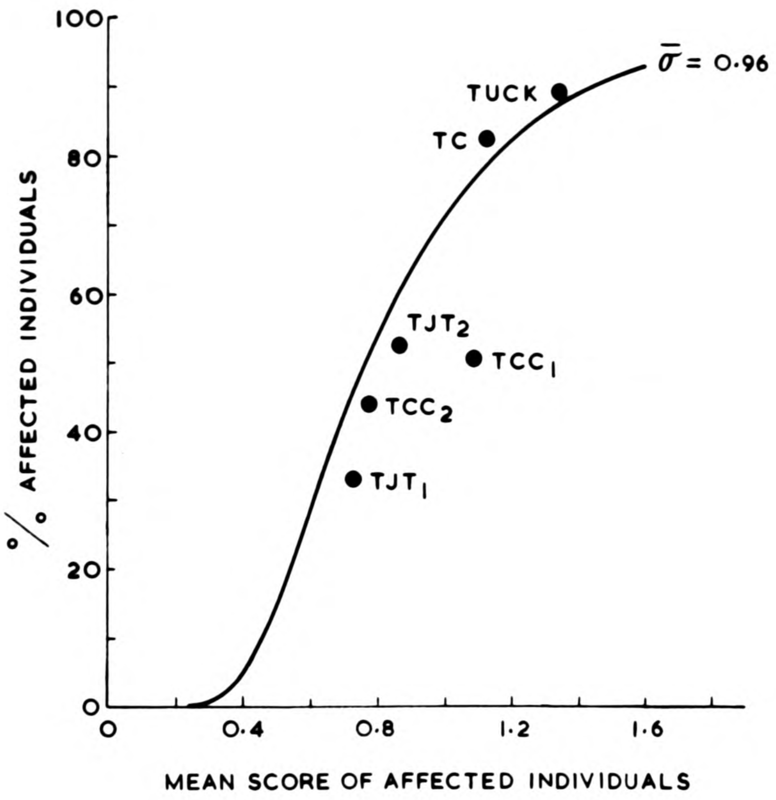


Fig. 10. The relationship between penetrance and expressivity - observed (points) and theoretical (line).

Reciprocal crosses were then considered separately. Values for σ and M ($M = \sigma x$), the distance of the distribution mean from the 0-1 threshold in threshold units, were calculated for each reciprocal group. Reciprocal crosses of the C57 groups showed a constant relationship in respect of both σ and M. The calculated values of both were lower for the progeny of C57 mothers. No such relationship was apparent between reciprocals of the JU crosses.

Reciprocal groups were then compared by a chi-square test using the actual numbers of animals scored in the three classes 0, 1 and 2 and above. The sexes were similarly compared, males of both reciprocal groups with females of both reciprocal groups within each cross. There was no difference between the sexes in any of the JU or C57 crosses. No differences were found between reciprocals of the JU crosses, but there were differences between reciprocals of two of the three C57 crosses ($p < 0.05$ and $p < 0.02$). Details of the reciprocals of the C57 crosses are shown in Table 2.

Table 2

Group code	Numbers scored in each category			χ^2	σ	M
	0	1	2 and above			
TCa	10	21	32	$\chi^2_2 = 3.45$ ($p > 0.1$)	1.03	+0.03
TCb	12	32	24		0.76	-0.29
TCC_1a	22	12	13	$\chi^2_2 = 6.19$ ($p < 0.05$)	1.52	-0.89
TCC_1b	17	13	2		0.68	-1.06
TCC_2a	105	61	27	$\chi^2_2 = 8.51$ ($p < 0.02$)	1.02	-1.10
TCC_2b	153	97	16		0.74	-1.15

If the difference between reciprocals were due to sex-linkage, consistent differences of a similar magnitude would be expected between the sexes. As these were not found the difference between reciprocals, if it was a real one, must be attributed to a maternal effect.

The difference between reciprocal F_1 groups was not significant, whereas between reciprocal first and second backcross groups it was. This could be related to the fact that both F_1 groups had parental strain mothers, whereas, in each backcross, one group had a C57 mother and its reciprocal had a hybrid mother.

The three groups of progeny of C57 mothers had lower means than those of Tuck and hybrid mothers. The animals expected to be the more vigorous mothers therefore produced more abnormal offspring. This seems to be at variance with previous findings (Grüneberg, 1951; Searle, 1954b; Deol and Truslove, 1957). However, the present abnormality is an addition to rather than a subtraction from the normal, and as such may only be expressed under optimum conditions.

The three groups of progeny of C57 mothers also had lower variances than their reciprocals. This could be explained in terms of narrower canalisation of the normal phenotype.

The behaviour of the supernumerary cusp therefore appeared to fulfil three criteria of quasi-continuous variation (Grüneberg, 1952). There was a marked variation between F_1 groups when affected Tuck animals were crossed to different strains; increased penetrance was accompanied by increased expressivity; and there were differences between reciprocal crosses. The behaviour of this part of a tooth is therefore analagous to the behaviour of whole third molars.

From the phylogenetic point of view it is perhaps of interest to note that Guthrie (1965), in his study of the molars of microtene rodents, found that the areas which are changing phylogenetically most rapidly are those which vary the most within a population. One of the most morphologically variable areas was found to be the anterior end of the lower first molar.

6. Conclusions

The supernumerary cusp exhibits quasi-continuous variation. No single major gene is responsible for the presence of the cusp. The expression of the cusp may be influenced by maternal physiology.

B. THREE MIMIC GENES WITH MAJOR EFFECTS ON THE COAT AND DENTITION - TABBY, CRINKLED, AND DOWNLESS.

I. Introduction

Two alleles of the sex-linked gene tabby, Ta and Ta^c, and two autosomal mimics of tabby, crinkled, cr (linkage group 14), and downless, dl (linkage group 4), produce very similar effects on the coat and dentition.

The superficial features of homozygotes and tabby hemizygotes include; abnormal texture of the coat, a bald patch behind each ear, a reduced complement of facial vibrissae, and, with the exception of Ta^c homozygotes and hemizygotes, more or less bald tails which are sometimes kinked towards the tip. The tails of Ta^c homozygotes and hemizygotes are generally completely covered with hair and are rarely kinked. All homozygotes and tabby hemizygotes have characteristically reduced molars, and incisors and third molars are sometimes absent.

Crinkled and downless heterozygotes are outwardly normal, though there is sometimes a faint suggestion of the mutant phenotype. Tabby heterozygotes which are phenotypically agouti show varying intensities of transverse striping, and are therefore immediately recognisable. They also show other definite signs of the mutant phenotype to a variable extent. The dentitions of all the heterozygotes may contain normal teeth, frankly mutant teeth, and teeth combining characteristics of both the normal and the mutant phenotypes. All three types of tooth may be present in the same animal. A further feature of the heterozygote dentition is the rare occurrence of an additional molar tooth.

The present investigation is concerned with the development of the dental abnormalities and with the mimicry exhibited by the different genes. An attempt has been made to fit the dental abnormalities into a single pedigree of causes, together with the hair defects, in terms of the principle of 'unity of gene action' (Grüneberg, 1943a).

II. Material

1. The Genes

(i) Crinkled

The first crinkled mouse appeared in the progeny of a male treated with nitrogen mustard. (Auerbach and Falconer, 1949).

The genetics of the crinkled gene and the development of abnormalities produced by it were studied by Falconer, Fraser and King (1951).

Crinkled has been assigned to linkage group 14 (King, 1956).

The teeth of crinkled mice have been described by Grüneberg (1965 and 1966a).

(ii) Tabby

The original tabby allele, here called Ta^F, arose in a strain selected for large size on a low plane of nutrition. The original tabby male was at first thought to be a crinkled mouse, but the new mutation was subsequently proved to be sex-linked (Falconer, 1953). The structure of the tabby coat was indistinguishable from that of crinkled and its development was presumed to be the same.

The teeth of tabby mice have been described by Grüneberg (1965 and 1966a).

Ta^C arose in a line selected for body weight (Mouse News Letter

35. p.24). Ta^{c} resembles another tabby allele of independent origin, Ta^{j} (Mouse News Letter 29, p.40).

Unlike Ta^{F} and $Ta^{F}Ta^{F}$ animals the tails of Ta^{c} and $Ta^{c}Ta^{c}$ mice are not bald. However, the tail hairs are not entirely normal and are more sparsely distributed than in the normal mouse.

Ta^{c} has not previously been studied in any detail.

(iii) Downless

Downless first appeared as an independent autosomal mutation resembling crinkled. Crossing with a crinkled mouse showed that the new mutation was not at the crinkled locus (Mouse News Letter 23, p.30).

Downless has been assigned to linkage group 4 (Mouse News Letter 34, p.32).

Downless homozygotes are indistinguishable from tabby (Ta^{F}) homozygotes and hemizygotes and from crinkled homozygotes.

Downless has not previously been studied in any detail.

Crinkled and both tabby alleles arose and have been maintained at the Institute of Animal Genetics, Edinburgh. Downless animals were obtained from the Radiobiological Research Unit, Harwell.

2. The Coat

Some of the work on the mutant coat, in relation to the mode of action of the genes, is reviewed here.

(i) Homozygotes

The development of crinkled mice was studied by Falconer, Fraser, and King (1951). The widespread abnormalities were considered to be secondary to two observed effects on the epidermis; the suppression of formation of new hair follicles between $12\frac{1}{2}$ and 17 days of gestation, and again from birth onwards; and a reduction of the rate of growth of follicles that did form.

The coat of normal mice is composed of four types of hair; guard hairs, awls, auchenes, and zigzags. The facial vibrissae are divided into two classes according to their distribution; the mystacials, around the snout; and the four secondary groups further posteriorly (supraorbital, postorbital, postoral and inter-ramal).

The coat of crinkled mice was found to contain only one type of hair resembling awls in general appearance. Falconer, Fraser and King studied the development of the coat and of the secondary groups of vibrissae in normal and crinkled mice. The results of this study, together with the appearance of the adult coat, led to the conclusion that, in normal mice, the type of fibre is related to the age at which the follicles are formed. In crinkled mice the first period of follicle suppression accounted for the absence of guard hairs, and the second for the absence of zigzags. The absence of these two types of hair was thought to be responsible for the general appearance of the coat, for the bald patch behind each ear, and for the bald tail. The first period of follicle suppression also accounted for the absence of some of the vibrissae. (Auchenes were not considered as a type distinct from awls).

Dun (1959) studied the development of mystacial as well as secondary vibrissae in normal, tabby, and ragged mice. In normal mice the mystacial vibrissae are initiated in a continuous sequence between 12 and $14\frac{1}{2}$ days. They are barely influenced by tabby, whereas the secondary groups, which start their development after the first and well before the last of the mystacials, are invariably affected. In ragged mice no follicle commencing after $12\frac{1}{2}$ days produced a functional fibre. There was almost perfect agreement between the incidence of vibrissae in the ragged mouse and the follicles of the $12\frac{1}{2}$ day normal foetus. The action of tabby is therefore not as precise or extreme as that of ragged.

Dun suggested a model for the action of the tabby and crinkled genes. In homozygotes and hemizygotes a substance needed for both initiation and growth of follicles is produced at a slower rate than normal. This substance has a threshold level which must be reached for initiation to occur. The supply is depleted by follicle growth and, as it is produced locally in the skin, the earliest developing follicles of a group have an advantage. At 12 days, when the follicles commence growth, this substance has a gradient of concentration, high anteriorly and low posteriorly. By 17 days sufficient of the substance has accumulated for a wave of follicle growth to occur, and new follicles are initiated until 20 days when the supply of substance falls below the critical level.

On the basis of the proportion of the different types of hair in the normal coat, and assuming that the tabby coat is, in fact, composed of awls alone, an expectation for follicle density in tabby animals can be calculated.

Grüneberg (1966b) made direct estimates of follicle density in normal and tabby mice. In the baby coat of the tabby mice hair density was nearly two and a half times that expected on the basis of the 'awls only' hypothesis. In the second hair generation of tabby the density was greater than in the baby coat and was not far below normal.

In normal mice the proportions of the various hair types do not vary significantly between first and second hair generations. It was therefore suggested that the reduced hair density in the baby coat of tabbies is probably due to general retardation, which is nearly completely corrected in the second hair generation. The structure of the coat was attributed to failure of differentiation associated with retardation of growth and reduction of hair calibre, rather than to the blocking of specific hair types.

(ii) Tabby heterozygotes

The coat of tabby heterozygotes has characteristic transverse stripes composed of black hairs which lack the agouti band. Falconer (1953) found that the stripes are most obvious in the baby coat, tending to become narrower and less conspicious with the growth of the second hair generation. A structural effect was also found, as some animals which were phenotypically non-agouti showed faint markings of a similar type. Examination of the hairs of heterozygotes revealed that in the black areas guard hairs were present as usual but zigzags were scarce or absent. Guard hairs and some zigzags have subsequently been found in the black areas by Lyon (1963) and Kindred (1967c). Both Lyon and Kindred also found that the agouti areas between the stripes differed from normal in that zigzags were reduced in number, though not to the same extent as in the stripes.

The black areas are therefore not typically mutant and the intervening agouti areas are not typically normal. Zigzags are the finest hairs in the coat so that if the basic effect of the tabby gene is to reduce hair calibre they are likely to be lost before any of the other hair types. The whole of the coat of tabby heterozygotes therefore shows an intermediate mutant phenotype, though the degree of abnormality is more severe in the stripes.

These findings were considered by Grüneberg (1966b) to be at variance with the Lyon hypothesis. He has suggested that the basis which underlies the striping is not a cell specific mosaicism but the transverse wrinkling of the skin which is present during the growth of the baby coat and again, to a lesser extent, during the growth of the second hair generation.

In a study of the tails of normal, tabby heterozygote, and tabby mice, Grüneberg (1966b) found that the scales of heterozygotes were uniformly intermediate in size between those found in normal and those found in tabby mice. There was no patchiness as might be expected on the basis of the Lyon hypothesis.

(iii) Summary

The general picture is one of a timed effect on the developing coat and secondary vibrissae. The reduction in number of hairs in the baby coat, and their reduction in calibre and lack of differentiation, are all thought to be associated with retarded growth. There is evidence to suggest that cell specific mosaicism does not occur in tabby heterozygotes.

3. The Teeth of Normal and Mutant Mice

(i) The Normal Mouse Dentition

The normal mouse dentition is composed of one continuously growing incisor and three molars of restricted growth in each quadrant. The upper molars are referred to as m^1, m^2, and m^3, and the lower molars as m_1, m_2, and m_3. As in Gaunt (1955) and Grüneberg (1965) the surfaces are referred to as anterior and posterior, buccal and lingual, and occlusal and basal; and the cusps are numbered as below. The crown of m^1 is composed of eight cusps. Numbered from anterior to posterior there are three central cusps, 1, 2, and 3; three buccal, B1, B2, and B3; and two lingual, L1 and L2. All the cusps present in m^1 except cusp 1 are represented in m^2. The crown of m_1 is composed of seven cusps. Numbered from anterior to posterior there are three buccal, B1, B2, and B3; three lingual, L1, L2, and L3; and a single central posterior cusp, 4. All the cusps present in m_1 except L1, and sometimes B1, are represented in m_2. The morphologies of the third molar crowns will not be considered.

Both m^1 and m^2 generally have three roots, anterior and posterior buccal roots and a single lingual root. Both m_1 and m_2 have two roots, one anterior and one posterior. The root form of the third molars will not be considered.

'I' is used to denote incisor.

(ii) The Mutant Mouse Dentition

The teeth of tabby (Ta^F) and crinkled mice have been described by Grüneberg (1965 and 1966a). The teeth of Ta^c and downless animals have not previously been described but have been found to show the same characteristics. The features described below therefore apply to all the genes.

(a) Homozygotes

Incisors may be reduced or absent.

The upper first molar crown is more bulbous and has more erect cusps than normal. Cusps B1 and B3 are regularly absent and L1 and L2 may be reduced. The separation between cusps 1 and L1 and between L1 and L2 is often reduced and sometimes completely absent. There may therefore be a single cusp lingually which may be more or less continuous with cusp 1. The root is usually single, though there may be two or even three roots. (No three rooted teeth were reported by Grüneberg but some were found in the present study).

The upper second molar crown is also more bulbous than normal, and B3 is regularly absent. B1 may be normal or increased in size. There is always a ridge of variable height running transversely across the anterior end of the crown connecting B1 and L1. Grüneberg has called this a 'rampart'. L1 and L2 may be reduced. All of the teeth examined by Grüneberg had a single root, but there were some in the present study which had two and more rarely three roots.

The lower first molar is reduced anteriorly and posteriorly. Cusps L1 and 4 are regularly absent, and B1 is often absent. The separation between cusps B3 and L3, and between B2 and L2, may be reduced or absent. The tooth usually has two roots, but may only have one. In some cases $m_1 < m_2$.

The lower second molar is also reduced anteriorly and posteriorly. Cusps B1 and 4 are regularly absent, and the separation between cusps B3 and L3, and between B2 and L2, may be reduced or absent. There may be one or two roots.

Upper and lower third molars are often absent.

(b) Heterozygotes

The mixed features of the heterozygote dentition, and the rare occurrence of an additional molar tooth, have already been mentioned. Grüneberg has called the latter phenomenon 'twinning', and has described three categories. In cases of overt twinning there are four molars in a row. The normal first molar is represented by two teeth and the anterior twin tends to be the smaller of the two. Incomplete twinning is recognisable by the presence of extra cusps and roots which may be accompanied by anteroposterior elongation and pinching in of the sides of the first molar crown. In one case described the twins had separate crowns, but there was one root that was common to both. Concealed twinning occurs when the two twin teeth are completely separate and when the third molar is absent. There are therefore only three teeth in the row, but the first two can usually be diagnosed as twins with reasonable certainty on the basis of the appearance of twins in the overt cases.

(iii) Additional situations

The material examined in this study included examples of additional situations not previously described. Grüneberg (1966a) suspected that twinning may take place in homozygotes. One example of overt twinning and two of incomplete twinning have been found in the lower molars of homozygotes.

In addition, one example of overt twinning and two of incomplete twinning have been found in the upper incisors of homozygotes. Further reference to these cases will be made later.

It is perhaps of interest to note here that the occurrence of supernumerary lower incisors, dependent on a number of genes, has been reported by Danforth (1958); and that the development of both upper and lower supernumerary incisors has been induced by hypervitaminosis A (Kalter and Warkany, 1961).

III. The Development of the Teeth of Tabby (Ta^F) Mice

1. Introduction

The normal development of mouse molars has been studied by Gaunt (1955 and subsequently), Cohn (1957) and Hay (1961); and the normal development of incisors by Hinrichsen (1959) and Hay (1961).

Grüneberg (1963) listed a number of cases of abnormal tooth development caused by single mutant genes. These were all secondary to some failure of normal connective tissue development. In the present case the primary effect appears to act on the dental epithelium itself.

The embryology of absent third molars in CBA mice and in mice homozygous for the crooked tail gene has been investigated by Grewal (1962). As third molars and also incisors are absent in tabby, crinkled and downless mice it was thought of interest to see whether the mechanisms involved are the same. Of greater interest, however, is the problem of the nature of twinning and how this phenomenon is related to the complete range of dental abnormalities. It was with these aims in view that the following investigation was carried out.

2. Material and Method

The A strain background has been found to favour the expression of molar abnormalities in heterozygotes as well as incisor abnormalities in homozygotes. Accordingly, material for sectioning was obtained as follows. A strain males mated to A strain females provided a control group of litters. A strain males mated to homozygous tabby females provided litters of mixed heterozygotes and tabby hemizygotes. The majority of homozygous tabby mothers were from stock, but a few were the result of one or two crosses to the A strain. It was originally intended to use these latter animals exclusively, but poor fertility made this impossible. The majority of litters examined were therefore heterozygous for the A strain background, but a few were to some extent homozygous. There were no obvious differences between these two types of litter.

Animals were caged one male to a maximum of three females. No suckling females were used. Matings were examined for births and females were examined for vaginal plugs between 9 and 10 a.m. Material was collected between 10 a.m. and mid-day. The day on which a plug was found was regarded as day zero. Litters were collected at two-day intervals from day 13 to day 29. Eight post-partum litters were used for which plug dates were not known. Birth was then used as the criterion of age and was taken to be at twenty days. (Of the 25 post-partum litters collected for which plug dates were known, one was born on day 18, 12 on day 19, 11 on day 20, and one on day 21.) Prospective mothers of post-partum litters were removed to separate cages before birth took place. The ages of litters collected before birth were checked by examination of the external features of all animals in the A strain litters, but of heterozygotes only in the mixed litters (Grüneberg, 1943b).

Tabby hemizygotes and heterozygotes of 13 day litters were separated on the basis of presence or absence of the postorbital tubercles. These are the first signs of the developing postorbital vibrissae which are very nearly always absent in hemizygotes and present in heterozygotes. There was no difficulty in separating the two types at this stage. For classification of older individuals additional criteria were adopted; the different degree of eruption of body hairs, the number of supraorbital vibrissae, and, in post-partum litters, the sex of the individual. Although a postorbital fibre is rarely present in tabby hemizygotes at birth, Dun (1959) found that at five days after birth there is invariably a small, slow growing, atypical fibre at this site. Such fibres are lost in the hair of the fully grown coat. Fibres of this type were found in the present material but were easily distinguishable from those of heterozygotes. The additional use of other criteria at this stage made the possibility of misclassification very remote.

All individuals were classified prior to fixation after examination under a dissecting microscope. The 13 and 15 day embryos were fixed whole. 17 day embryos were decapitated and the heads only were fixed. The classification of these embryos was checked again after fixation and prior to further processing. Individuals of 19 days and older were decapitated and the heads were skinned prior to fixation. Classification of these animals could therefore not be checked subsequently. There were very few cases where classification was in doubt. These were mainly instances where a postorbital fibre was present on one side but not on the other. Animals of this type were rejected. Examination of the prepared material, in the light of what is known to occur in adult animals, provided no evidence to suggest that any misclassification had been made.

All litters were fixed in Bouin's fluid. Litters of 19 days and older were decalcified in 5% nitric acid. All material was embedded in paraffin wax, serially sectioned at 10 microns in the sagittal plane, and stained with haematoxylin and eosin. A total of 127 animals from 65 litters were prepared and examined. The numbers of each genotype sectioned at each stage are shown in Table 3.

Table 3. (For explanation see text)

Genotype	No. of animals followed in brackets by no. of litters from which taken		
	A Strain	Ta^F+	Ta^F
Stage			
13 Days	5(3)	5(2)	5(2)
15 Days	5(3)	5(4)	5(3)
17 Days	5(3)	9(3)	5(3)
19 Days	5(2)	7(3)	5(3)
21 Days	5(3)	9(3)	5(3)
23 Days	2(2)	5(3)	4(2)
25 Days	2(1)	2(1)	5(3)
27 Days	3(1)	5(3)	4(2)
29 Days	0	5(2)	5(2)

3. Results

The findings are considered in four sections:

(i) Incisors

(ii) Lower first and second molars

(iii) Upper first and second molars

(iv) Third molars

In each section a summary of the findings is followed by photographs and brief descriptions.

The magnification of the photographs is as follows:

X65 Numbers 1 - 95 (excluding 1, 23, 31 and 75), and numbers 116 - 119.

X195 Numbers 1, 23, 31, and 75, and numbers 96 - 115

Unless otherwise stated the left of each illustration is anterior and the right posterior.

Where control animals, heterozygotes and tabby hemizygotes are being compared, comparable planes of section were selected as far as this was possible.

Occasional reference is made here to the findings in the fully formed dentitions of the four week old animals examined. These data are discussed fully in subsequent sections.

Abbreviations used in the illustrations are as follows:

LL	labial lamina
DL	dental lamina
IE	internal enamel epithelium
EE	external enamel epithelium
O	odontoblasts
D	dentine
PA	pre-ameloblasts
E	enamel
S	supernumerary tooth germ

(i) Incisors

Heterozygotes showed no differences from the controls and are therefore not considered here.

At 13 days there was no definite difference between the tooth rudiments of Ta and control animals except that, on average, the Ta rudiments were probably a little smaller. At 15 days a definite difference was apparent. The Ta tooth germs were obviously smaller than the controls and were barely invaginated. At 17 days and subsequently some of the lower incisor germs showed signs of degeneration. The relatively small size of the Ta germs that did develop was maintained at all stages examined, and was associated with delayed histodifferentiation. Developing Ta incisors generally appeared to be abnormal in shape. Examination of serial sections suggested that this may have been partly due to abnormal angulation. There was no sign of twinning at any stage.

There was a striking difference in intensity of abnormality between upper and lower jaws. This is consistent with what has been found in fully formed dentitions. All the upper incisor germs examined looked as if they would have formed teeth. By contrast, there was a wide range of expression in the lowers varying from near normality to degeneration. From 17 days two, and from 19 days three distinct categories of lower incisor germ were discernible;

(a) More or less well differentiated, though variable in size and shape.

(b) Poorly differentiated. The internal enamel epithelium was undergoing degeneration and no enamel was formed. Odontoblasts were abnormal but some dentine was formed. (It is generally accepted that the internal enamel epithelium induces the underlying mesenchyme to form odontoblasts. Induction must then occur before degenerative changes in the epithelium take place).

(c) Undifferentiated. The epithelium of the tooth germ was undergoing degeneration, though growth did not appear to cease completely in every case judging by the size of some of the examples at later stages.

Table 4 shows the frequency of these categories of lower incisor germ at different stages.

Table 4 (For explanation see previous page)

	(a)	(b)	(c)
17 days	5	0	3
19 days	5	1	4
21 days	0	0	10
23 days	2	1	5
25 days	5	2	3
27 days	3	1	4
Total	20	5	29

It is possible that some 17 day germs in category (c) may have subsequently developed sufficiently to justify inclusion in category (b).

It can therefore be concluded that, in Ta animals, growth and histodifferentiation of developing incisor germs may be retarded; that in more severely affected cases, found only in the lower jaw, the internal enamel epithelium is the first tissue to suffer degeneration; and that, in the most severely affected cases there is complete lack of histodifferentiation, and epithelial growth very nearly, if not completely, ceases.

Lower Incisors - Illustrations

1. A strain. 13 days. Early labial lamina and dental lamina. Ta animals at this stage showed the same features.

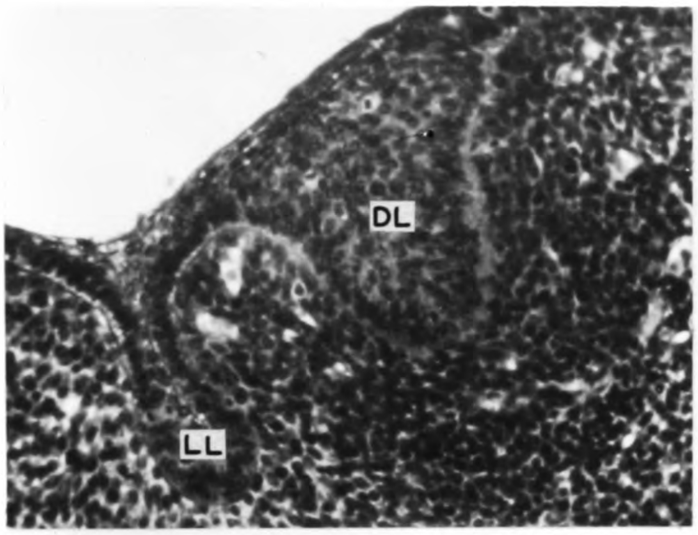
2. A strain. 15 days. Further downgrowth of labial lamina. Tooth germ in early bell stage with differentiation of internal and external enamel epithelia.

3. Ta. 15 days. Representative example. Downgrowth of labial lamina comparable with control (2). Almost no invagination of the tooth germ (I) has taken place.

4. A strain. 17 days. Further growth of labial lamina. Well defined bell. External enamel epithelium becoming reduced. Internal enamel epithelium becoming well differentiated. Odontoblasts developing.

5. Ta. 17 days. The most normal example at this stage. Downgrowth of labial lamina comparable with the control. Bell small and of a different shape to that of the control (4). Internal enamel epithelium becoming fairly well differentiated. Odontoblasts developing.

6. Ta. 17 days. The least normal example at this stage. Downgrowth of labial lamina comparable with the control (4). Degeneration of epithelium of the tooth germ (indicated by arrow).



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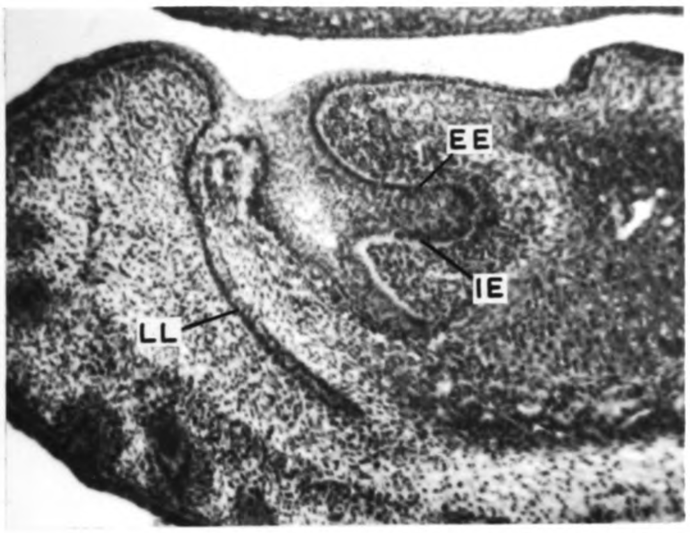

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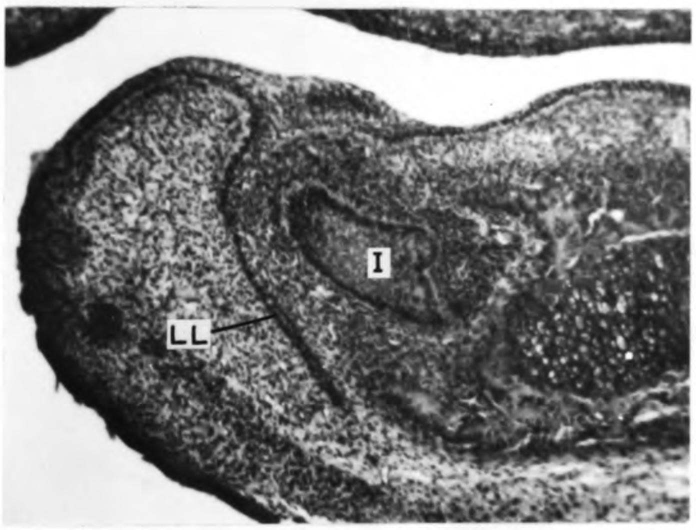

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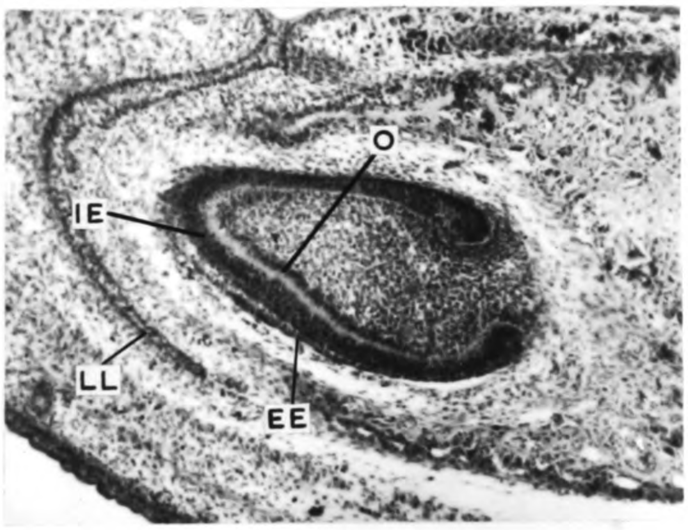

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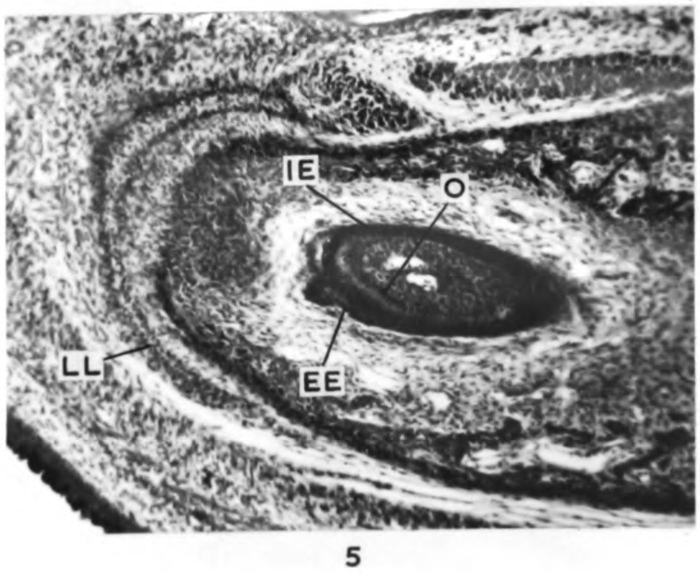

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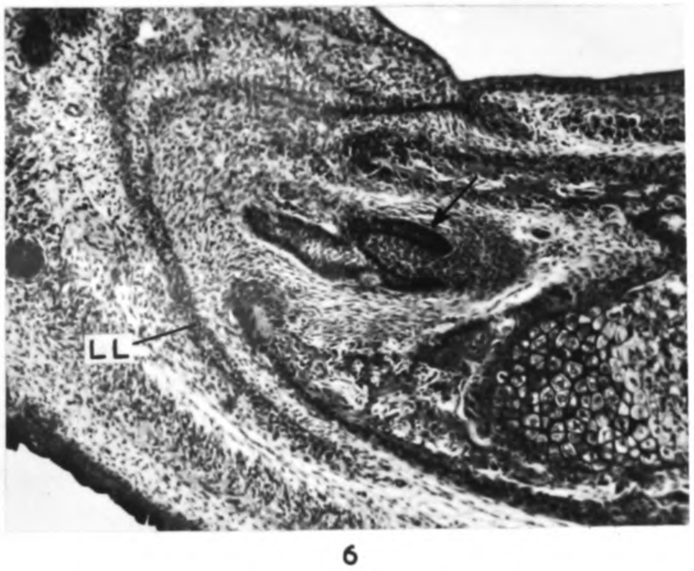

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7. A strain. 19 days. Dentine formation has begun. Pre-ameloblasts well differentiated.

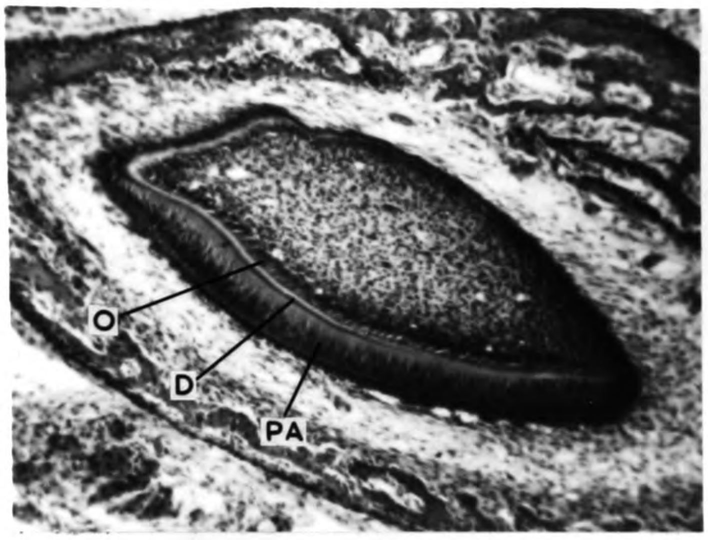
8. Ta. 19 days. The most normal example at this stage. Tooth germ smaller and of a different shape to that of the control (7). Odontoblasts and pre-ameloblasts less well differentiated.

9. Ta. 19 days. The least normal differentiated example at this stage. Tooth germ very small and narrow. Odontoblasts and pre-ameloblasts less well differentiated than in the control (7).

10. Ta. 19 days. Poorly differentiated example. Internal enamel epithelium degenerating. Odontoblasts abnormal, but some dentine has been laid down.

11. The same example as in 10. Further buccally to show the considerable extent of epithelial downgrowth (indicated by arrow) in spite of the abnormal structure of the tooth germ.

12. Ta. 19 days. The least normal example at this stage. Degeneration of epithelium of the tooth germ (indicated by arrow).



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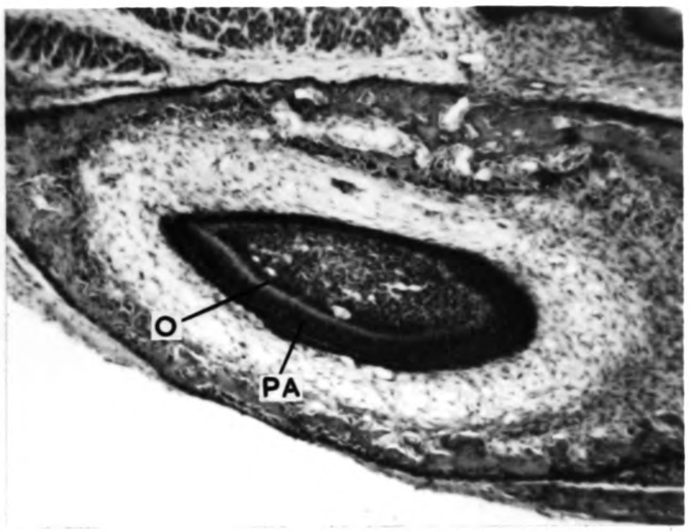

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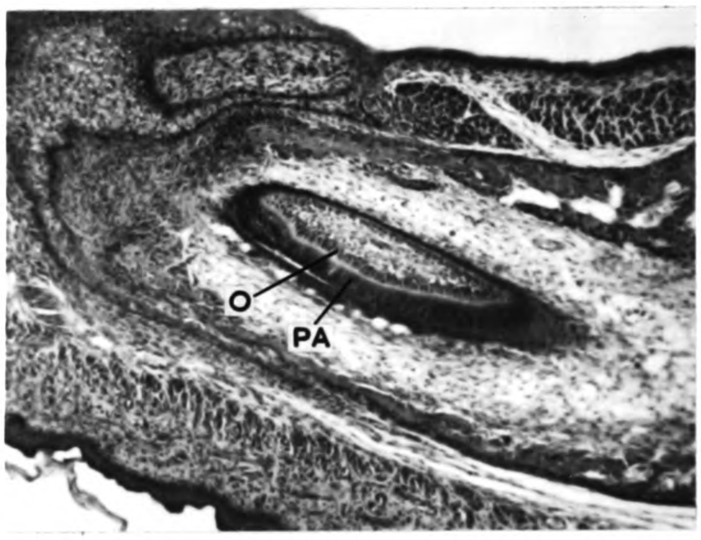

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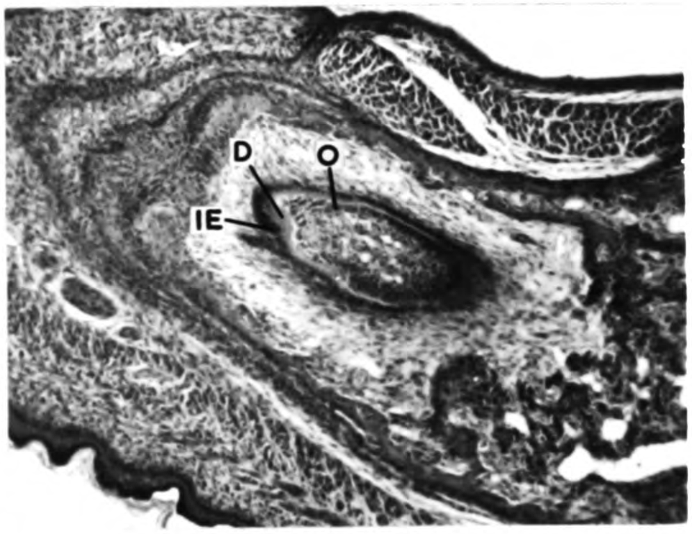

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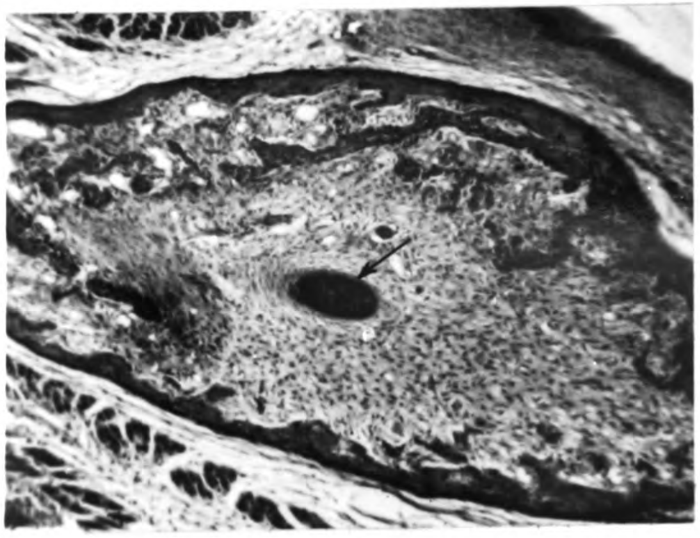

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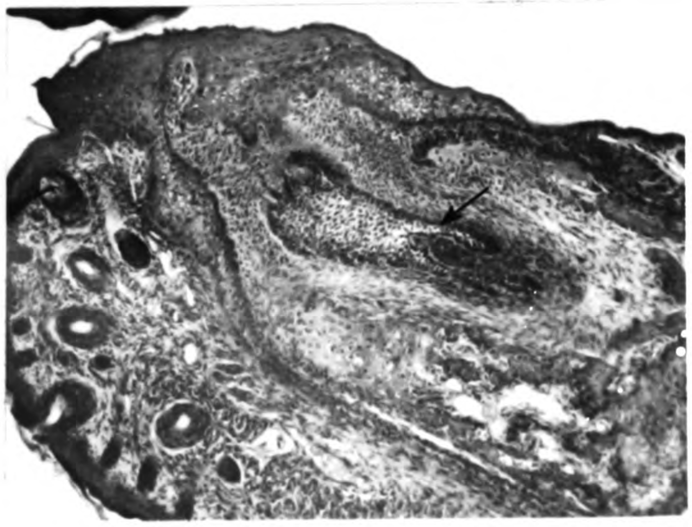

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13. A strain. 21 days. Dentine formation advanced.

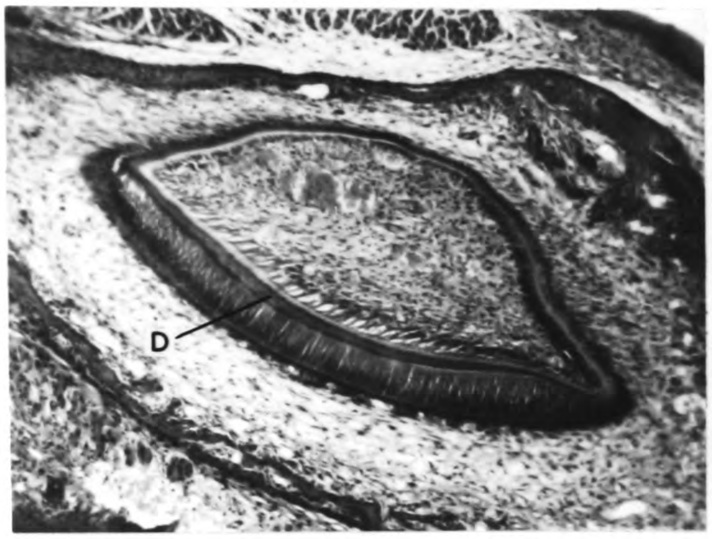
14. Ta. 21 days. The most normal example at this stage. Degeneration of epithelial remnants of the tooth germ (indicated by arrow).

15. A strain. 23 days. Enamel formation has started.

16. Ta. 23 days. The most normal example at this stage. Shape of developing tooth is different from that of control (15). Enamel formation has started.

17. Ta. 23 days. Poorly differentiated example. Internal enamel epithelium degenerating. Abnormal odontoblasts, but some dentine has formed.

18. Ta. 23 days. The least normal example at this stage. Further degeneration of epithelial remnants of the tooth germ (indicated by arrow).



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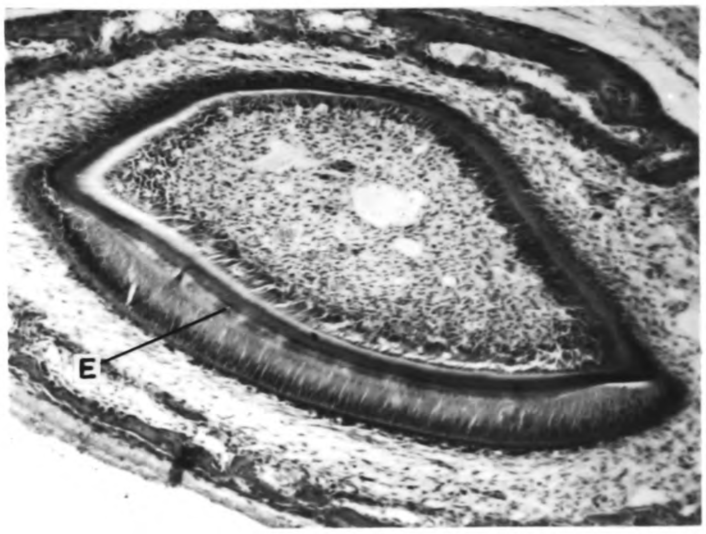

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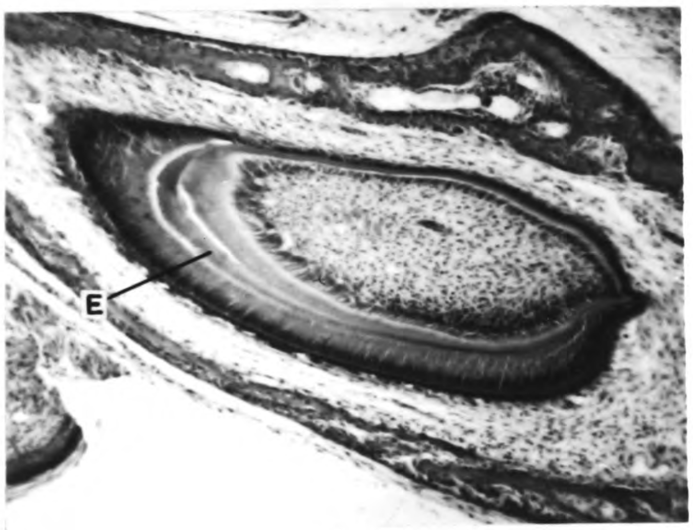

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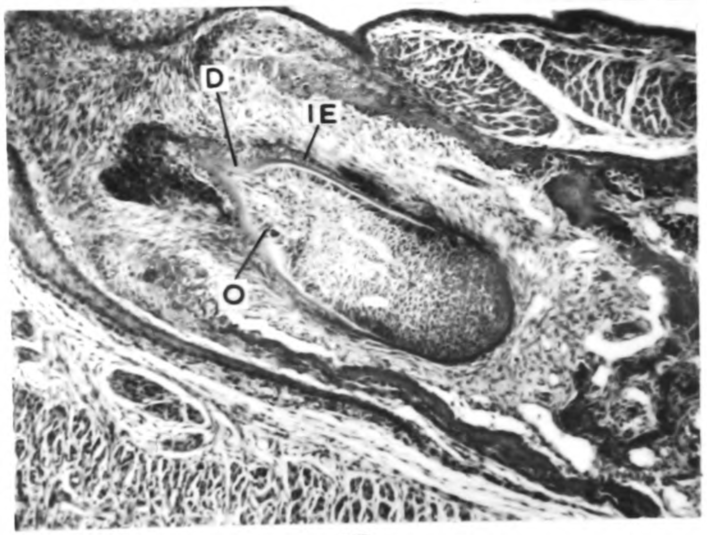

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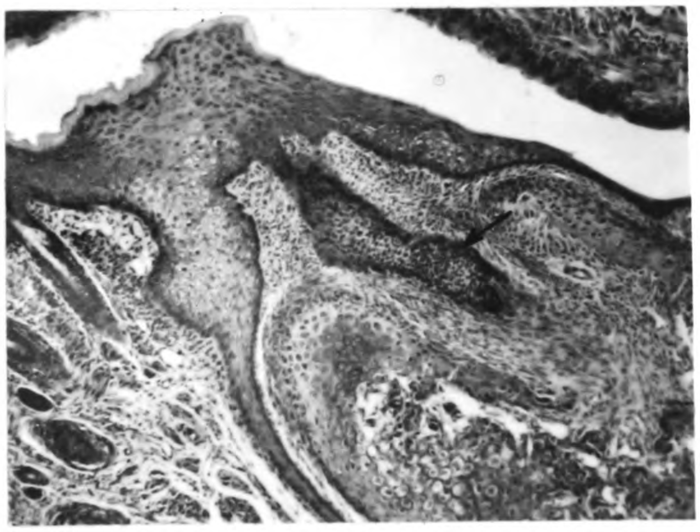

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19. Ta. 25 days. Poorly differentiated example. Very little trace of internal enamel epithelium except for basally. Abnormal odontoblasts, but some dentine has been formed.

20. Ta. 25 days. The least normal example at this stage. Degenerating epithelial remnants of the tooth germ still present (indicated by arrow).

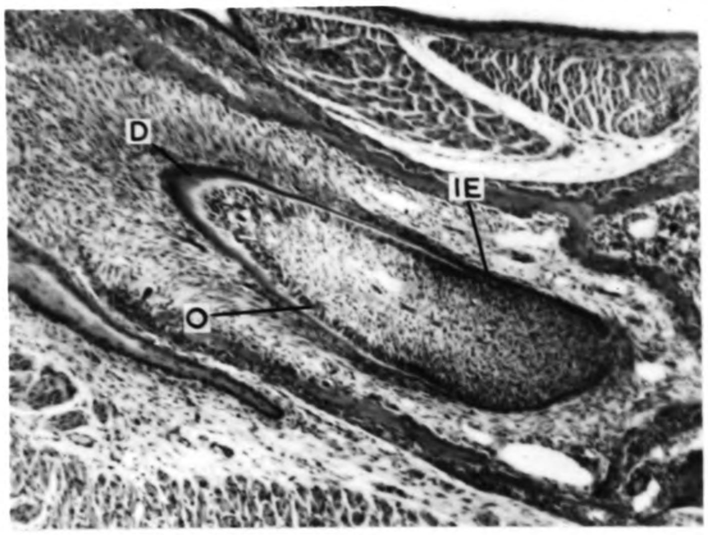
21. Ta. 27 days. Poorly differentiated example. Barely any trace of internal enamel epithelium except for basally. Abnormal odontoblasts, but some dentine has been formed.

22. Ta. 27 days. The least normal example at this stage. Degenerating epithelial remnants of the tooth germ still present (indicated by arrow).

Upper Incisors - Illustrations

23. A strain. 13 days. Dental lamina. Ta animals at this stage showed the same features.

24. A strain. 15 days. Tooth germ in early bell stage with differentiation of internal and external enamel epithelia.



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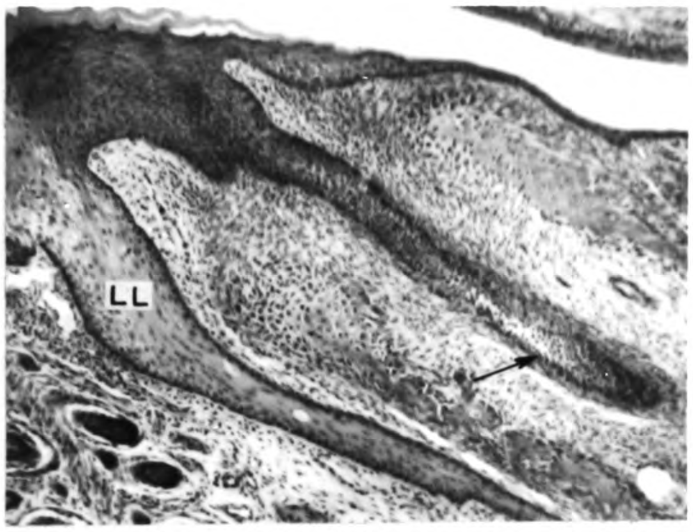

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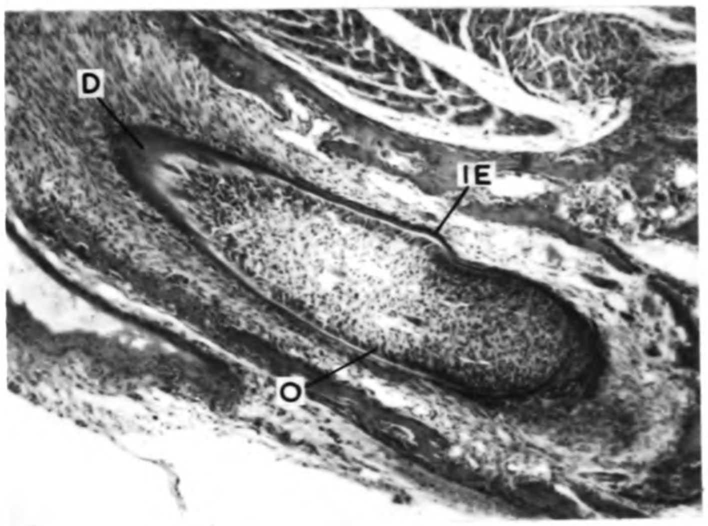

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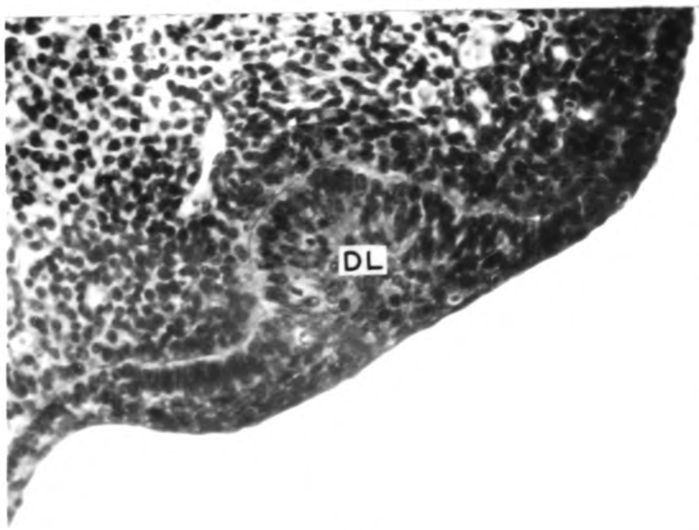

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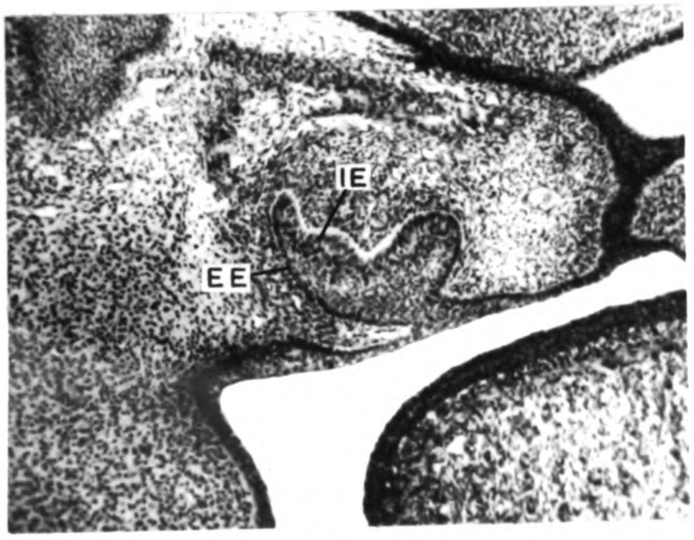

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25. Ta. 15 days. Representative example. Bell smaller and not as well invaginated as that of the control (24).
Internal and external enamel epithelia becoming differentiated.

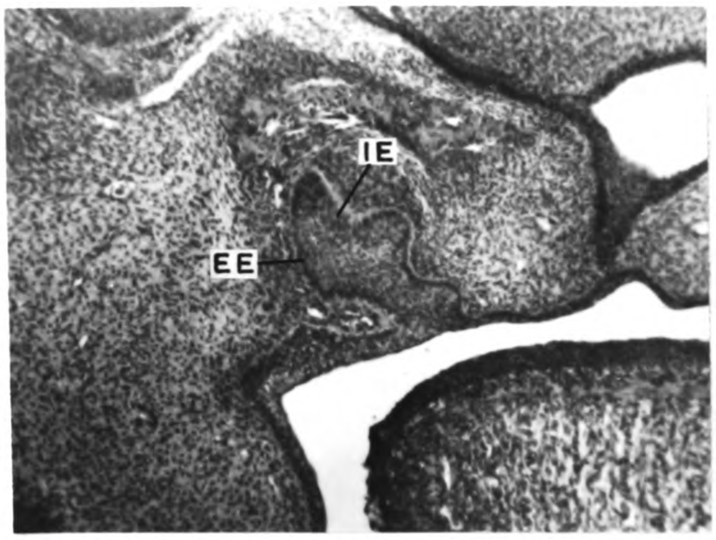
26. A strain. 17 days. Well defined bell. External enamel epithelium becoming reduced. Internal enamel epithelium becoming well differentiated. Odontoblasts developing.

27. Ta. 17 days. Representative example. Bell much smaller and less advanced than that of the control (26). External enamel epithelium still well defined. Odontoblast layer just appearing.

28. A strain. 19 days. Dentine formation has begun. Pre-ameloblasts well differentiated.

29. Ta. 19 days. The most normal example at this stage.
The developing tooth is different in shape to the control (28) but almost equally advanced.

30. Ta. 19 days. The least normal example at this stage. A very small tooth, well differentiated for its size, but not as far advanced as the control (28).



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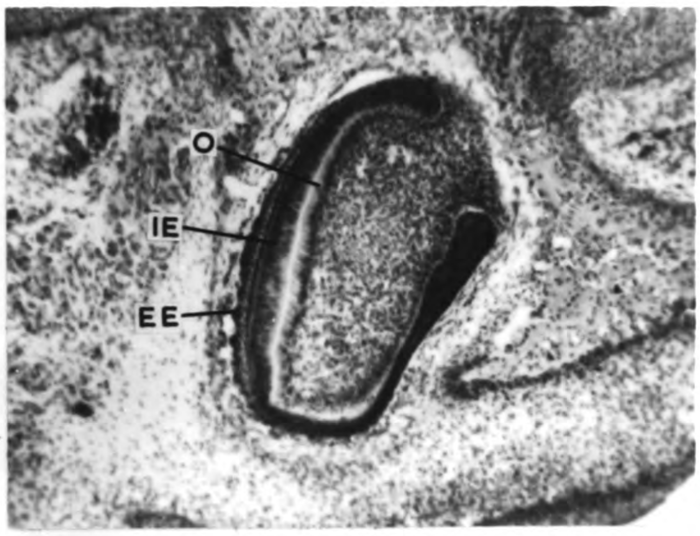

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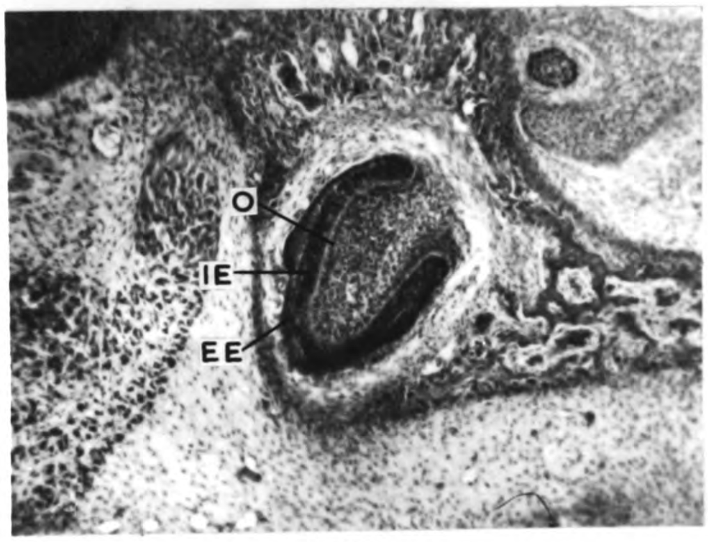

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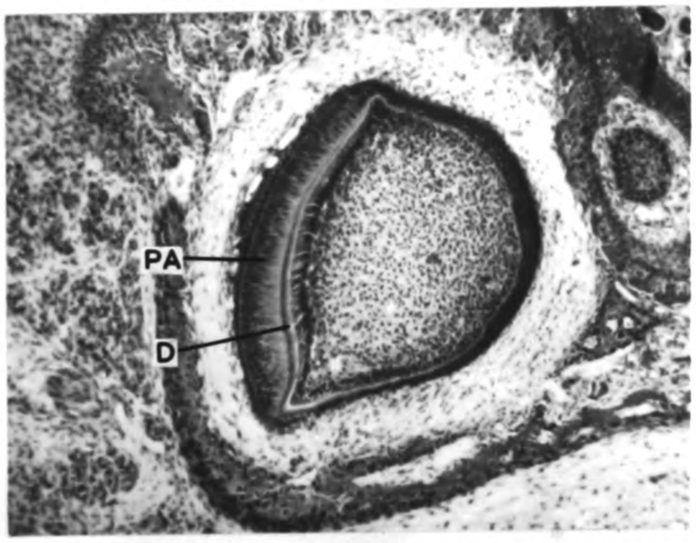

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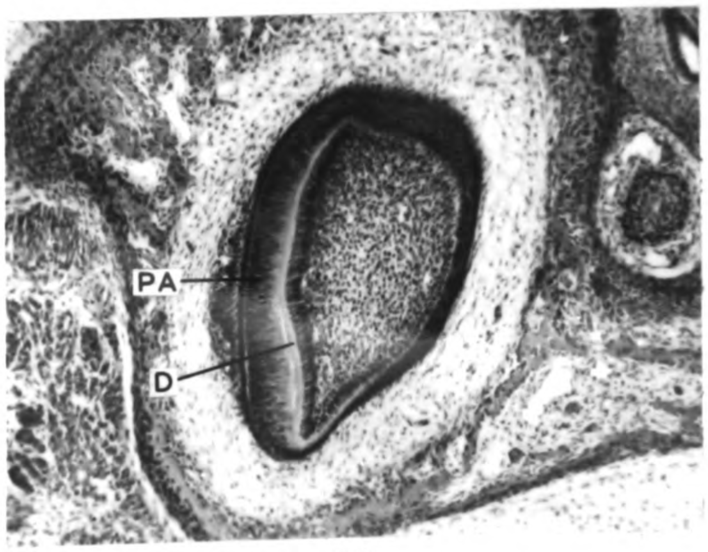

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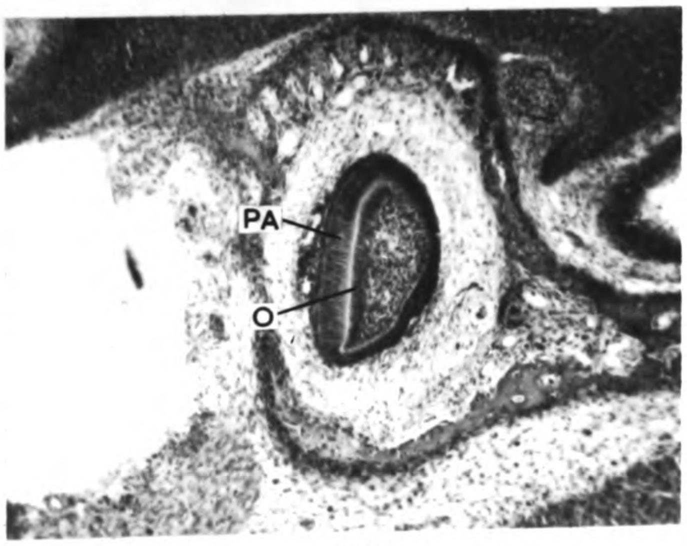

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(ii) Lower First and Second Molars

At 13 days there was no detectable difference between the tooth rudiments of Ta, Ta+, and control animals. At 15 days differences became apparent. At this and subsequent stages Ta tooth germs were generally smaller than the controls and of a different shape. Small size was sometimes associated with delayed histodifferentiation. Similar abnormalities, but of a lesser degree, were present in some Ta+ animals, although the majority were more or less normal in this respect.

There were examples of interaction between the developing first and second molars. Poor development of m_1 was sometimes associated with an enlarged m_2 in which differentiation was sometimes more advanced than in the control m_2. However, m_2 never appeared to be as advanced as m_1.

At no stage was there any evidence of division of the first molar germ in either Ta or Ta+ animals.

A feature of the control animals was a small extension of the dental lamina anteriorly from the point of origin of the first molar germ. In a few Ta+ and fewer Ta animals there was proliferation of the epithelium of this extension of lamina to form a finger-like downgrowth anterior to the developing m_1. In some of these cases a supernumerary tooth germ was formed, and in others the epithelial downgrowth appeared to regress. There was evidence of interaction between the supernumerary germ and m_1 and m_2, as was observed between m_1 and m_2 in cases where there was no supernumerary. The presence of a supernumerary, or even a degenerating epithelial downgrowth, was associated with a small m_1 and a small m_2.

Whether the most anterior germ was a first molar or a supernumerary was decided after comparison of all the molar tooth germs on that side, of the affected side with the opposite side (which was generally more normal), and of the affected animal with other animals at the same stage. It was considered that a supernumerary germ could never be larger or more advanced than m_1, although, after 19 days, histodifferentiation in these two teeth appeared to be about equally advanced. It was also considered that m_1 would always be in a more advanced state of differentiation than m_2. In spite of the difference in differentiation between m_1 and m_2 they were sometimes observed to be of almost equal size, and in one case (73 and 74) the tooth taken to be m_1 (on the basis of its developmental state) was smaller than m_2.

Table 5 shows the total numbers of developing Ta+ and Ta first molars examined at different stages (N), the numbers of cases where proliferation of the anterior lamina was observed (P), and the numbers of cases where a supernumerary tooth germ was formed (S).

Table 5

	Ta+			Ta		
Stage	N	P	S	N	P	S
15 days	10	2	0	10	0	0
17 days	17	3	0	9	1	0
19 days	14	1	2	10	1	1
21 days	18	1	0	10	0	0
23 days	10	0	0	8	0	1
25 days	4	0	1	10	0	0
Total	73	7	3	57	2	2

(For explanation see previous page)

Lower First and Second Molars - Illustrations

31. A strain. 13 days. Dental lamina. Ta+ and Ta animals at this stage showed the same features.

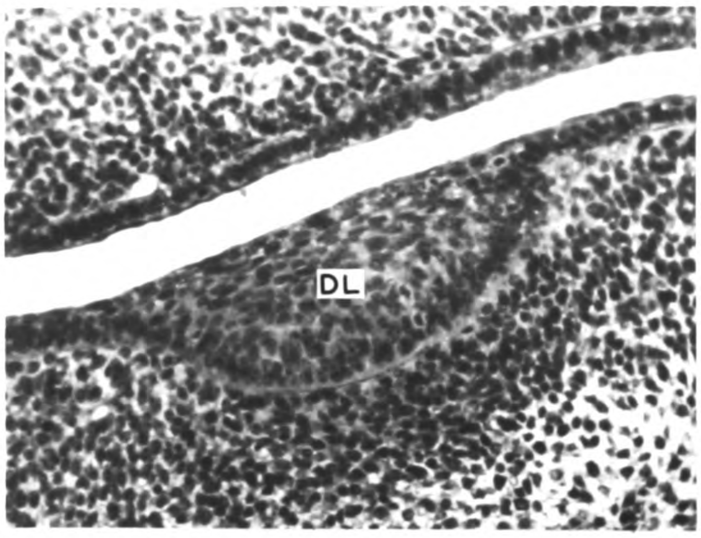
32. A strain. 15 days. m_1 in early bell stage. Differentiation of internal and external enamel epithelia.

33. Ta+. 15 days. m_1 in early bell stage. Extra tongue of dental lamina anteriorly (indicated by arrow).

34. Ta. 15 days. Representative example. m_1 in early bell stage. Bell smaller and more bulbous than in the control (32).

35. A strain. 17 days. m_1 showing early morphodifferentiation and development of odontoblasts. m_2 in early bell stage.

36. A strain. 17 days. m_1 sectioned further lingually to show the anterior extension of dental lamina (indicated by arrow).



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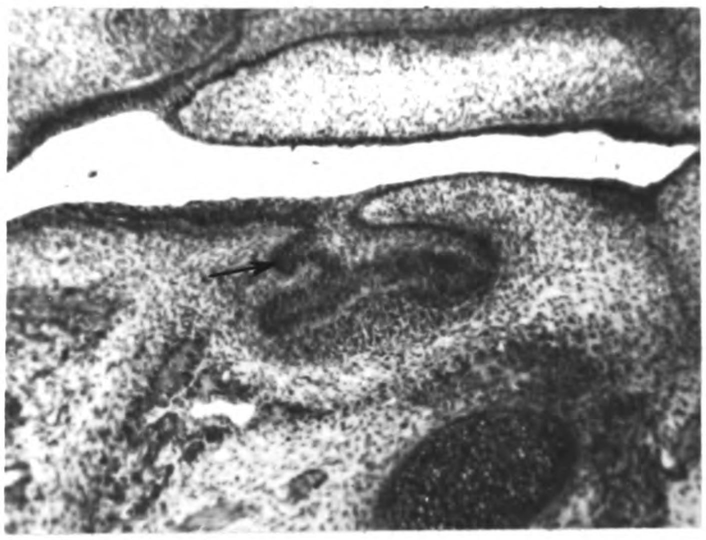

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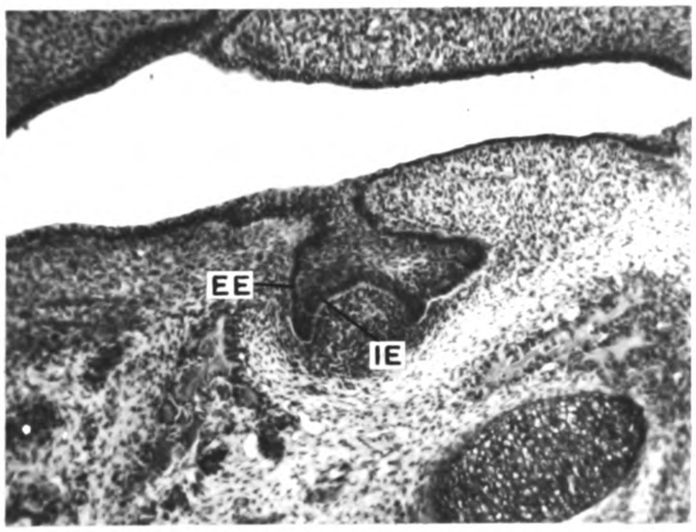

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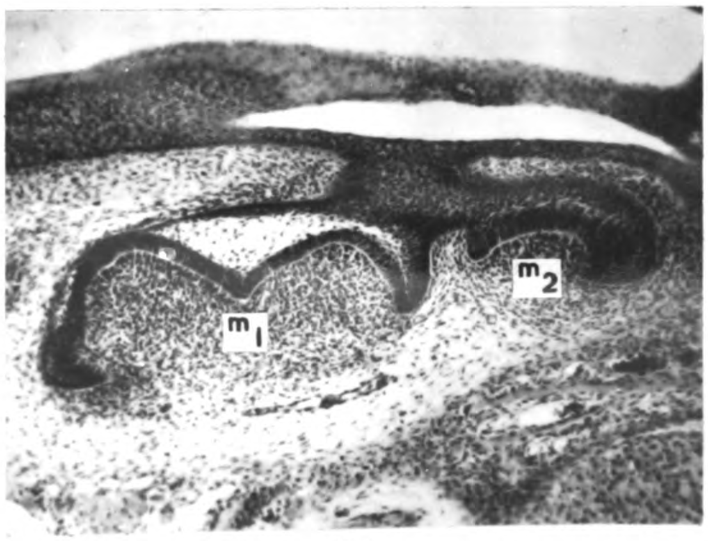

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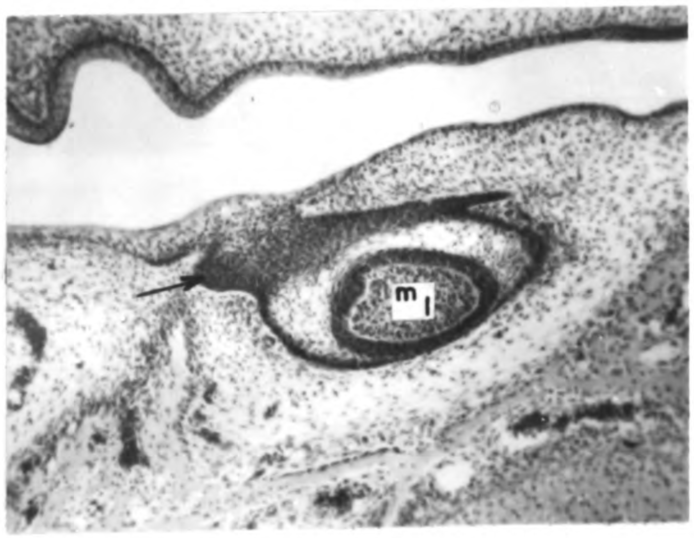

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37. Ta+. 17 days. m_1 sectioned lingually. Large bud of dental lamina anteriorly (indicated by arrow). Compare with control (36).

38. The same example as in 37. Sectioned further buccally to show m_1 with early development of odontoblasts and m_2 at the cap stage. m_1 and m_2 are smaller than in the control (35).

39. Ta. 17 days. The most normal example at this stage. m_1 showing early development of odontoblasts, but the bell is smaller and more bulbous than in the control (35).

40. The same example as in 39. m_1 sectioned further lingually. Minimal extension of dental lamina anteriorly (indicated by arrow). Compare with control (36).

41. The same example as in 39. Sectioned further buccally to show the maximum diameter of m_2. m_2 similar in size to the control m_2 (35).

42. Ta. 17 days. m_1 sectioned lingually. m_1 smaller than the control m_1 (36). Anterior bud of dental lamina showing some invagination (indicated by arrow).

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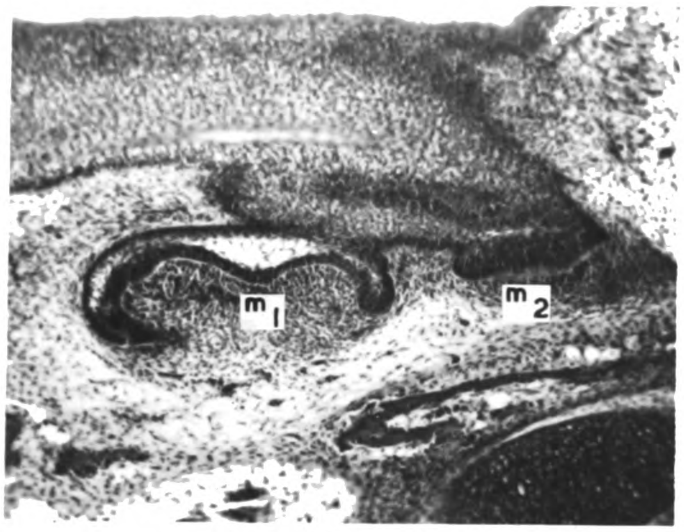

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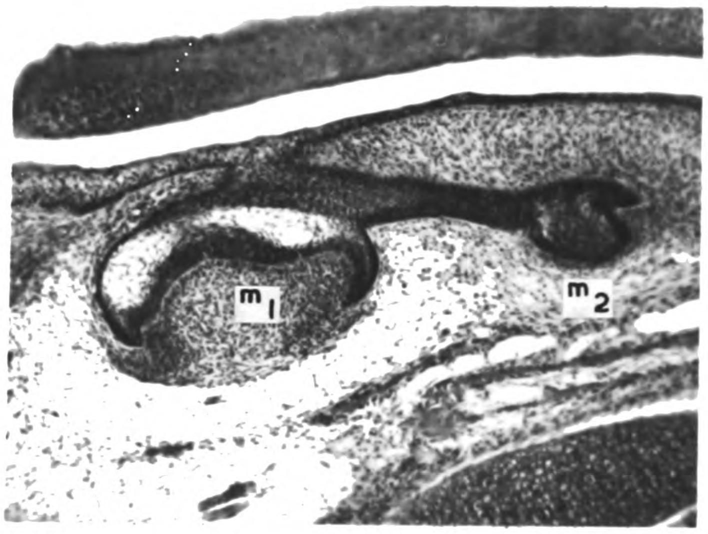

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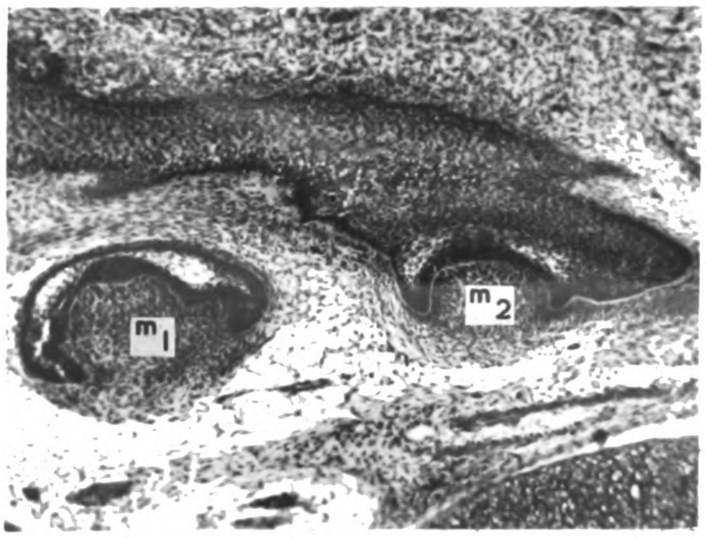

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43. The same example as in 42. Sectioned further buccally to show m_1 and the maximum diameter of m_2. m_2 much smaller than the control m_2 (35) and still in the cap stage.

44. Ta. 17 days. The maximum diameter of the smallest m_1 found at this stage. Very much smaller than the control (35) but odontoblasts developing.

45. The same example as in 44. Sectioned further buccally to show the maximum diameter of m_2, comparable in size with the control m_2 (35).

46. A strain. 19 days. The maximum diameter of m_1. Morphodifferentiation well advanced.

47. The same example as in 46. Sectioned further buccally and posteriorly to show the maximum diameter of m_2.

48. A strain. 19 days. m_1 sectioned lingually to show the anterior extension of dental lamina at this stage (indicated by arrow).

43

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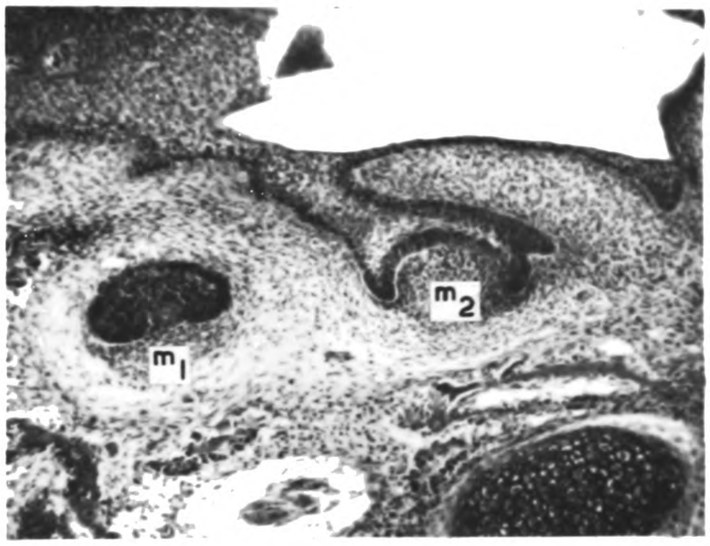

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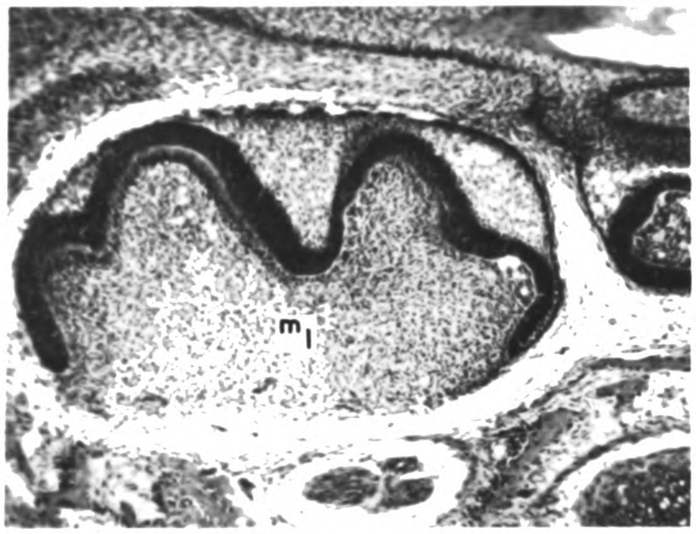

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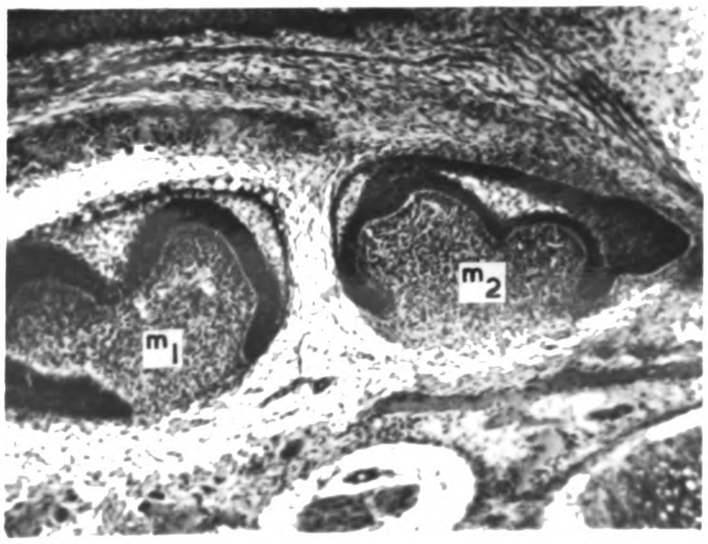

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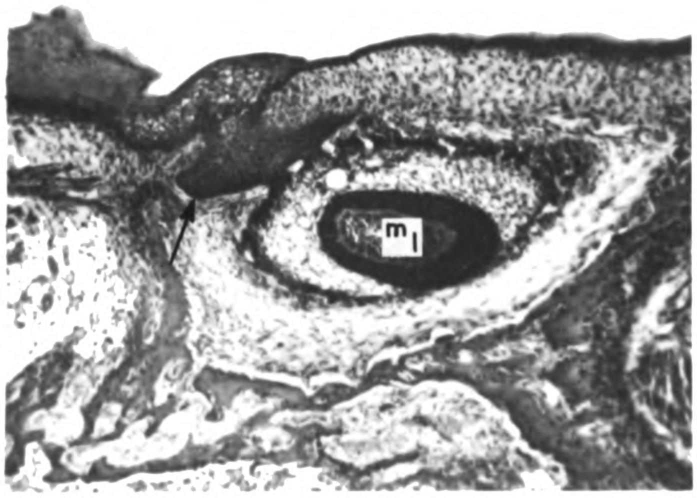

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49. Ta+. 19 days. m_1 sectioned lingually. Small supernumerary germ with laminal connections.

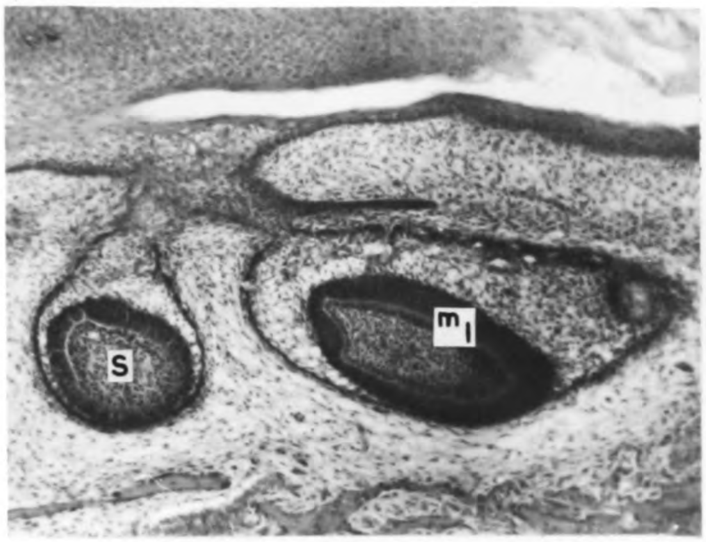
50. The same example as in 49. Sectioned further buccally and posteriorly to show the maximum diameter of m_1 and m_2. m_1 smaller and less well differentiated than the control (46).

51. The same animal as in 49 but the opposite side. Considerable epithelial downgrowth anteriorly (indicated by arrow). Comparison with the opposite side suggests that this was an unsuccessful attempt to produce a supernumerary germ.

52. Ta. 19 days. The most normal example at this stage. m_1 much smaller and less well differentiated than the control (46).

53. Ta. 19 days. The maximum diameter of an anterior supernumerary tooth germ. m_1 sectioned lingually. Odontoblast development of the supernumerary is comparable with that shown by the maximum diameter of m_1 (54).

54. The same example as in 53. Sectioned further buccally and posteriorly to show the maximum diameter of m_1 and m_2. m_1 and m_2 smaller than m_1 and m_2 of the most normal Ta example (52). m_2 is still at the cap stage but is comparable with the m_2 of another Ta example where there was an attempt to form a supernumerary (56).



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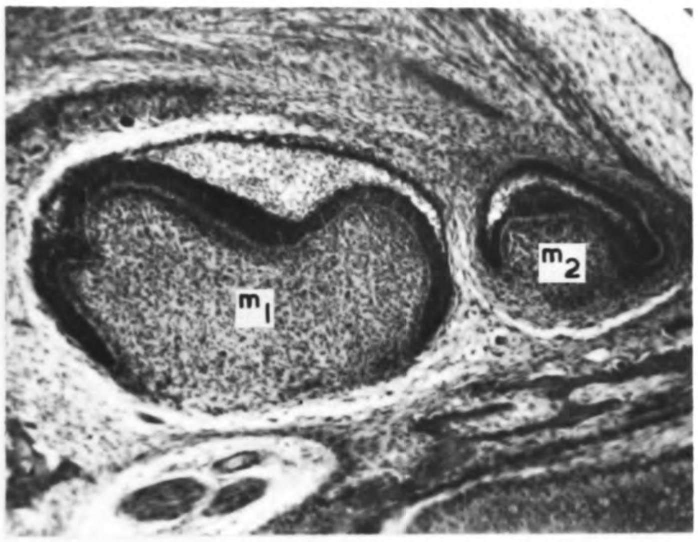

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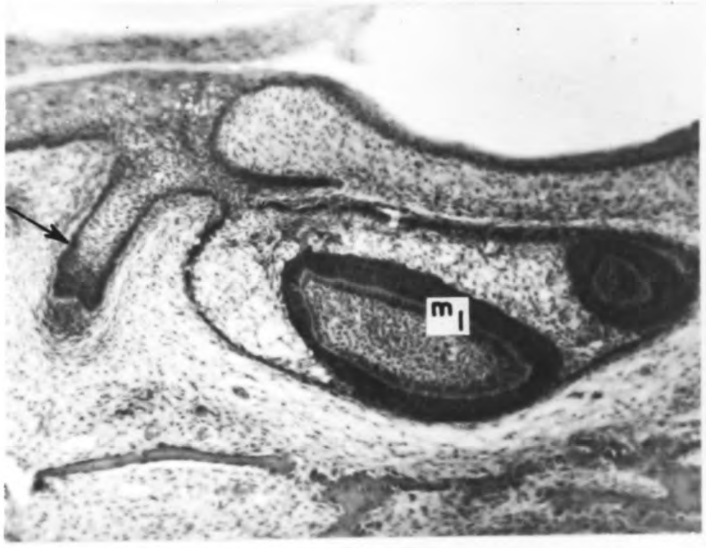

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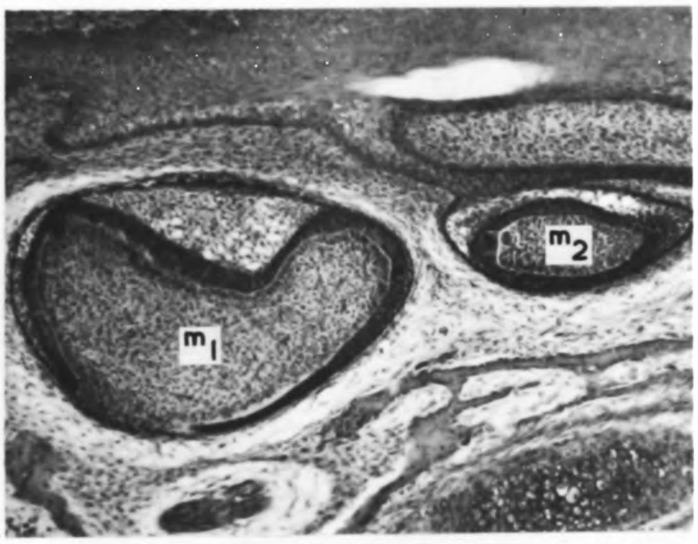

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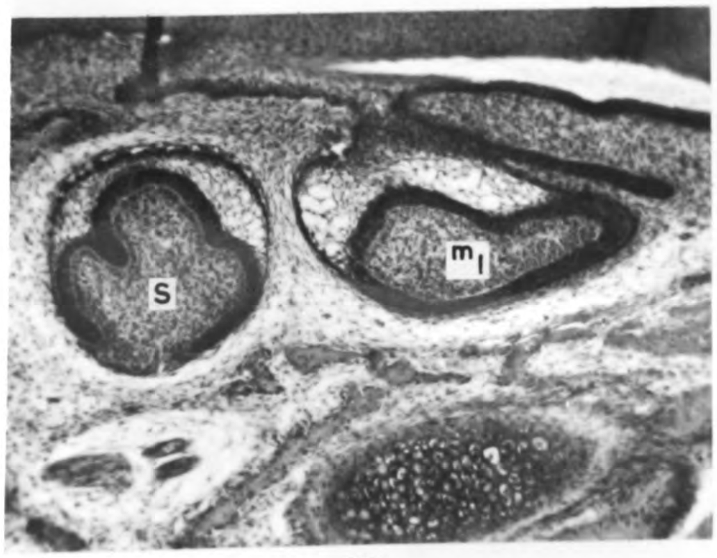

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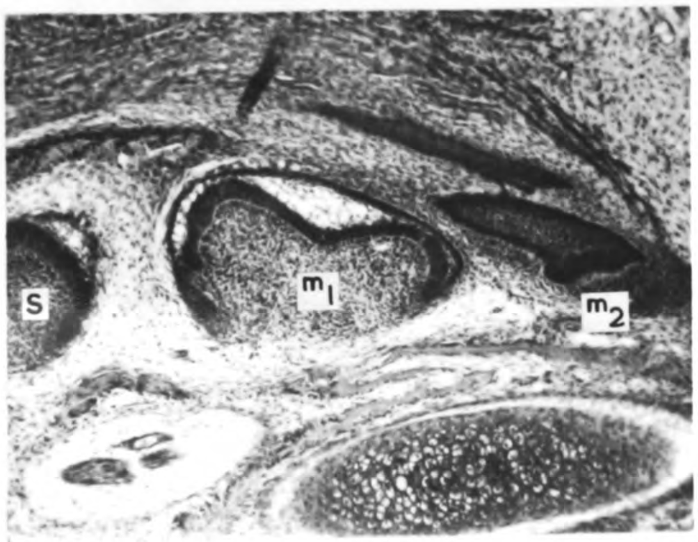

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55. Ta. 19 days. m_1 sectioned lingually. Anterior downgrowth of dental lamina (indicated by arrow). Presumably an unsuccessful attempt to form a supernumerary germ.

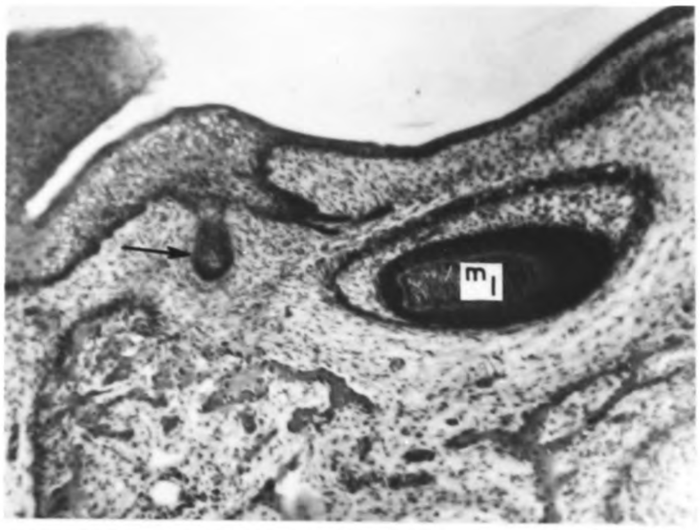
56. The same example as in 55. Sectioned further buccally to show the maximum diameter of m_1 and m_2. m_1 smaller than the control (46). m_2 much smaller than the control (47) and still at the cap stage.

57. A strain. 21 days. The maximum diameter of m_1. Dentine formation has begun.

58. The same example as in 57. Sectioned further buccally and posteriorly to show the maximum diameter of m_2.

59. Ta+. 21 days. m_1 sectioned lingually. Degenerating anterior downgrowth of dental lamina (indicated by arrow).

60. Ta+. 21 days. The maximum diameter of the smallest Ta+ m_1 found at this stage. Much smaller than the control (57) but with dentine formed over the whole crown.



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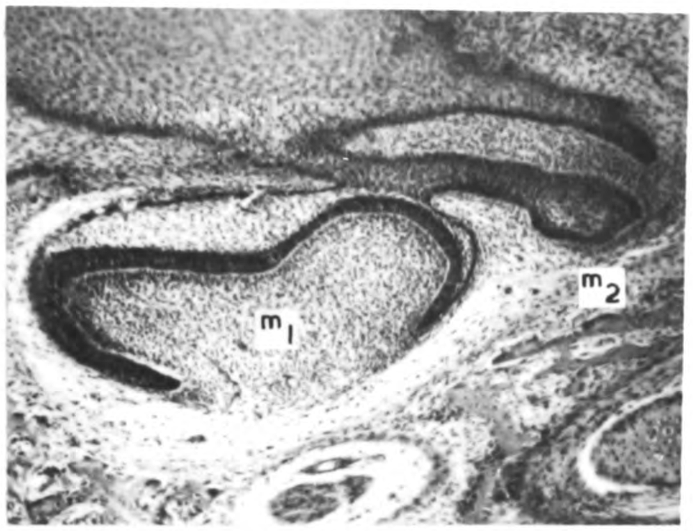

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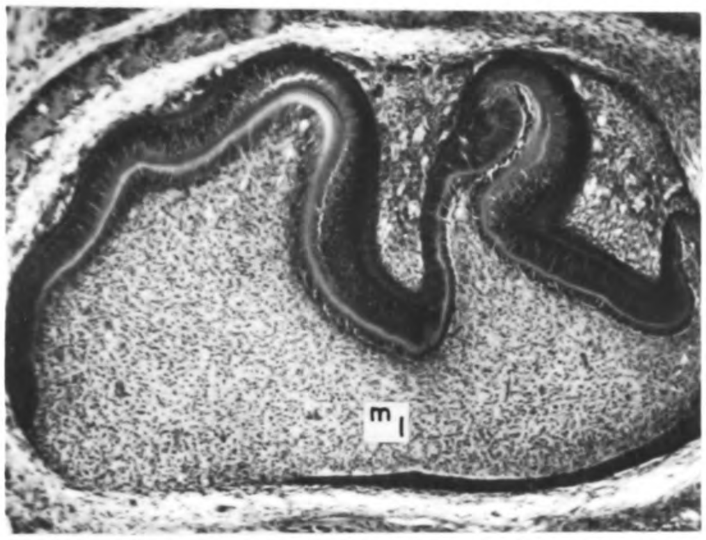

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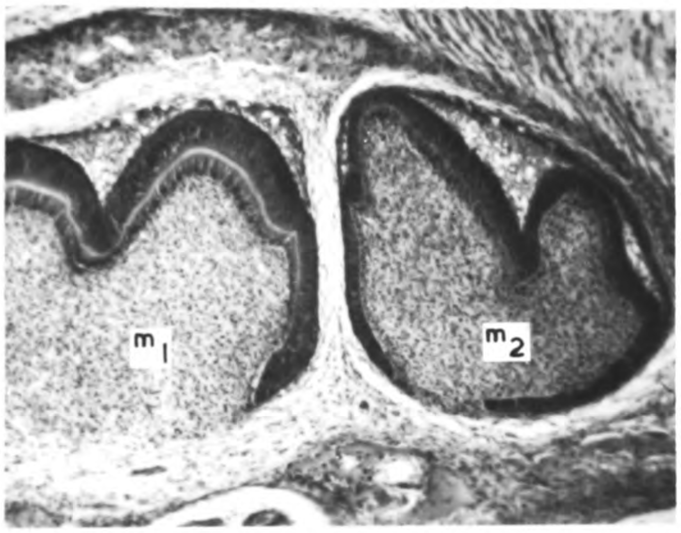

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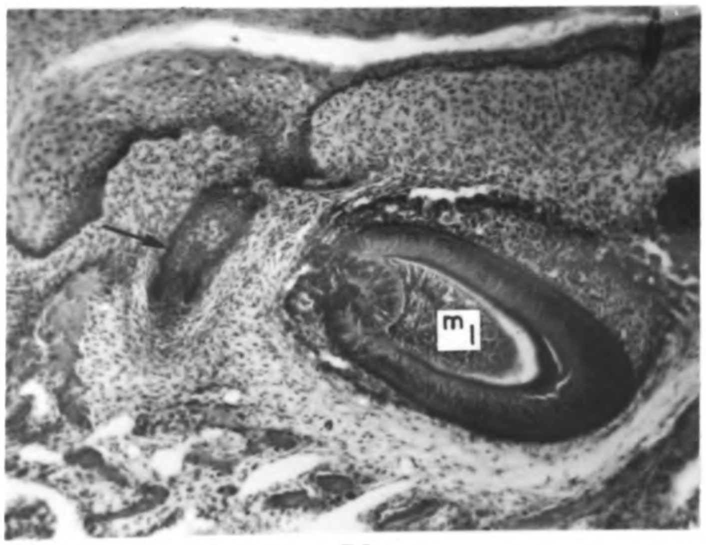

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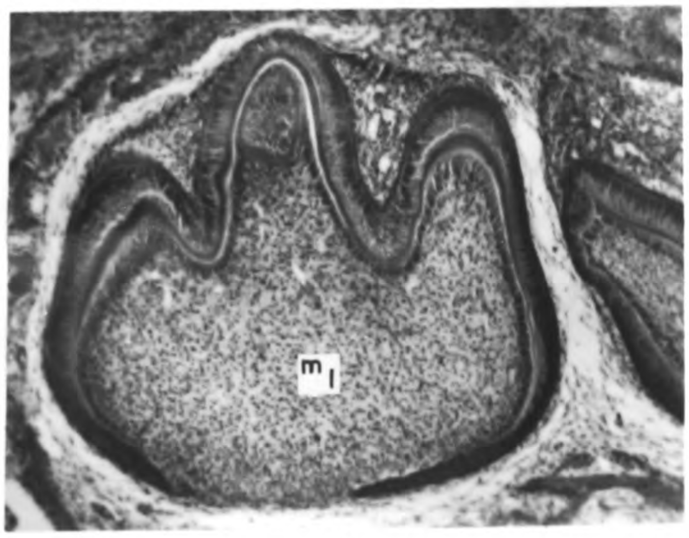

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61. The same example as in 60. Sectioned further buccally and posteriorly to show the maximum diameter of m_2 which is larger and more advanced than the control (58). Dentine formed in part of the crown. Early development of m_3.

62. Ta. 21 days. The most normal example at this stage. m_1 much smaller and less advanced than the control (57).

63. A strain. 23 days. The maximum diameter of m_1. Enamel formation has started.

64. The same example as in 63. Further posteriorly to show m_2 and the rudiment of m_3. This is not the maximum diameter of m_2.

65. Ta. 23 days. The most normal example at this stage. Maximum diameter of m_1. m_1 smaller and different in shape to the control (63). Enamel formation has just started.

66. The same example as in 65. Further posteriorly to show m_2 and the rudiment of m_3.

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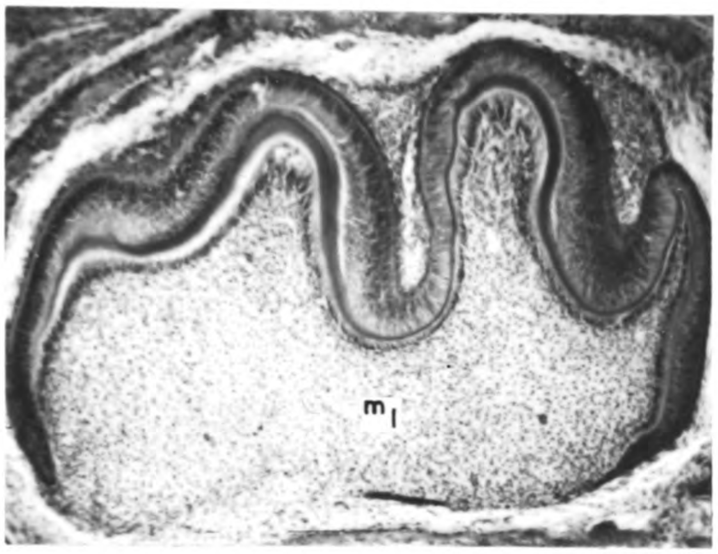

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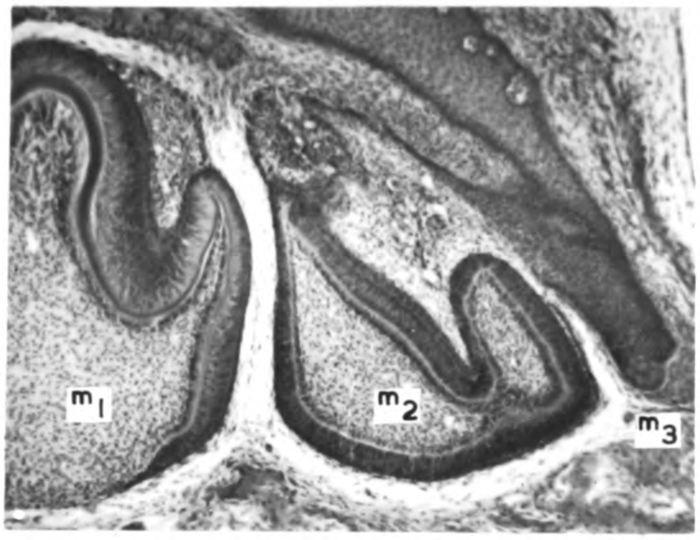

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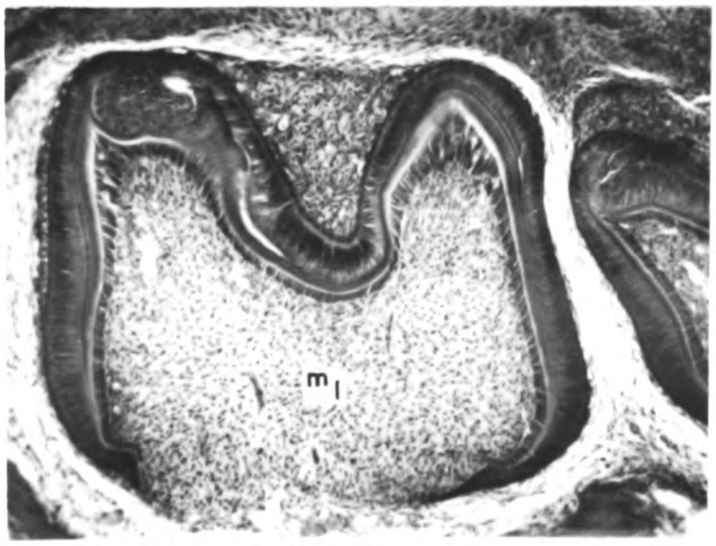

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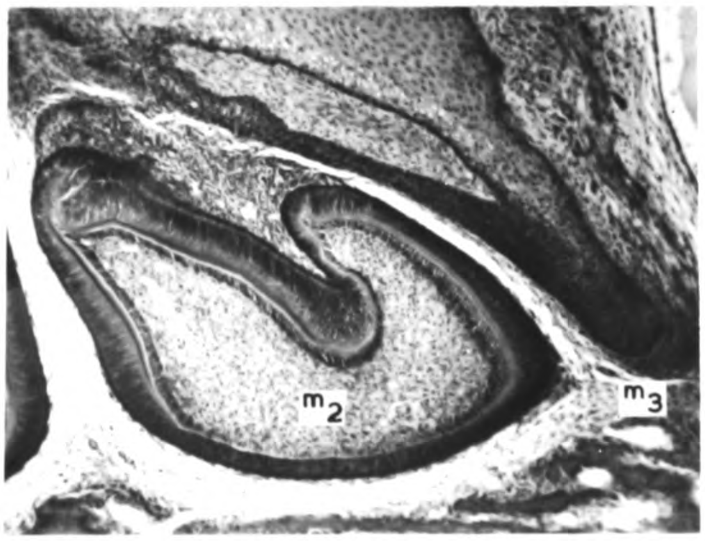

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67. Ta. 23 days. The laminal connection of an anterior supernumerary. m_1 sectioned lingually. Dentine formation of supernumerary at a similar stage to that shown by the maximum diameter of m_1 (68).

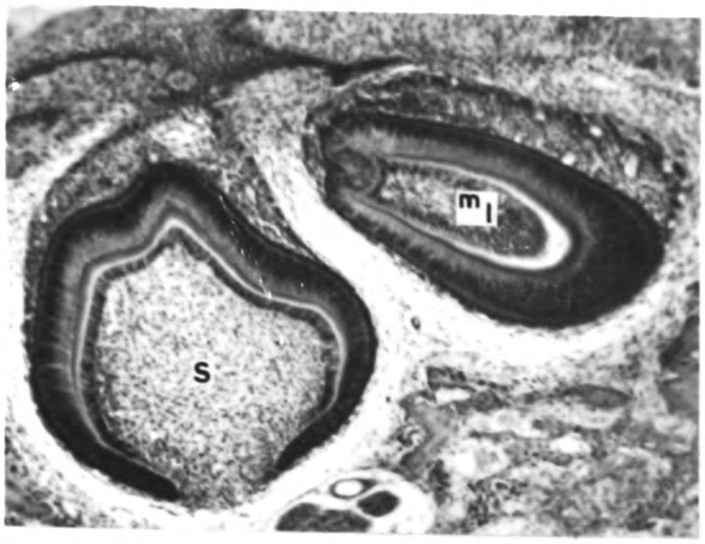
68. The same example is in 67. Maximum diameter of m_1. A little smaller than m_1 of the opposite side (70) but a comparable state of development.

69. The same example as in 67. Maximum diameter of m_2. m_2 much smaller than m_2 of the opposite side (70) and very much smaller than the control (64).

70. The same animal as in 67 but the opposite side. m_1 and a very small m_2. There was no sign of a developing supernumerary anteriorly.

71. A strain. 25 days. The maximum diameter of m_2 and the rudiment of m_3.

72. Ta+. 25 days. m_1, m_2, and the rudiment of m_3. m_2 is much smaller than the control (71). There was a supernumerary tooth developing anteriorly. Comparison with the control (71) makes it seem likely that the rudiment of m_3 would not have formed a tooth.



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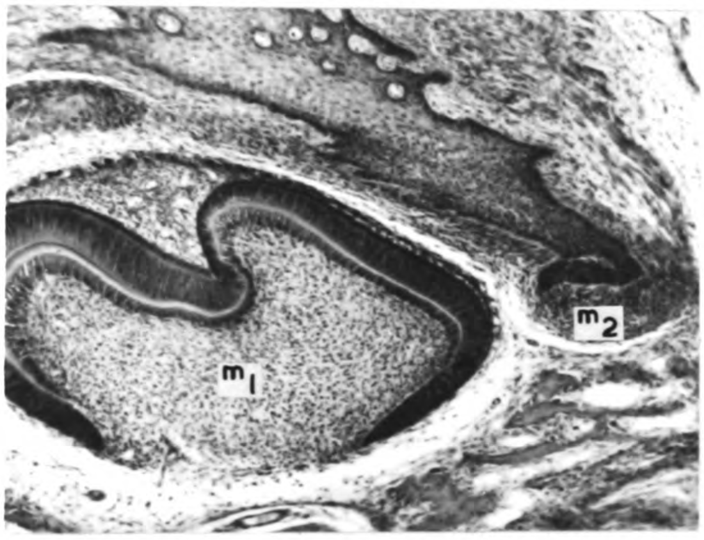

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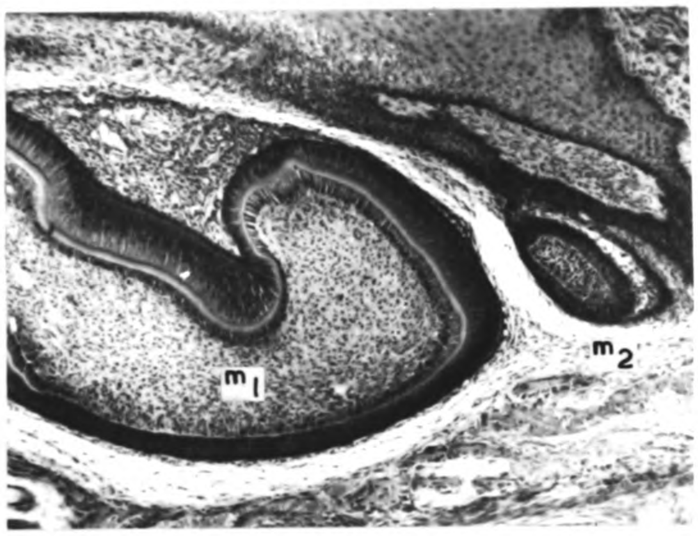

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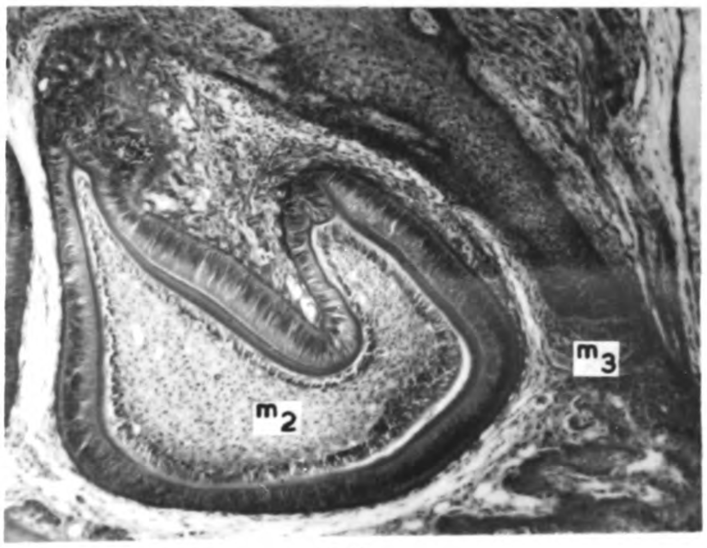

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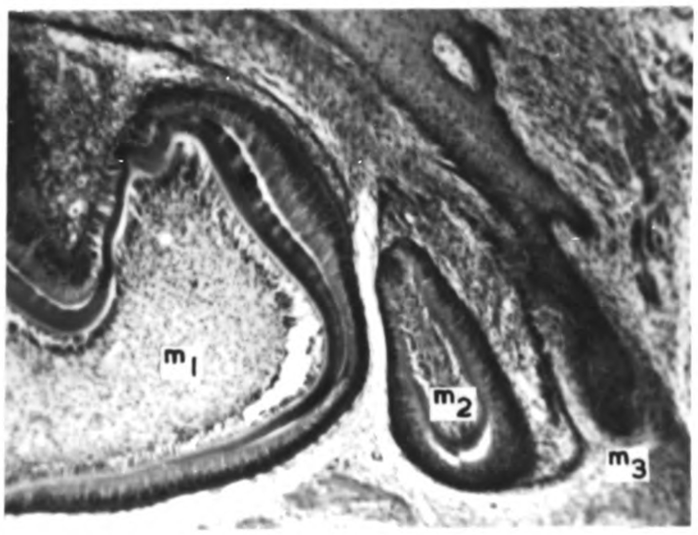

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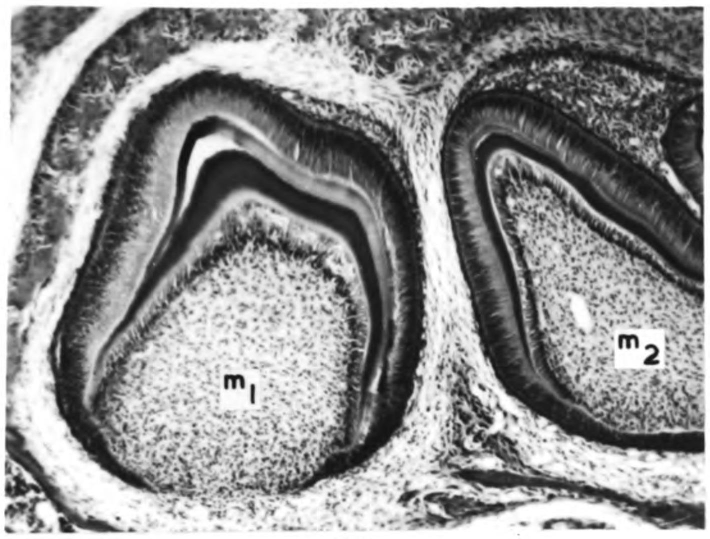
73. Ta. 25 days. The maximum diameter of m_1. m_1 extremely small but with enamel formation well advanced.

74. The same example as in 73. Further posteriorly to show the maximum diameter of m_2 and the rudiment of m_3. Enamel formation in m_2 has just started.



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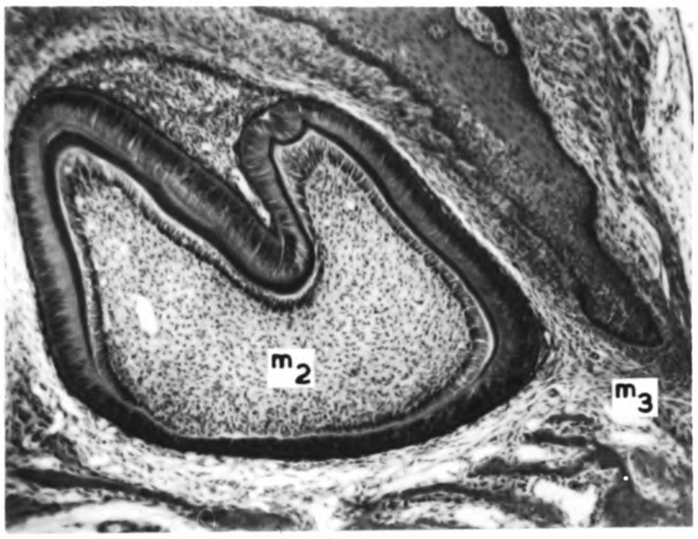


74

(iii) Upper First and Second Molars

Abnormalities of the upper molars were less striking than those of the lowers. As with the incisors and lower molars no differences between the three groups were detectable at 13 days. At 15 days and subsequently Ta m^1 germs were generally smaller than the controls and of a different shape. Ta m^2 germs were more variable and some appeared to be a little larger and more advanced than the controls at some stages. The only abnormalities found in the heterozygote group were three instances of supernumerary tooth germ development. Ta+ animals were otherwise indistinguishable from the controls. There was no evidence of supernumerary development in hemizygotes. The cases of supernumerary development showed size interaction between the teeth similar to that found in the lower molars. The rampart was an obvious feature of developing tabby second molars.

Upper First and Second Molars - Illustrations

75. A strain. 13 days. Dental lamina. Ta+ and Ta animals at this stage showed the same features.

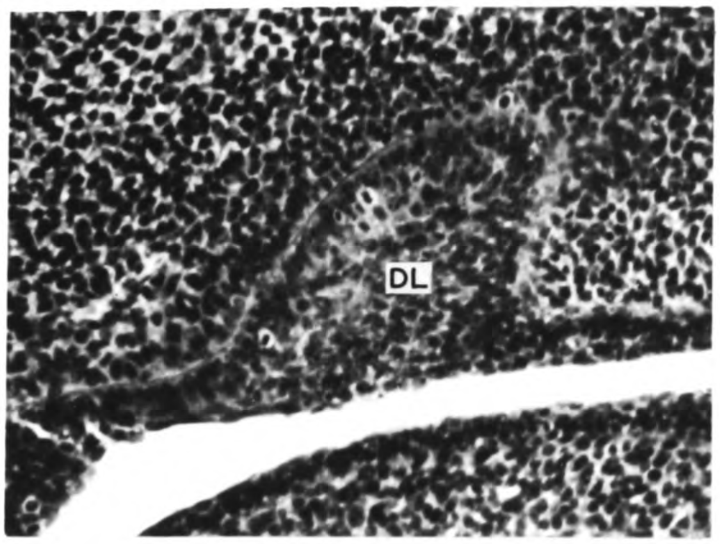
76. A strain. 15 days. m^1 in early bell stage. This section shows the maximum concavity of the bell.

77. The same example as in 76. Sectioned further buccally to show the maximum height of the buccal margin of the bell (indicated by arrow).

78. Ta+. 15 days. Two mesenchymal condensations visible above the differentiating lamina (indicated by arrows).

79. The same example as in 78. Sectioned further buccally to show the buccal margins of the two bells (indicated by arrows).

80. Ta. 15 days. Representative example. Bell of m^1 very much smaller than the control (76).



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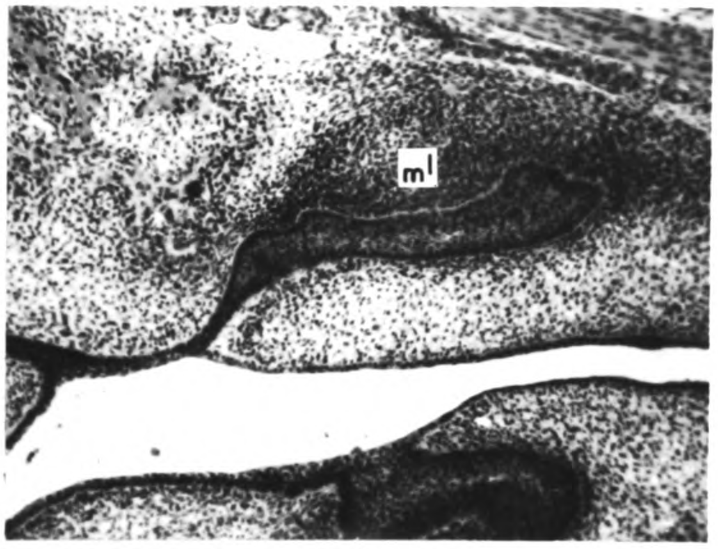

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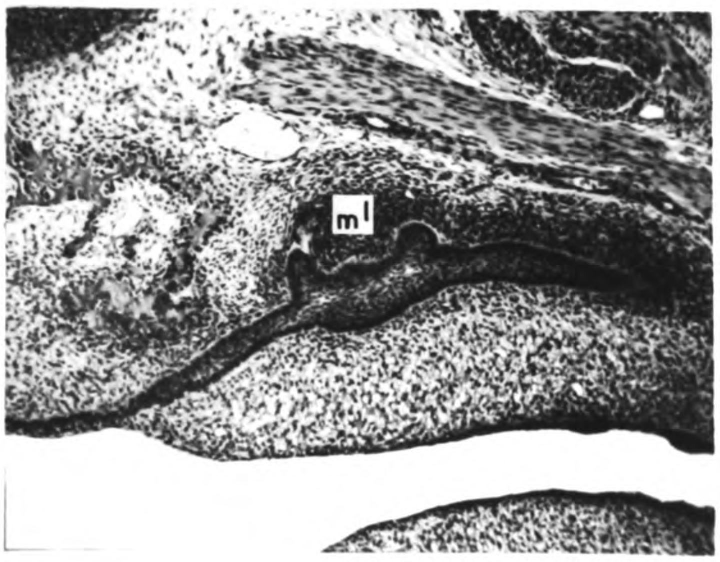


80

81. A strain. 17 days. Early morphodifferentiation of m^1. m^2 at bell stage. Ta+ animals at this stage all showed the same features.

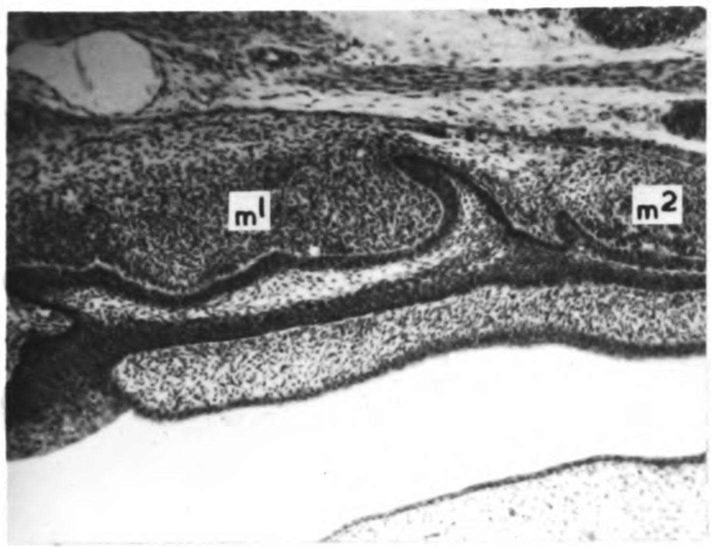
82. Ta. 17 days. m^1 smaller and more bulbous than the control (81). m^2 at bell stage.

83. A strain. 19 days. Morphodifferentiation of m^1 well advanced. Odontoblast layer appearing.

84. The same example as in 83. Further posteriorly to show m^2.

85. Ta+. 19 days. Developing anterior supernumerary and m^1. Both are about equally well differentiated.

86. The same example as in 85. Further posteriorly to show m^1 and m^2 which are both much smaller than m^1 and m^2 of the control (83 and 84).



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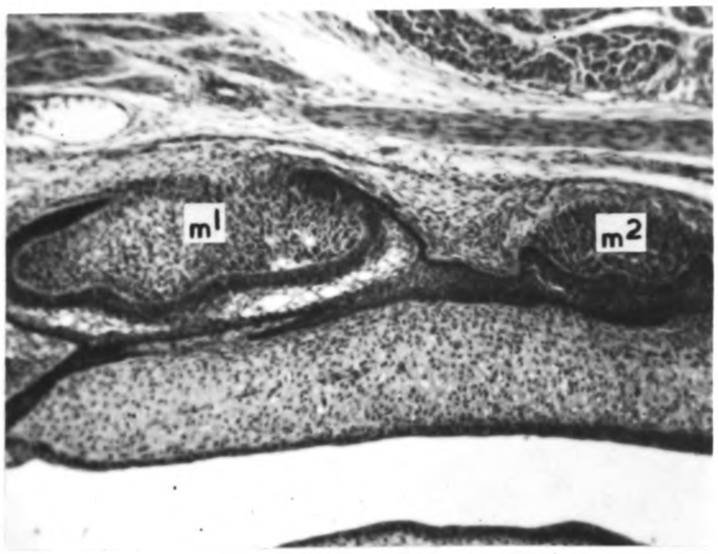

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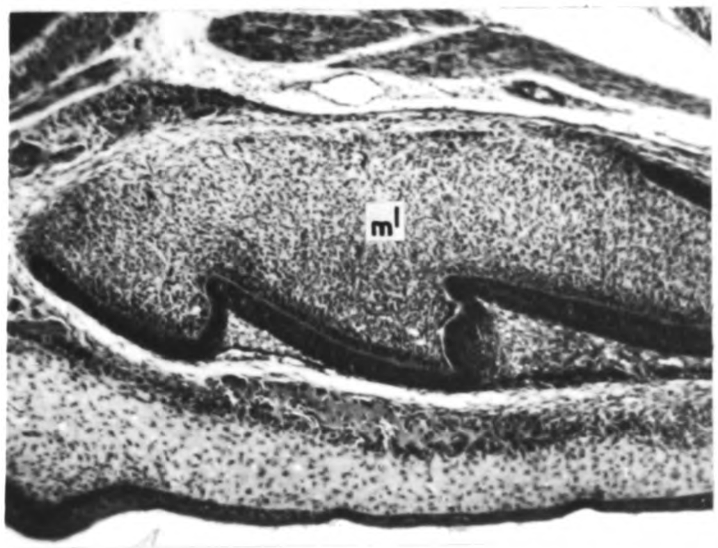

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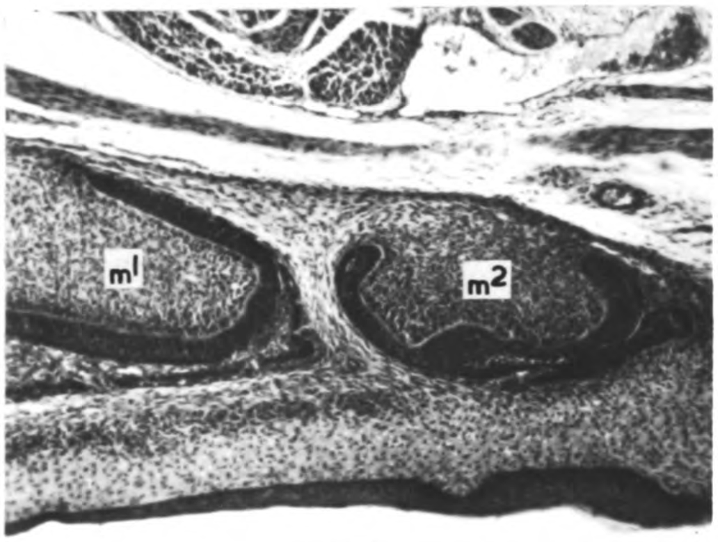

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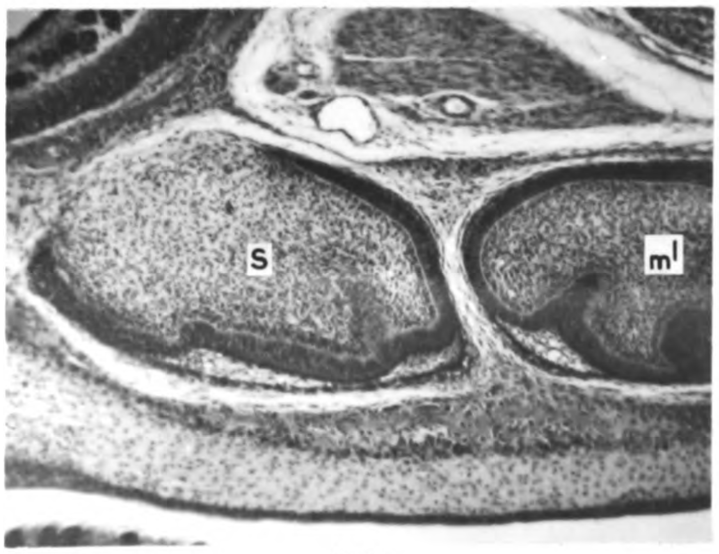

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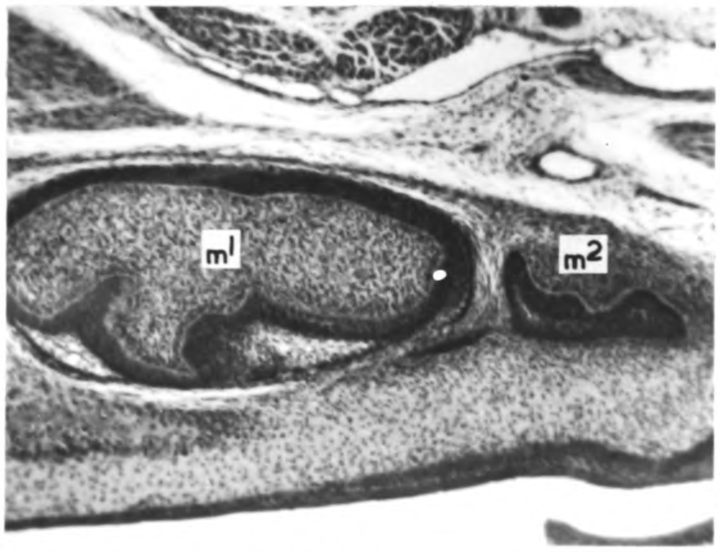

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87. Ta. 19 days. Representative example of m^2. The first sign of a developing rampart (indicated by arrow).

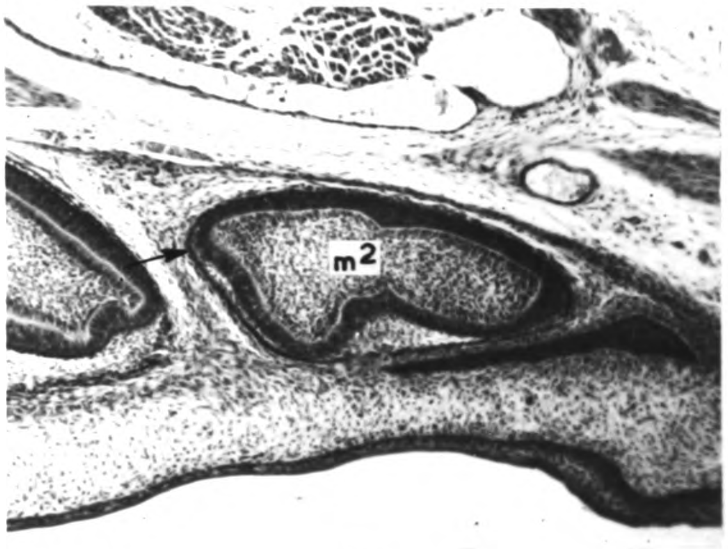
88. A strain. 21 days. Morphodifferentiation of m^2 fairly advanced. Ta+ animals at this stage showed no abnormalities.

89. Ta. 21 days. m^2. Further development of the rampart (indicated by arrow) which is absent in the control (88). The anteroposterior diameter of m^2 is greater than that of the control and the odontoblasts appear to be better differentiated.

90. A strain. 23 days. m^1. Dentine formation well advanced.

91. The same example as in 90. Further posteriorly to show m^2. Well differentiated odontoblast layer.

92. Ta+. 23 days. Developing anterior supernumerary. Dentine formation well advanced.



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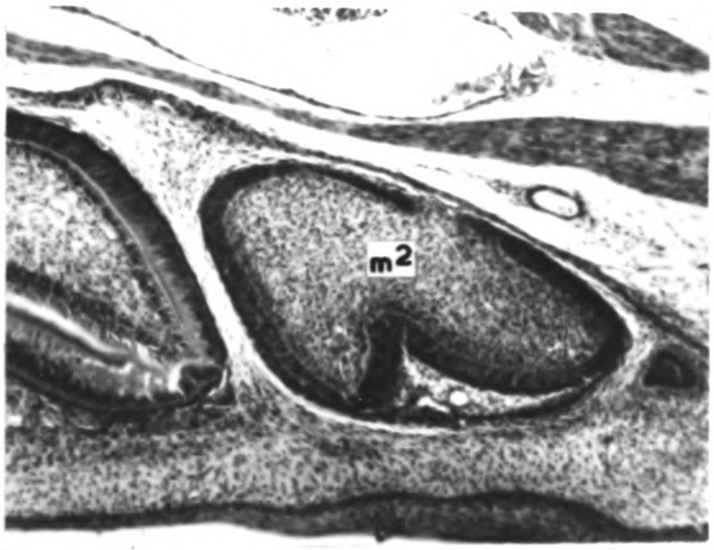

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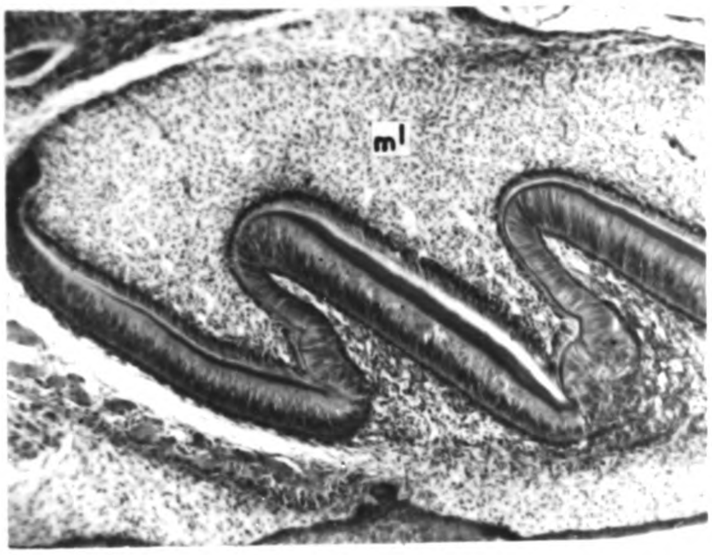

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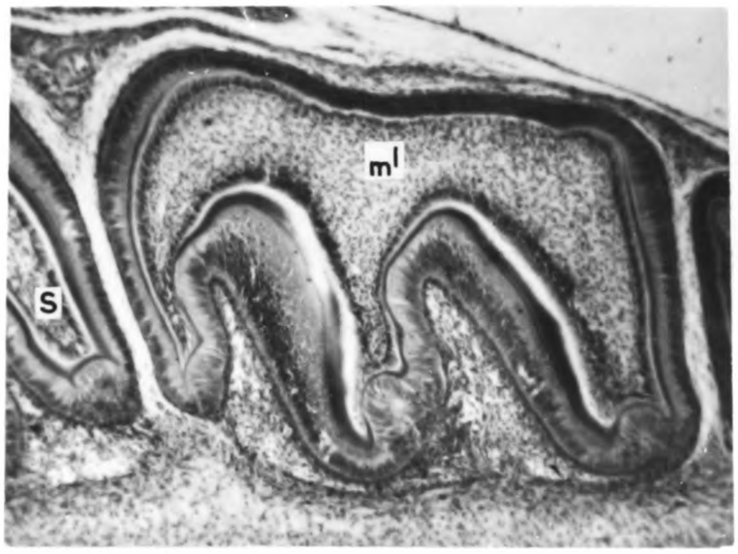
91

92

93. The same example as in 92. Further posteriorly to show m^1. The degree of dentine formation is similar to that of the supernumerary.

94. The same example as in 92 and 93. Further posteriorly to show m^2 and the rudiment of m^3. m^2 is smaller than the control (91) but has a well differentiated odontoblast layer.

95. Ta. 23 days. m^2 larger and more advanced than control (91). Dentine formation has started. Further development of the rampart (indicated by arrow) which is not present in the control.



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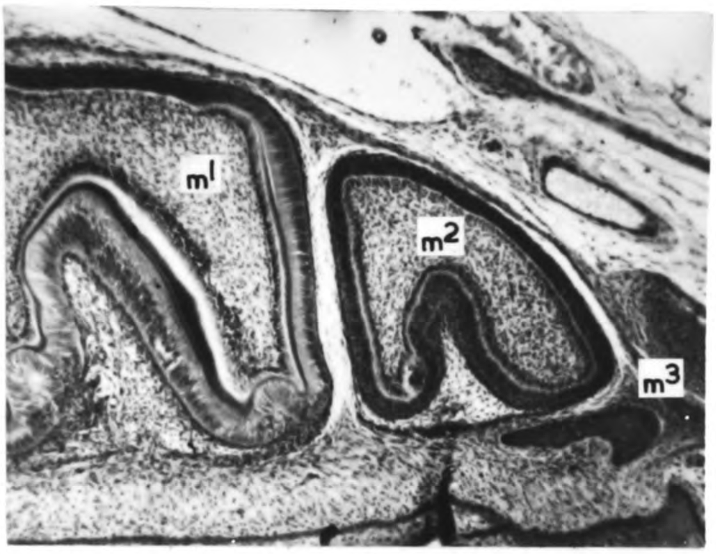

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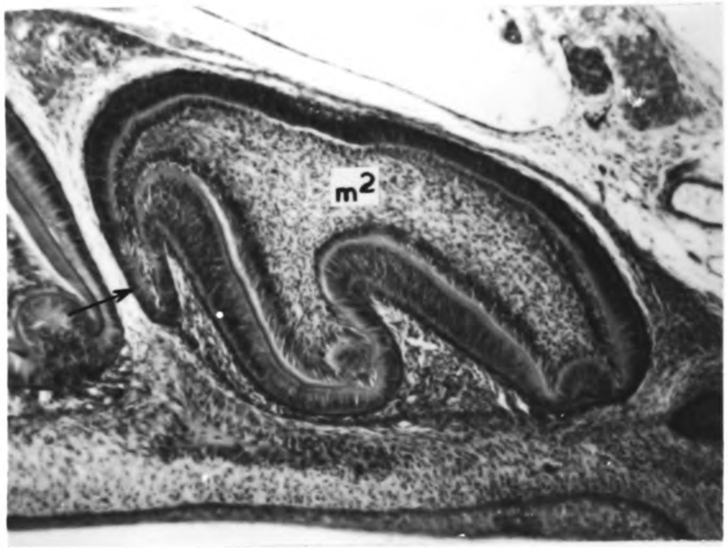

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(iv) Third Molars

A difference between rudiments which were presumed to be destined for regression and those which looked as if they would form teeth started to be detectable at 25 days and was definite at 27 days. The rudiments which were destined for regression did not invaginate to form bells. No cases of regression were found in the controls, though absence of lower third molars does occur in the A strain at a low frequency. No bell was formed by any of the Ta m_3 rudiments at 27 and 29 days. About half the Ta m^3 rudiments had formed bells at these stages. Most of the Ta+ m_3 rudiments and all of the Ta+ m^3 rudiments had formed bells at these stages.

The findings are similar to those of Grewal (1962).

Lower Third Molars - Illustrations

96. A strain. 23 days. Early m_3 rudiment.

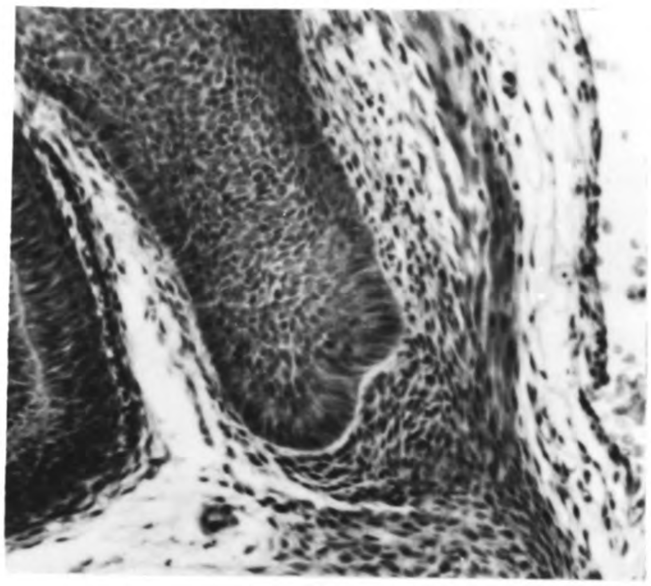
97. Ta. 23 days. Representative example. Early m_3 rudiment.

98. A strain. 25 days. Marked mesenchymal condensation beneath a flattened m_3 rudiment.

99. Ta. 25 days. No flattening of m_3 rudiment and no marked mesenchymal condensation.

100. A strain. 27 days. Early bell stage.

101. Ta+. 27 days. Early bell stage.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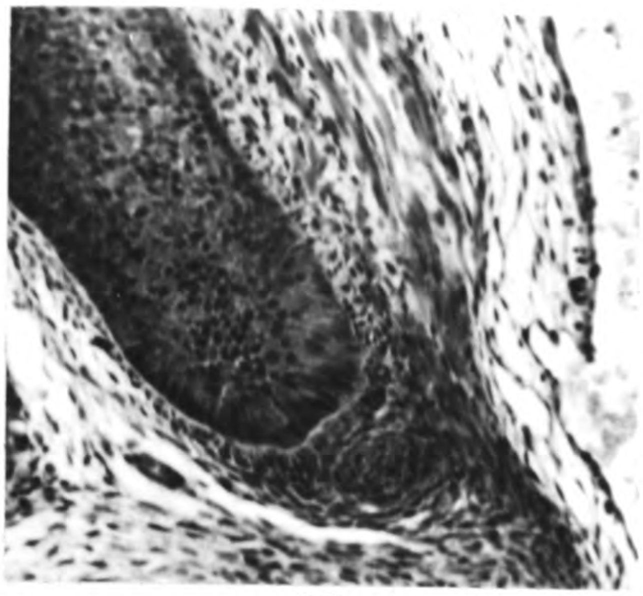
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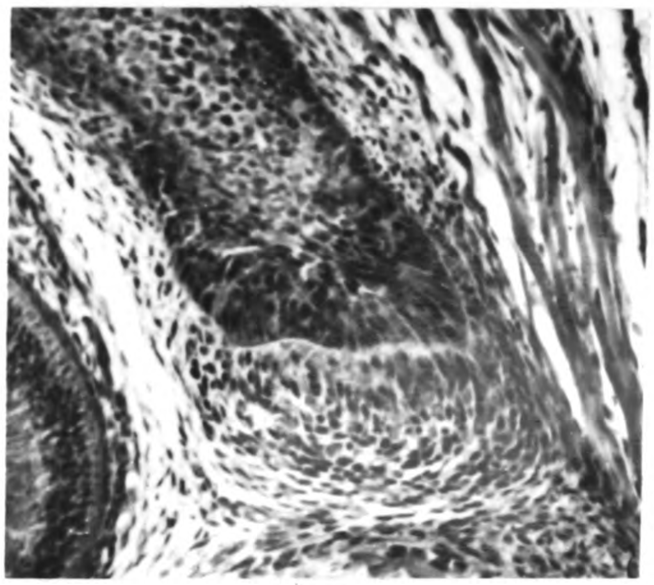
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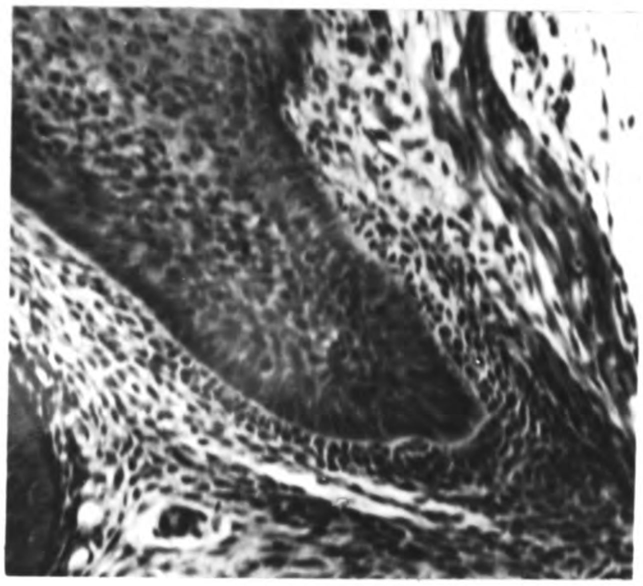
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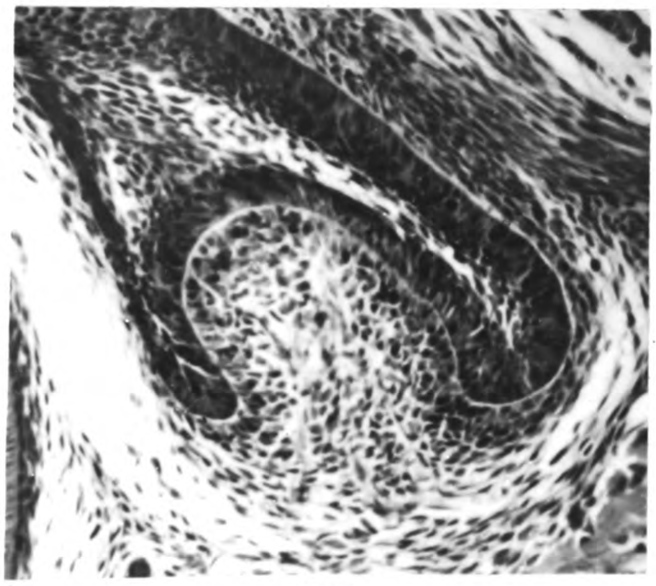
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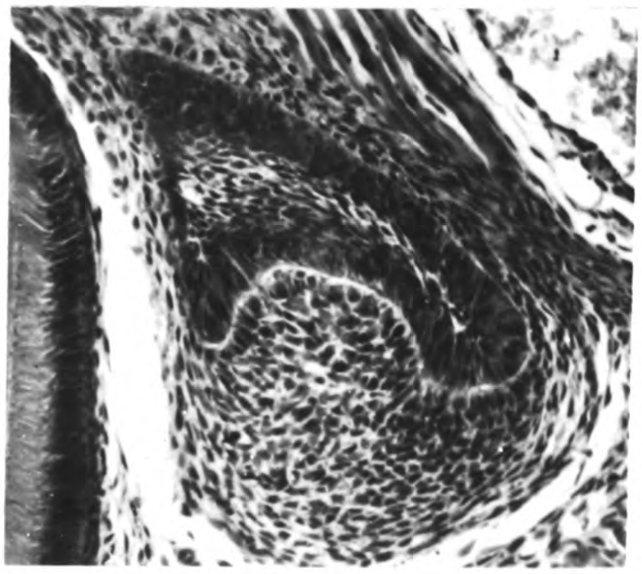
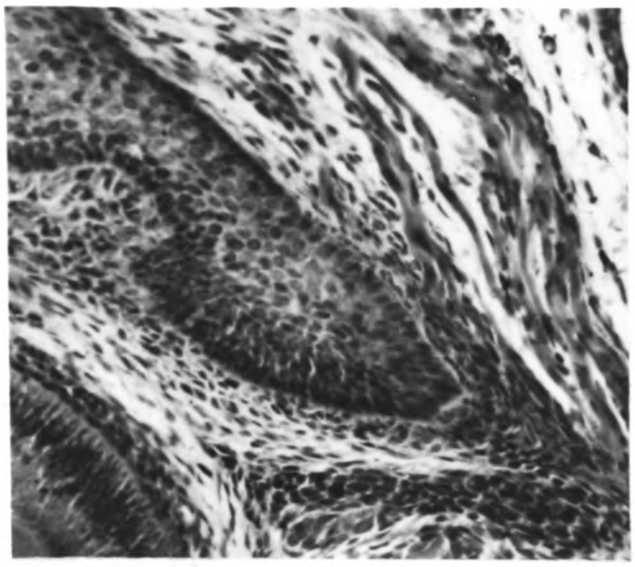

101

102. Ta+. 27 days. No invagination has taken place.

103. Ta. 27 days. No invagination has taken place.

104. Ta+. 29 days. Advanced bell stage.

105. Ta. 29 days. Degeneration.



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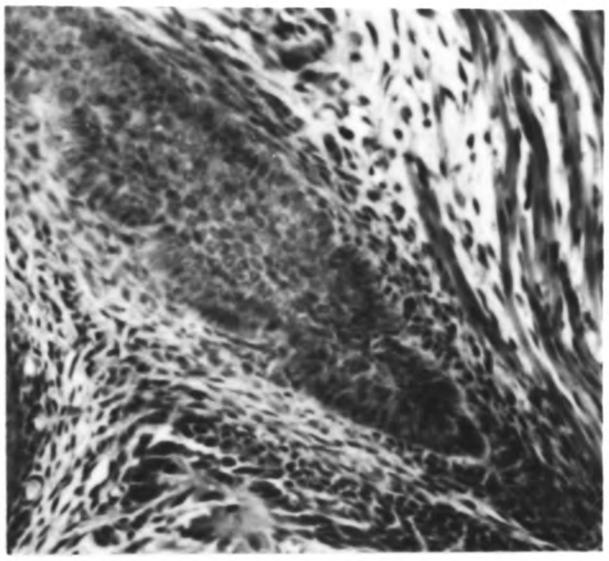

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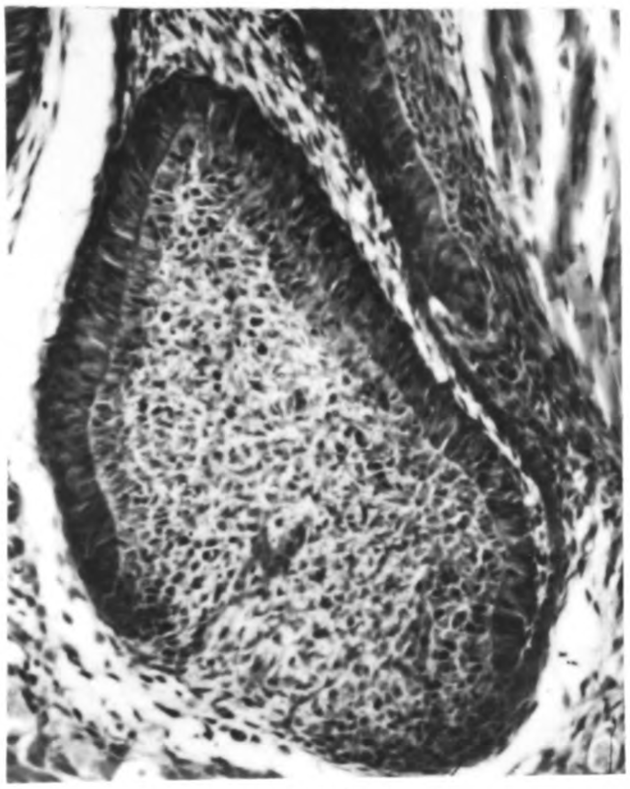

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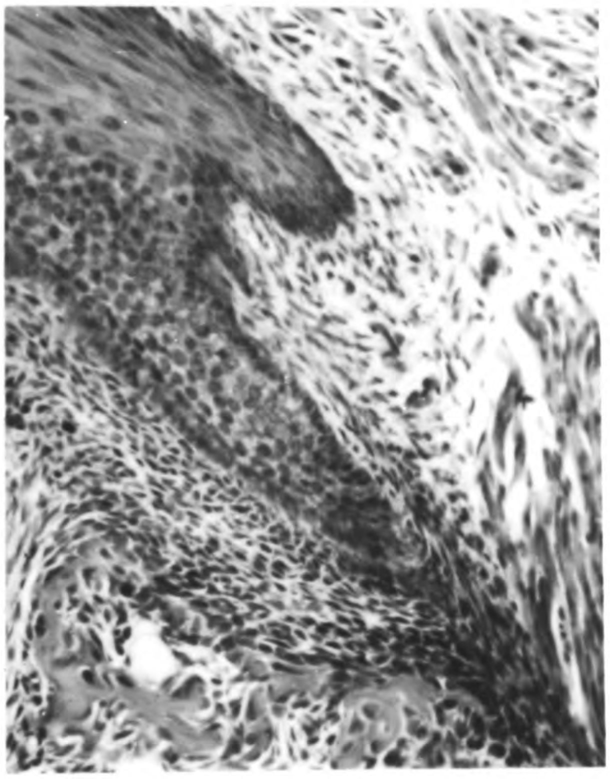

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Upper Third Molars - Illustrations

106. A strain. 23 days. Early m^3 rudiment.

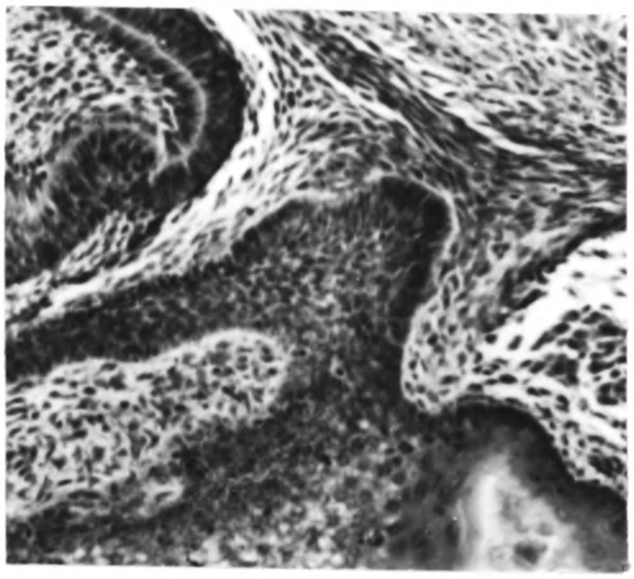
107. Ta. 23 days. Representative example. Early m^3 rudiment.

108. A strain. 25 days. Slight increase in size of the m^3 rudiment compared with 106.

109. Ta. 25 days. Representative example. Little different from 107.

110. A strain. 27 days. Early bell stage.

111. Ta. 27 days. Early bell stage. Bell more bulbous than the control (110).



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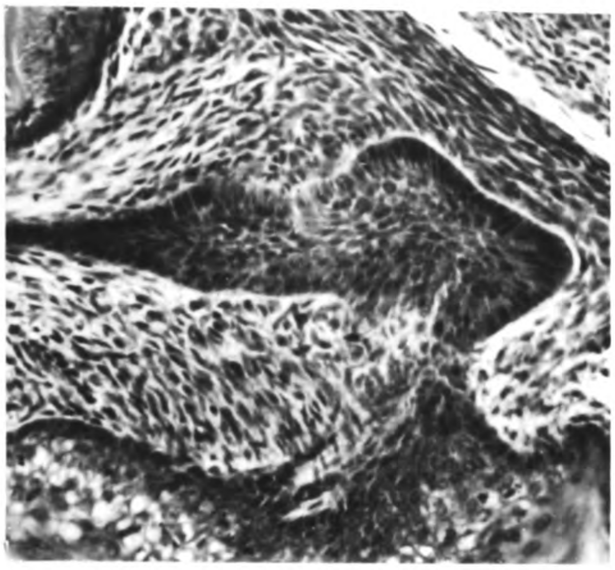

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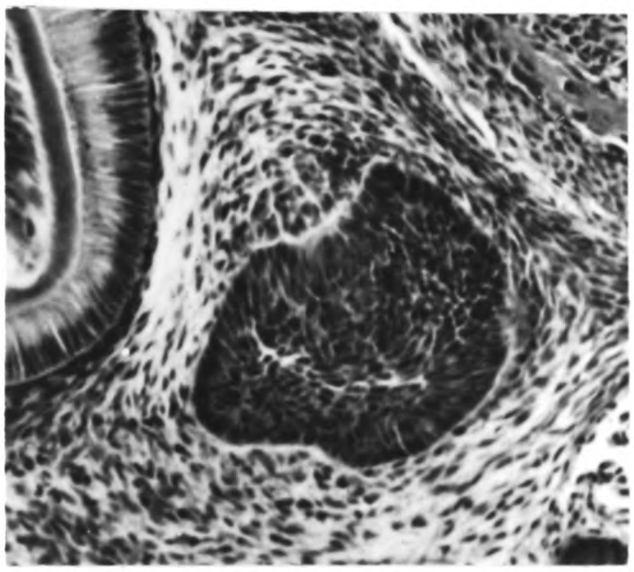

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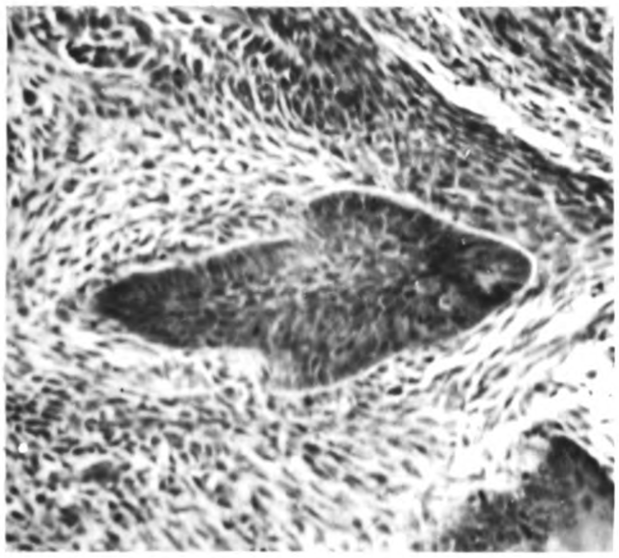

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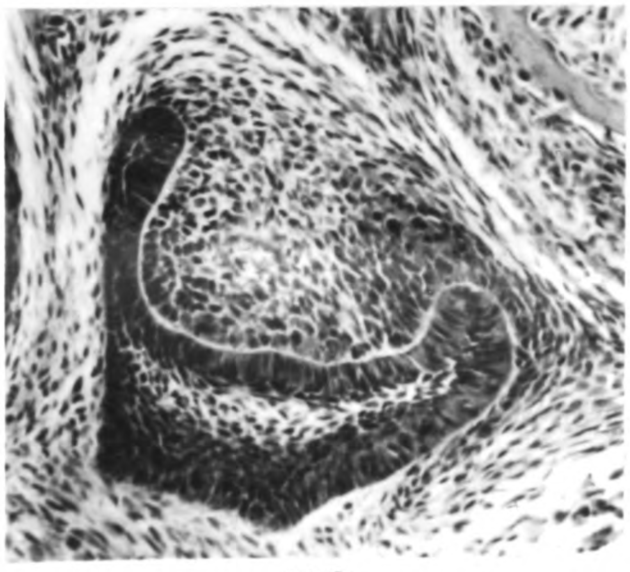

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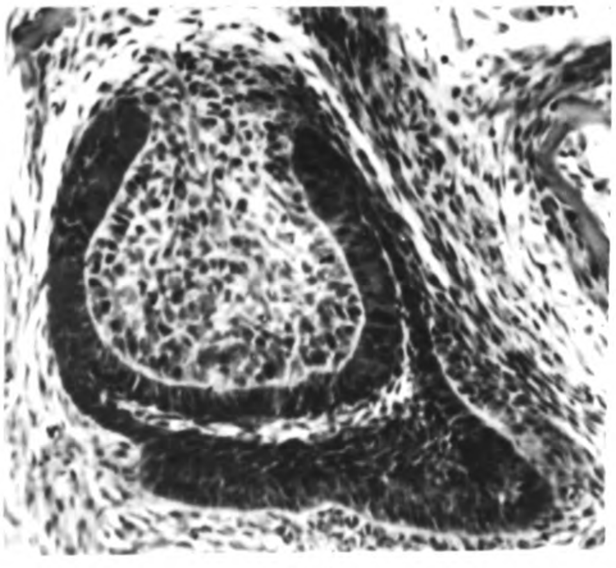


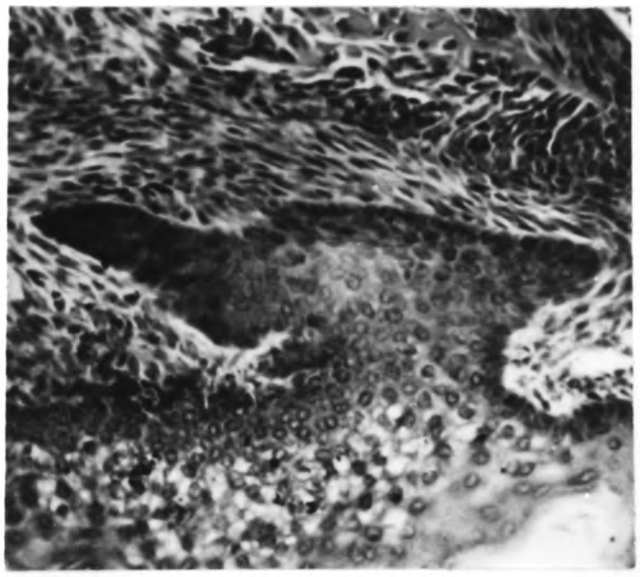
111

112. Ta. 27 days. No bell has been formed.

113. Ta+. 29 days. Advanced bell stage.

114. Ta. 29 days. Bell stage. Bell is smaller and less advanced than in 113.

115. Ta. 29 days. Degeneration.



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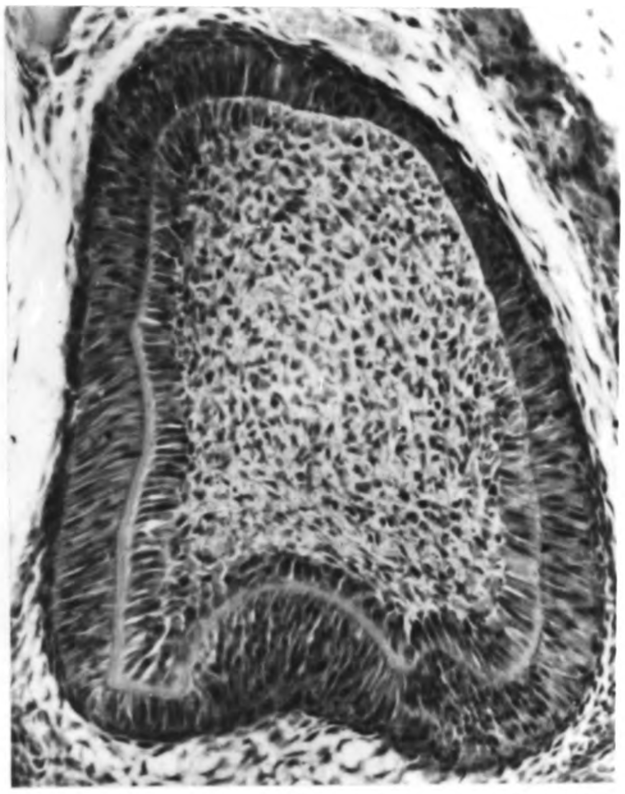

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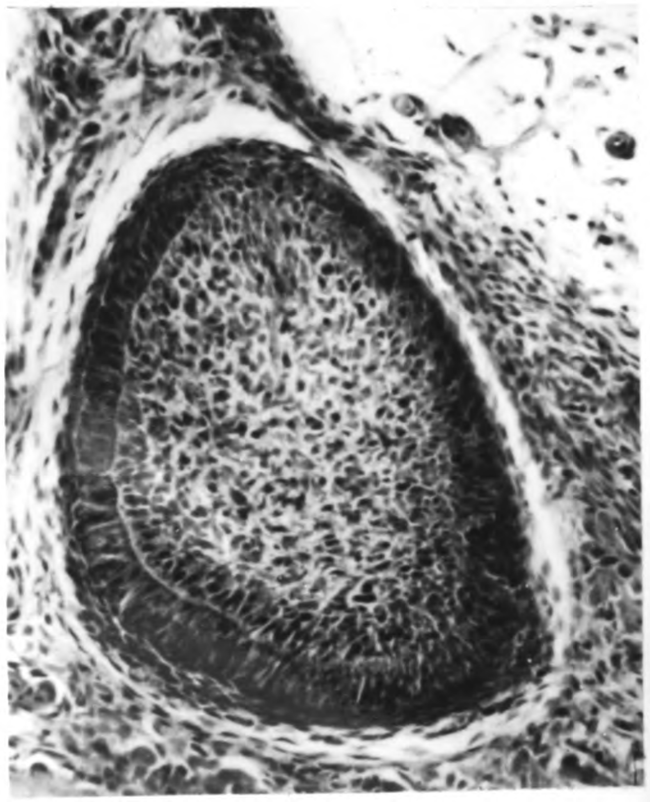

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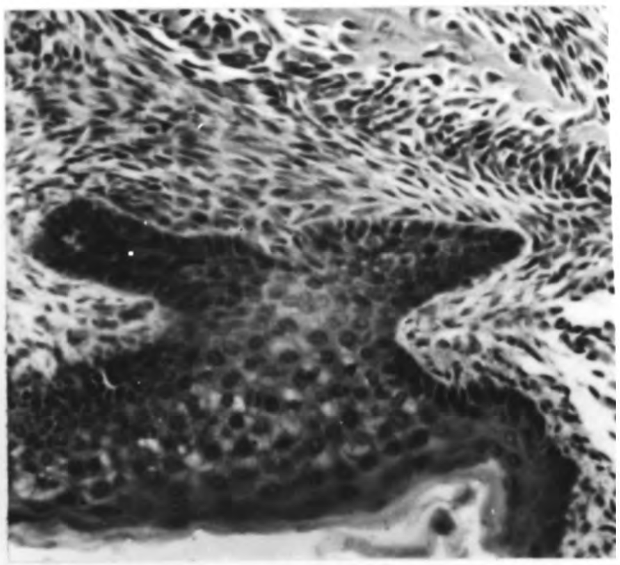

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4. Discussion

(i) General

The development of the teeth of the control animals was comparable with the findings of other workers (Gaunt, 1955 and subsequently; Cohn, 1957; Hinrichsen, 1959; and Hay, 1961).

In tabby hemizygotes the general effect on the teeth appeared to be one of reduced rate of growth and delayed histodifferentiation. The effect on the lower incisors was the most severe. Sometimes no tooth at all was formed, and sometimes there was an intermediate condition where dentine but no enamel was formed. In the case of the molars, however, there was no evidence to suggest that enamel formation is ever prevented or that a first molar is ever completely suppressed. It did seem likely that complete suppression could be the rare fate of some lower second molar germs when a supernumerary tooth was developing. Such cases were in fact found amongst the fully formed dentitions examined. As far as absent third molars are concerned, the findings here were similar to those of Grewal (1962).

Similar but less severe effects were observed in the molars of some heterozygotes.

'Overt twinning' was found to be produced by the de novo development of a supernumerary tooth from an overgrowth of a normal anterior extension of dental lamina, and not by division into two of a first molar germ. The failure of third molar rudiments to form bells was observed many times, so it is reasonable to assume that 'concealed twinning' does occur. In such cases the development of a supernumerary tooth would be followed by the regression of the third molar rudiment in that quadrant. Direct evidence of this has been found in only one case in this study (compare illustrations 71 and 72), and in one case by Grüneberg (1966a). Examples of supernumerary development were found in both upper and lower jaws of heterozygotes, and in the lower jaw only of hemizygotes.

The picture formed is therefore one of a generalised partial suppression of growth and differentiation of dental epithelium with occasional localised points of abnormal overgrowth. The greatest variation was found in the lower molars. These will now be considered in more detail in the light of what is known of the development of the coat, and what has been observed in the fully formed dentition.

The first period of hair follicle suppression, from 12½ to 17 days, is just that during which the first molar develops from a small bud of epithelium to an early stage of morphodifferentiation and histodifferentiation. Within this period, at 15 days, only the earliest sign of epithelial overgrowth was observed (illustration 33). At 17 days, the beginning of the phase of follicle formation, overgrowth was more advanced but had not yet proceeded very far (illustrations 37 and 42). By contrast, at 19 days, towards the end of the follicle formation phase, the overgrowth had developed into a tooth germ in which histodifferentiation was almost as advanced, if not equally advanced, as in the first molar posterior to it (illustrations 49 and 53). Subsequently the various stages of histodifferentiation appeared to proceed together in the supernumerary and first molar germs.

(ii) Stabilisation of Length of the Tooth Row

The interpretation offered for these observations is based on the premise that there is a tendency for the length of the tooth row to be stabilised. Because of its retarded growth the first molar fails to occupy all the space allotted to it. As a consequence there is an overgrowth of the dental lamina to form an additional tooth germ to occupy the vacant space. The fact that overgrowth appears to start during the first period of follicle suppression could be accounted for by a difference in sensitivity to the Ta gene between the differentiating epithelium of the first molar germ and the undifferentiated epithelium of the dental lamina. However, whereas growth had not proceeded far before 17 days, rapid development of the supernumerary germ occurred during the follicle formation phase.

A release of inhibition, which is taken to occur at the end of the first period of follicle suppression, would no doubt affect the first molar as well as the potential supernumerary; though possibly not to the same extent, on account of their different states of differentiation. This could explain why epithelial downgrowths which failed to produce supernumerary germs were found. A sudden increase in size of the first molar could, presumably, prevent the downgrowth from developing further. The second molars, also in a less differentiated state than the first, would also be likely to react more strongly to a release of inhibition. This would then be the basis of the size interaction observed between first and second molars, especially noticeable in cases where no supernumerary was present.

The existence of this size interaction in the fully formed dentition has already been recognized (Grüneberg, 1965).

In the cases where a supernumerary does materialise the usual result, possibly due to the postulated release of inhibition at 17 days, is that the supernumerary and first molar together occupy more space than the first molar alone would have done. The majority of cases of 'twinning' found in the fully formed dentitions were 'concealed' and in the few 'overt' cases the third molars were small. Thus, the addition of tooth substance to one end of the row is followed by removal from the other.

There is some evidence from other sources in favour of the concept of stabilisation of the length of the tooth row. An obvious fact is the existence of a large space between incisor and first molar in which teeth normally never develop. Grüneberg (1951) pointed out that in CBA mice, in which m_3 is much reduced, m_1 is relatively large. The data of Grewal (1962), showing the sizes of upper and lower molars of the strains A, C57 and CBA, demonstrate a negative interaction between the combined lengths of the first two molars and that of the third. Van Valen (1962) has demonstrated a similar negative interaction in the dentition of Peromyscus.

(iii) Anteroposterior Restriction caused by Tabby?

Although no measurements have been made, the length of the molar tooth row in tabby animals is certainly reduced. This is due to a reduction in both number and size of the teeth. It is now suggested that this might be due, not only to poor growth of dental epithelium, but also to a primary reduction of the space available.

Such an effect could be a specific one, affecting the tooth bearing regions alone, or it could be the result of a more general abnormality such as a generalised reduction in growth rate. During the collection of material for this investigation hemizygote embryos were generally smaller than their heterozygote litter mates. After birth this difference became very obvious. Falconer, Fraser and King (1951) found that at birth crinkled mice were on average 5% lighter than their normal litter mates. The difference increased steadily to 20-30% three weeks after birth.

A reduction of available space could be partially responsible for the characteristic shape of the tabby upper molars, in particular the erect cusps, and the rampart in the case of m^2. Anteroposterior restriction of a developing tooth would tend to make the cusp slopes more vertical, as occurs with the folds of a concertina bellows when the two sides are being pushed together. This is well illustrated by the case shown in illustrations 92 - 94 where the presence of a supernumerary is associated with apparent squashing up of the teeth. As a consequence these teeth have narrower more vertical cusps than the control (90 and 91).

The development of the rampart, which could be regarded as a reaction to the small size of the first molar (suggested by Grüneberg, 1965) starts as an anterior outgrowth (87), which subsequently becomes bent occlusally as the space between the developing teeth closes (89 and 95).

Support for anteroposterior restriction causing a difference in shape of the teeth is given by Butler (1956). He quoted the studies of Osborn (1902) who found that increasing brachycephaly was associated with broadening of the teeth. Butler also pointed out that the teeth of mammals in which the jaws are long and the tooth germs widely spaced during development tend to be proportionately longer than those of mammals with crowded dentitions. The report of Grainger, Paynter and Shaw (1959) on morphological differences between the teeth of a caries resistant and a caries susceptible strain of rats included diagrams of midsections through upper first molars of the two strains. In the smaller of the two teeth the cusps appeared to be slightly more erect than in the larger.

(iv) A Basis for the Abnormalities

If the interpretations of the observations are correct, then it is basically the size of the developing first molar at and before 17 days, together with the potential length of the tooth row, which are responsible for the ultimate number, size and form of the molar teeth. The final size of the first molar is not a good indication of its status at 17 days, as recovery or further suppression could take place after 17 days and before its final form is decided by the onset of hard tissue formation. A slight difference in size between left and right first molars at 17 days could result in the successful formation of a supernumerary tooth germ on one side but the suppression of a potential counterpart on the other. Thus small differences in local conditions at a critical stage of development could be responsible for formidable asymmetry in the adult dentition.

(v) The Incidence of Supernumerary Tooth Formation

In the lower molars the incidence of supernumerary formation in the embryological material was lower than was expected on the basis of the cases diagnosed in the fully formed dentitions examined. Assuming that all the cases of proliferation of the anterior extension of dental lamina detected at and before 17 days would have formed supernumerary teeth (probably an over-estimate), and all those detected at and after 19 days would not, the incidence observed in heterozygotes was 8/73 = 11%, and in hemizygotes was 3/57 = 5% (see Table 5). The levels that were expected were respectively around 20% and around 10% (see Fig. 32).

The likelihood of misdiagnosis of supernumeraries in adult heterozygotes was remote as there was usually a definite difference of size and form between the supernumerary and the first molar. Comparison with the opposite side, which was usually more normal, clarified the doubtful cases. In hemizygotes, however, misdiagnosis could have been a source of considerable error. Diagnosis here was based solely on the relative sizes of the first two standing molars. If the first was smaller than the second it was classified as a supernumerary. The case shown in illustrations 73 and 74 demonstrates that this is not a reliable criterion. In this instance a tooth which could fairly definitely be called a first molar was very much smaller than the second.

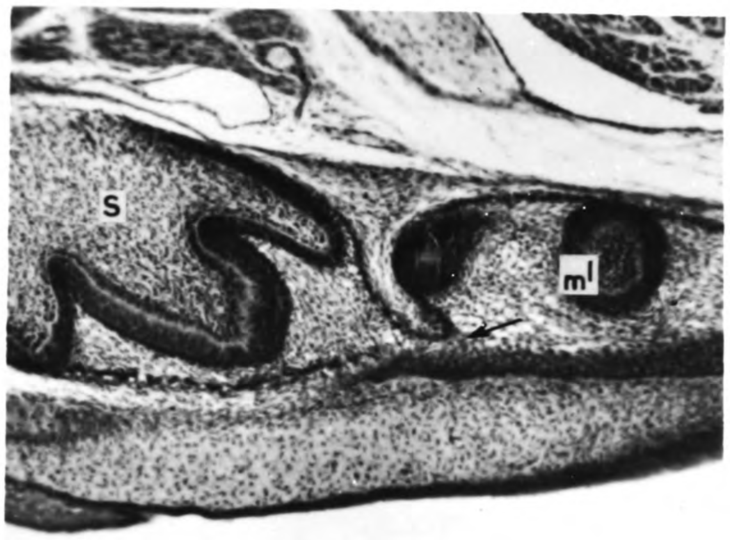
The only other possible source of the discrepancy was the difference in background genotype between the two sets of material. Whereas the majority of the embryological material had a heterozygous genetic background, the adult specimens were to some extent homozygous for the A strain background.

(vi) '<u>Incomplete Twinning</u>'

'Overt twinning' and 'concealed twinning' have already been discussed, but no mention has so far been made of 'incomplete twinning'. If the extra teeth found in the first two cases arise independently, then in the third, the rare compound teeth observed must be the consequence of fusion rather than of incomplete fission. Hitchin and Morris (1966) showed that fusion of the developing incisors of the dog, or connation as they called it, is related to the persistence of dental lamina between the two incisor germs. Rapid growth of adjacent germs was thought to cause the external enamel epithelium to be stripped off the persisting interdental lamina. As a result, the stellate reticulum of the two germs becomes confluent, the internal enamel epithelia of the two germs come into contact, and fusion takes place. In addition to connation of two adjacent incisors of the normal series there were two examples of connation of a first incisor with a supernumerary tooth.

Illustration 116 is of a case already shown in a different section (85). The external enamel epithelium between the supernumerary and the first molar has just become separated from the underlying dental lamina (indicated by arrow). This illustration is comparable with one in the paper by Hitchin and Morris, though in their case separation was more extreme.

Stripping of the external enamel epithelium from persisting interdental lamina would not only be a function of rapid growth of adjacent tooth



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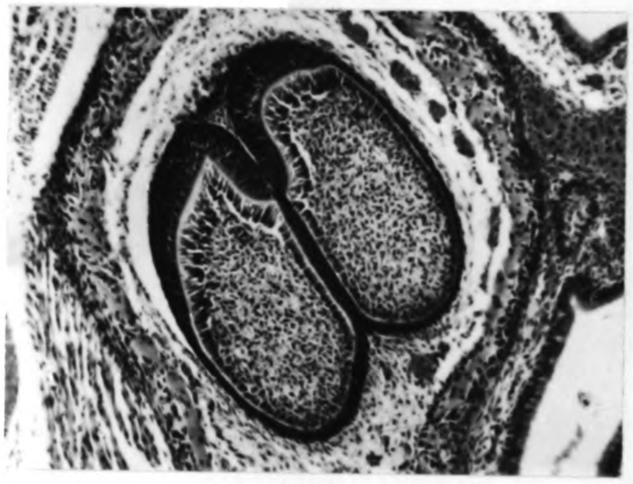

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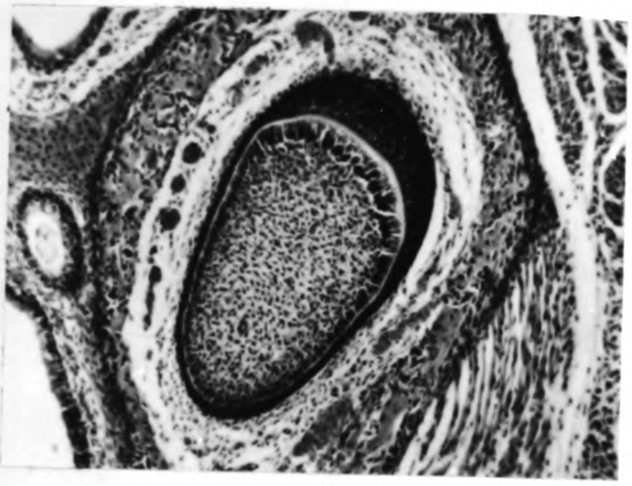

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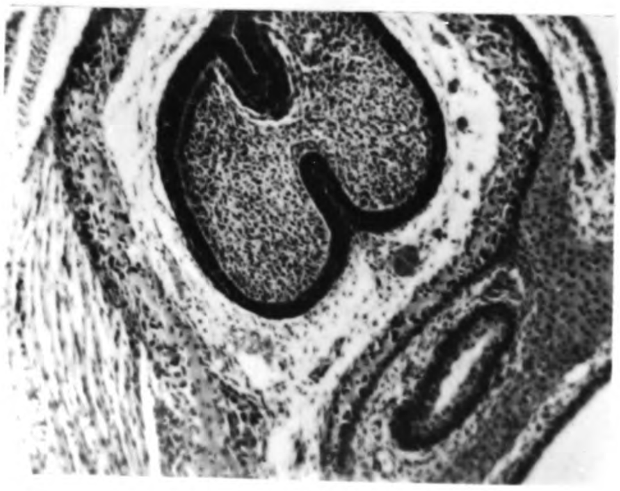


119

germs, but also of their proximity. The more tightly squeezed together the developing germs the greater the tension on the external enamel epithelium and the greater the likelihood of its separation from the underlying lamina. With this in mind 'incomplete twinning' would then be related to antero-posterior restriction placed on the developing germs. It is conceivable that this has been a mechanism whereby reduction in tooth number has occurred during phylogeny.

In the trials made prior to the main investigation some of the material was sectioned transversely. Illustrations 117 - 119 are of a tabby hemizygote or homozygote at about 21 days. Anteriorly on the left there was a single incisor germ (118). Anteriorly on the right there were two incisor germs with their internal enamel epithelia in intimate contact (117). Further posteriorly on the right there was a connection between the pulp cavities of the two germs (119). The anterior end of a developing incisor is the first to form, whereas the posterior end is the youngest region where proliferation continues throughout life. The example shown in 117 and 119 must therefore have started out as two separate germs which fused subsequently. An example of a fully formed upper incisor of this sort is shown in Fig. 11. It can be appreciated that once such a tooth has been subjected to wear the nature of its origin would be obscured. The only example of complete separation between a normal and a supernumerary incisor is shown in Fig. 12.

A similar argument can be used to explain the origin of composite molars with separate crowns and common roots. The crown develops before the root, so if the crowns are separate and the root common there must originally

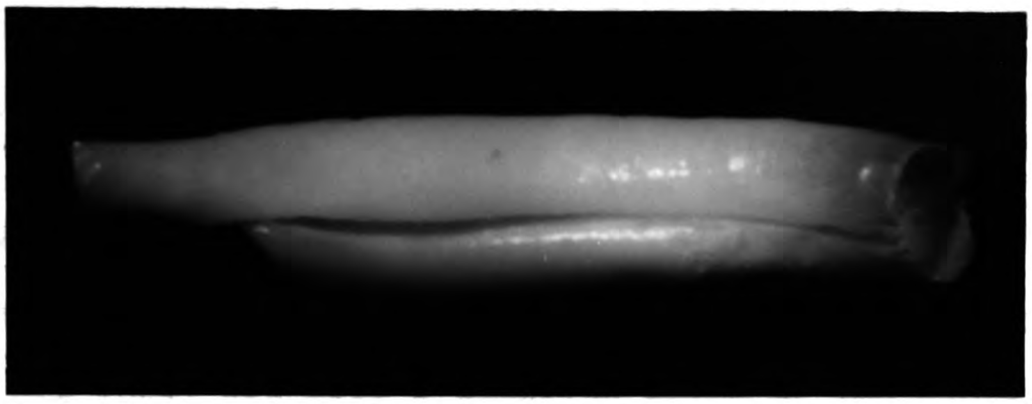


Fig. 11. A composite upper right incisor (X25)

Fig. 12. Complete separation between supernumerary and upper right incisor (X5.5)

Fig. 13. Lingual view of a composite lower right first molar (X25)

have been two germs which fused after the formation of the crowns was complete. Such a case is illustrated in Fig. 13.

Further evidence for the origin of fusion being associated with restriction of space comes from the study of artificially induced malformations. Knudsen (1965 and 1966) has made a detailed study of the dental malformations induced in mice by teratogenic agents. All these abnormalities were associated with exencephaly. There were various degrees of fusion of the two incisors within each jaw, and also intermediate cases where the pulp cavities of the two germs were separate but their stellate reticulum was confluent. Upper incisor fusion occurred in 68% of treated embryos but lower incisor fusion occurred in less than 2%. Ritter (1963) induced lower incisor fusion, and fusion of the lower molars of one side with those of the other, by x-radiation. These mandibular fusions were associated with mandibular micrognathy. Knudsen (1966a) reported on the molar malformations of exencephalic embryos. There were amazing cases of fusion of upper molar germs with lower molar germs on the same side. All these cases of fusion appear to have been associated with a reduction in the amount of connective tissue which normally separates the individual developing tooth germs.

5. Conclusion

It has been suggested that all the abnormal features of the tabby (Ta^F) dentition discussed have their origin in a single primary effect of the mutant gene, a reduction in the rate of growth and histodifferentiation of dental epithelium. A subsidiary effect, a reduction in the length of the tooth row, has also been suggested as a possibility. There is no reason to suppose that Ta^c, crinkled, or downless, are fundamentally any different.

'Overt' and 'concealed twinning' have been found to be due to the de novo development of a supernumerary tooth, and it seems most likely that 'incomplete twinning' is caused by the fusion of supernumerary and first molar germs.

IV. The Reaction of the Genes to Two Inbred Backgrounds

1. Introduction

Genes do not act in isolation but interact with each other. The effect produced by a gene in one genetic situation may be modified considerably in another. This has been shown by the response of mutant phenotypes to selection (e.g. Dun and Fraser 1959), and by the differences in manifestation of mutant phenotypes produced by crossing to different inbred strains (e.g. Green 1957). Critical comparison of genes with similar effects can therefore only be made in a standard genetic environment.

The activities of the wild type alleles of mutant mimic genes must be fairly intimately related on the biochemical level. The genes are, however, unlikely to be identical if homozygosity of any one alone is sufficient to produce the mutant phenotype. The simplest explanation is that the wild type allele of each gene is responsible for one of a number of related steps, either in series or in parallel, towards the formation of a single end product which is necessary for the development of a normal phenotype.

Complete blockage at any point along an isolated pathway would be expected to produce an identical result. If blockage is incomplete, or if there are cross connections with other pathways, as seems more likely, differences between the genes may be detectable.

The background genotype may interact with a gene either at a fundamental level, or at some stage along the ramifications that lead to the final phenotype. In the first case all the pleiotropic effects produced by the gene would be expected to be influenced in the same direction, whereas in the second case local reactions would tend to be more or less independent of each other (Grüneberg, 1963). Previous studies have shown that the different manifestations of a gene tend to be independently modified by genetic background (e.g. Carter, 1951; Green, 1957).

As the primary site of action of each mimic gene is distinct it is probable that each will have a unique set of interactions with the background genotype. If background modification acts on the blocked steps of the pathway the different mimics would then be expected to react differently. If, however, modification acts on the final product of the pathway, the reaction of the different mimics would tend to be the same.

An analysis of the reactions of mimic genes to different backgrounds could, therefore, pinpoint the level at which background modification operates. Modification at the primary site of action of each gene would be likely to result in a different reaction of each gene to the same background, but within each gene the different pleiotropic effects would all be influenced in the same direction. Modification acting on the end product of the pathway, provided it is always qualitatively the same, would cause all genes to react in the same way and all the pleiotropic effects to be influenced in the same direction. Modification acting between the end product of the pathway and the final phenotype would produce different reactions in different aspects of the phenotype within each gene. If there were also differences between genes it would indicate that the nature of the end product is not independent of the position of the genetic lesion on the pathway.

Such speculative interpretation of gross phenotypic differences in biochemical terms is unlikely to be fruitful if taken too literally. However, a general consideration of this sort could help to throw light on the nature of complex developmental processes.

In the present case there was an additional consideration; a comparative study of autosomal and sex-linked genes with similar effects. Such a study is relevant to the current ideas of dosage compensation in mammals. A comparison between tabby (Ta^F) and crinkled has already been made (Grüneberg, 1966a). The present work is extended to include Ta^c and downless, and has the additional dimension of variation in the background genotype.

2. Material

Isaacson (quoted by Grüneberg, 1965) found that the incidence of incisor abnormality in tabby mice was influenced by genetic background. Homozygous tabby females from stock were crossed to a number of inbred strains and the F_1 tabby males were examined. The two most extreme degrees of manifestation were 85.5% abnormal in the A strain F_1, and 11.0% abnormal in the JU F_1. Strains A and JU were consequently chosen as the backgrounds on which the genes were to be compared.

The main body of the material examined was composed of five classes of animals according to their background genotype: the stock background; the backgrounds after one cross to JU ($\frac{1}{2}$JU), and after two crosses to JU ($\frac{3}{4}$JU); and the backgrounds after one cross to A ($\frac{1}{2}$A), and after two crosses to A ($\frac{3}{4}$A). All of these animals were the progeny of heterozygous mothers and homozygous (or hemizygous) fathers, and in each mating both parents had the same level of background genotype.

Within each background class there were four subclasses, one for each of the genes. Within each tabby subclass there were four groups according to genotype; TaTa, Ta+, Ta, and + ; and within each crinkled or downless subclass there were two groups, one of heterozygotes and one of homozygotes, in which the sexes were balanced as far as possible. An average of six matings were made within each subclass, and in most cases material was drawn from several litters of each mating. Unfortunately the two subclasses tabby (Ta^F) $\frac{3}{4}$JU and downless $\frac{3}{4}$JU were not completed because of poor fertility, in spite of the fact that a considerably larger than average number of matings was set up in each case. They are therefore not included in the following discussions. There were two additional groups, one of A strain and one of JU animals. The number of individuals in each group varied between 21 and 26, and in all about 1,300 animals were examined.

Animals were collected at four weeks of age, by which time all the teeth are fully formed, except that root length has not yet reached a maximum.

The following data were recorded:

(i) The numbers of vibrissae of the secondary facial groups.

(ii) An assessment of the degree of striping of tabby heterozygotes.

After sacrifice the animals were decapitated and the heads skinned and prepared by the method of Luther (1949). The dentitions were examined subsequently.

The counting of the vibrissae and the examination of the dentitions were carried out under a dissecting microscope (magn. 25X).

The findings were considered under two headings: Vibrissa Number, and The Dentition.

3. Vibrissa Number

(i) Review

(a) Vibrissa Number in the Normal Mouse

On each side of the face of the normal mouse there are two supraorbital, one postorbital, and two postoral vibrissae. Beneath the chin is a single group of three inter-ramals. The supraorbital, postorbital, and postoral vibrissae of both sides are respectively called groups A, B, and C; and the inter-ramals are called group D.

A survey by Dun (1958) and additional data of Dun and Fraser (1959), together comprising about 6,000 mice from a variety of stocks, showed that variation in vibrissa number is normally very limited. Most of the variability that was present was restricted to group D, which occasionally had two rather than three vibrissae. Group B was absolutely invariant. The variation of inter-ramal score was at first thought to be due to sensitivity of this group to environmental fluctuation. However, crossing inbred strains which differed in respect of their inter-ramal score produced an intermediate F_1, and selection for high and low inter-ramal score was effective in both directions (Dun, 1958). The variation was thus shown to be genetic in nature.

The two inbred strains used in the present study, A and JU, differed in respect of both the C and D groups. The effect of crosses between the two strains on the scores at these two sites is shown in Fig. 14. In each parental group there was a high score at one site and a low score at the other. High score or low score is therefore not necessarily a property common to both groups together. In group C high score appeared to be almost completely dominant over low score, whereas in group D high score and low score had about equal weight. These observations suggest that the two groups are to some degree under separate genetic control. There are other indications of the existence of separate genetic systems influencing the different groups (Fraser, Nay and Kindred, 1959; Fraser and Kindred, 1962).

(b) Variation of Vibrissa Number in Mutant Mice

Reduction in the number of secondary vibrissae in crinkled and tabby mice was first noticed by Falconer, Fraser and King (1951) and Falconer (1953). Not only is the total number reduced, but variability is increased (Fraser, Nay and Kindred, 1959), and this variation responds to selection (Dun and Fraser, 1959). Analysis of the effects of selection showed that there was a single primary zone of canalisation at the maximum normal

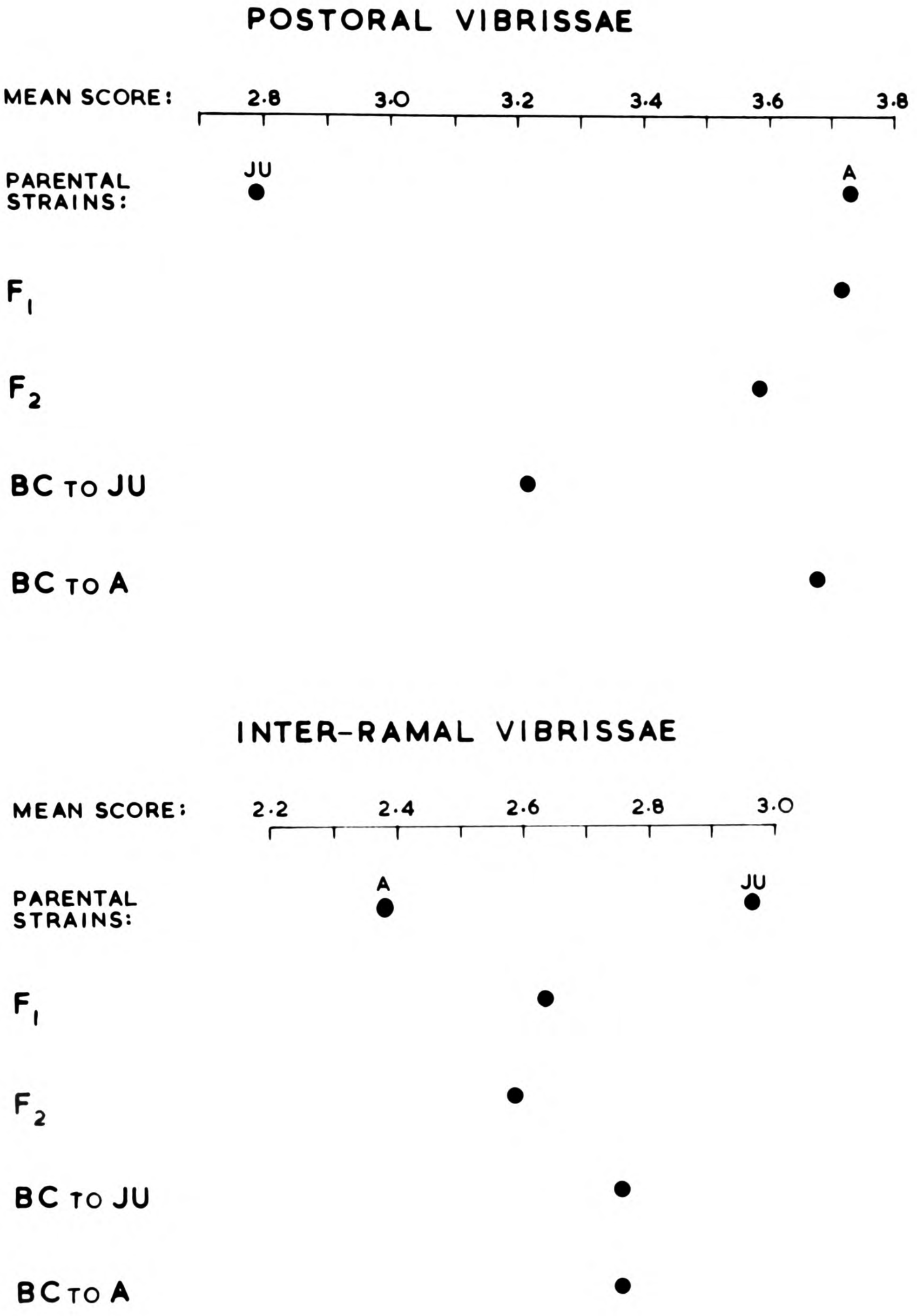


Fig. 14. Postoral and inter-ramal vibrissa scores of strains A and JU and the progeny of crosses between them.

vibrissa number, a secondary zone at the minimum level, shown in tabby animals, and a number of weak intermediate zones. These intermediate zones were thought to have been related to a tendency towards the preservation of symmetry in the paired groups (Fraser and Kindred, 1960; Kindred, 1963).

A number of other observations were made by Fraser, Nay and Kindred (1959). Tabby heterozygotes were found to be intermediate for total vibrissa number, but the different groups showed different degrees of dominance. Crinkled behaved as a complete recessive. The mean total vibrissa number in Ta and crcr mice was similar, but this similarity did not hold for the separate groups. Crinkled animals also showed a much wider range of variation than tabbies. Crosses between tabby and crinkled mice suggested that all these differences were due to background genotype. There was thought to be no evidence of interaction between the tabby and crinkled genes. No sex difference was found between TaTa and Ta mice and between crcr females and males.

Kindred (1967a) crossed crinkled into the stocks that had been selected for high and low vibrissa number in tabby animals. The extreme phenotypes of tabby and crinkled were found to be identical, but crinkled heterozygotes on the low selection line background showed a significant deviation from complete recessivity. Similarly there was a detectable difference between the coats of ++ and cr+ animals on the low, but not the high selection line background.

In some of the work reviewed here the abnormal postorbital vibrissae, detectable in tabby animals 5 days after birth, were scored.

These fibres are lost with the growth of the coat and, as scoring in the present study was at four weeks of age, only vibrissae with detectable sinus hair follicles were recorded.

(c) Correlated Characters and Maternal Effects

Although the individual groups of secondary vibrissae appear to be to some extent under independent control, selection for total vibrissa number produced correlated responses in other aspects of the tabby phenotype. Dun (1959) demonstrated a negative correlation between vibrissa number and the degree of striping of the coat of tabby heterozygotes. Dun and Fraser (1959) found that the tabby gene depressed growth more markedly in the low selection line than in the high selection line. Fraser and Kindred (1962) showed that the number of mystacial vibrissae in tabby mice, which is invariant in unselected stocks, responded slightly to selection practiced on the secondaries alone.

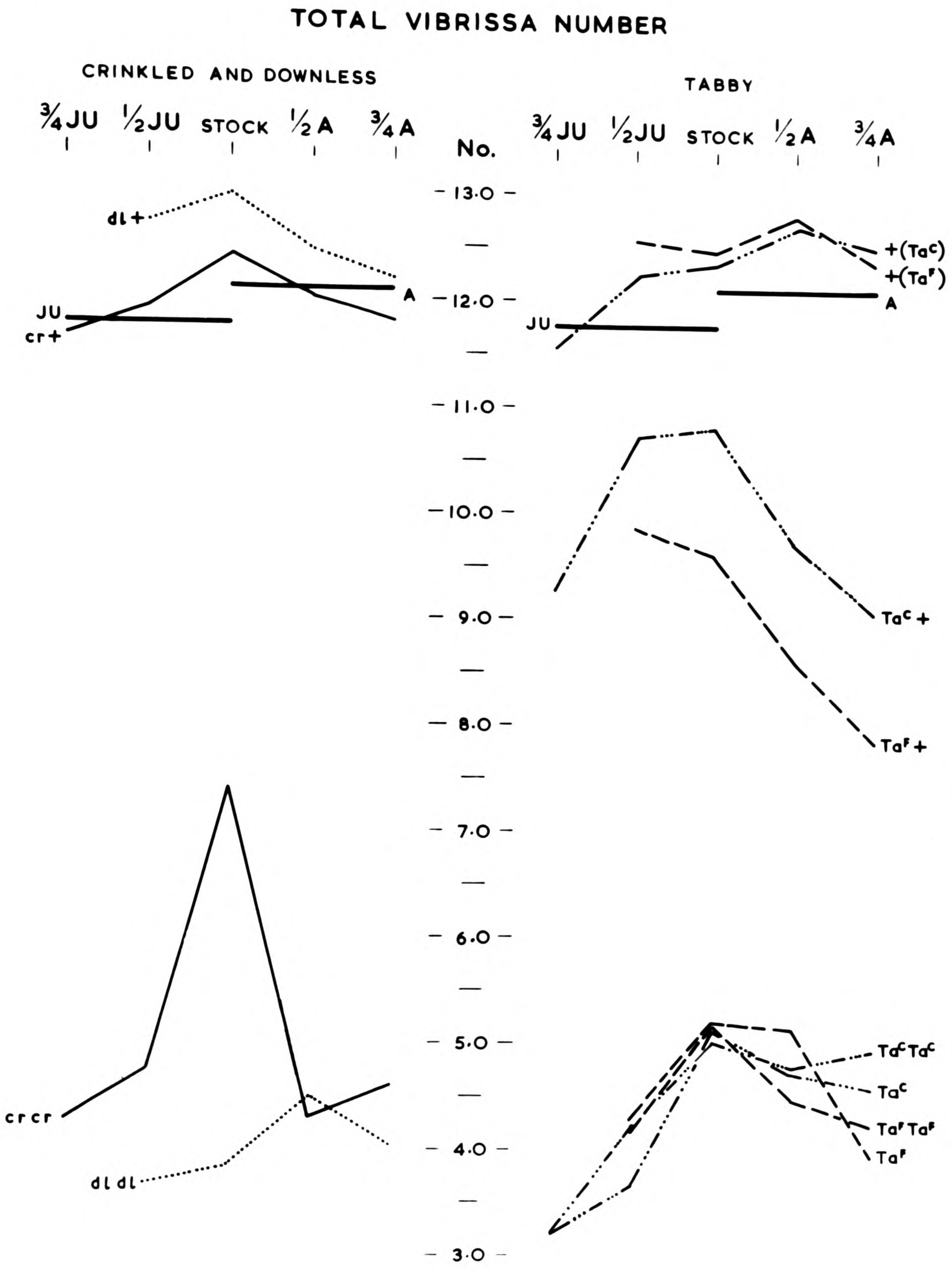
Two maternal effects on the total secondary vibrissa score in tabby heterozygotes have been reported. Ta+ progeny of Ta+ x + matings had higher scores than Ta+ progeny of ++ x Ta matings. There was no difference between the Ta+ progeny of TaTa and Ta+ mothers (Kindred, 1961). Ta+ progeny of brown mothers had slightly but consistently higher scores than Ta+ progeny of black mothers (Kindred, 1962). In the present study no account was taken of any colour genes. Brown, non-agouti, and albino animals were represented in most groups.

(ii) Results

The results are presented diagrammatically. Each point represents the mean vibrissa number for that group. The mean levels of the A and JU pure strain groups are shown as horizontal lines. Throughout, crinkled and downless are compared with the two tabby alleles.

Fig. 15. Total Vibrissa Number

Both crinkled and downless appeared to behave as complete recessives. By contrast, both tabby alleles showed an intermediate level of dominance. There were no apparent differences amongst homozygotes and hemizygotes. Crinkled homozygotes, which had a high score on the stock background, dropped to the common homozygote - hemizygote level on the inbred backgrounds. Tabby heterozygotes were more sensitive to changes of the background genotype than their wild type and homozygote and hemizygote litter mates.



TOTAL VIBRISSA NUMBER
CRINKLED AND DOWNLESS
TABBY
¾JU
½JU
STOCK
½A
¾A
No.
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
dl+
JU
cr+
A
crcr
dldl
+(Ta^c)
+(Ta^F)
Ta^c +
Ta^F +
Ta^cTa^c
Ta^c
Ta^FTa^F
Ta^F

facing p. 81

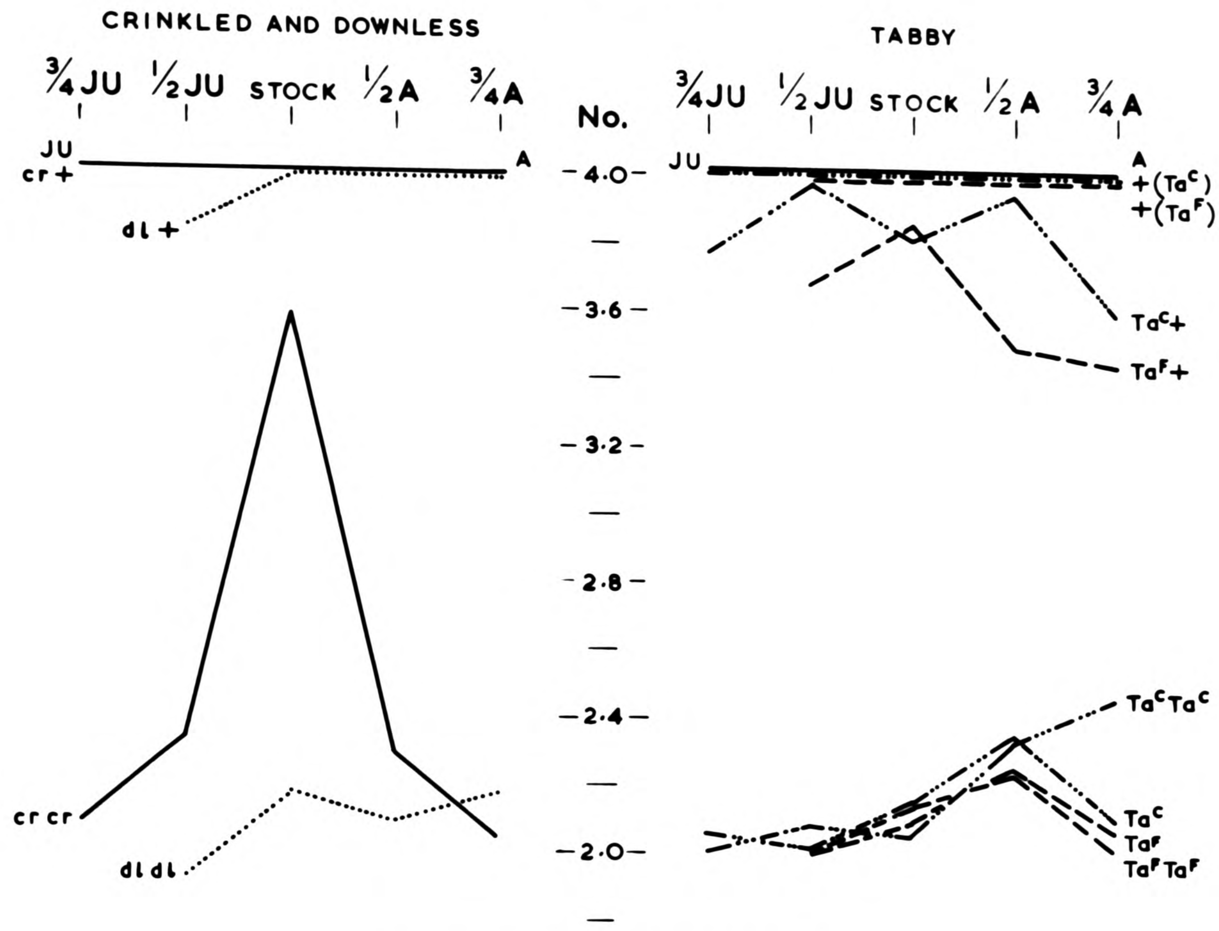
Fig. 16. Supraorbital Vibrissae

Crinkled was completely recessive. Downless showed a slight deviation from complete recessivity in one of the groups. Tabby was incompletely recessive. There were no marked differences amongst homozygotes and hemizygotes. Crinkled homozygotes, which approached the normal score on their stock background, fell dramatically to the common homozygote-hemizygote level on the inbred backgrounds.

Fig. 17. Postorbital Vibrissae

Crinkled and downless were completely recessive. Tabby was incompletely recessive. There were no marked differences amongst homozygotes and hemizygotes.

SUPRAORBITAL VIBRISSAE



POSTORBITAL VIBRISSAE

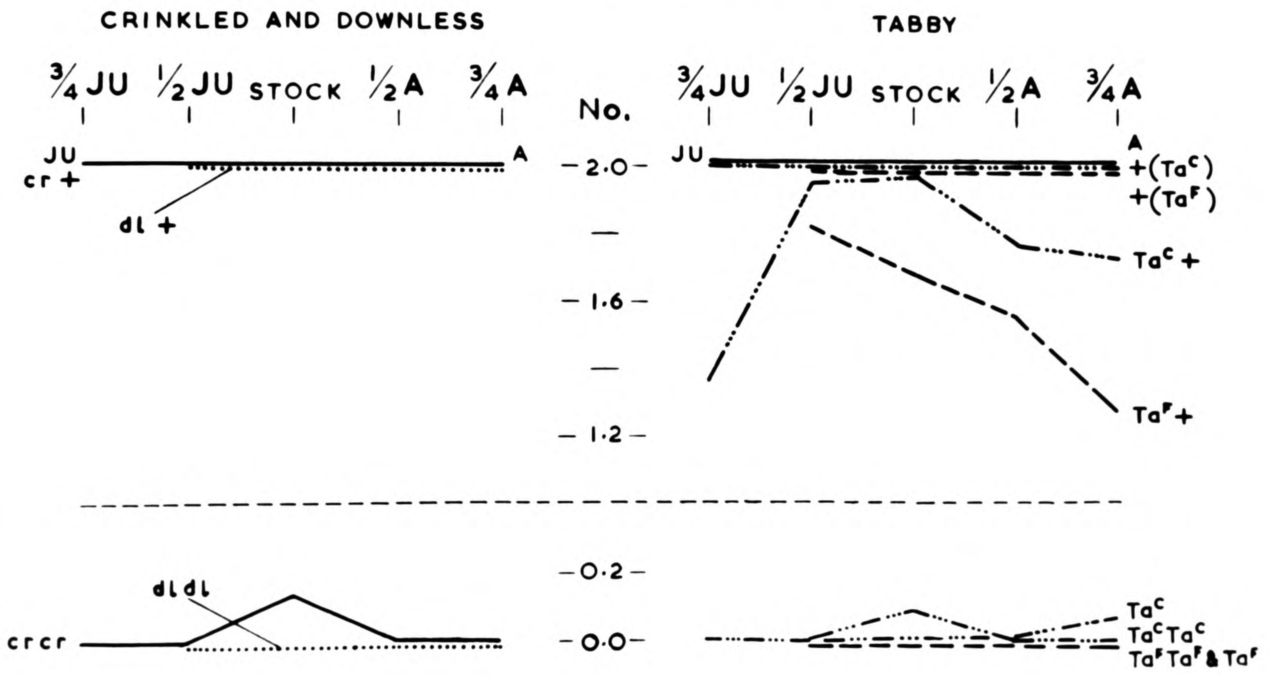
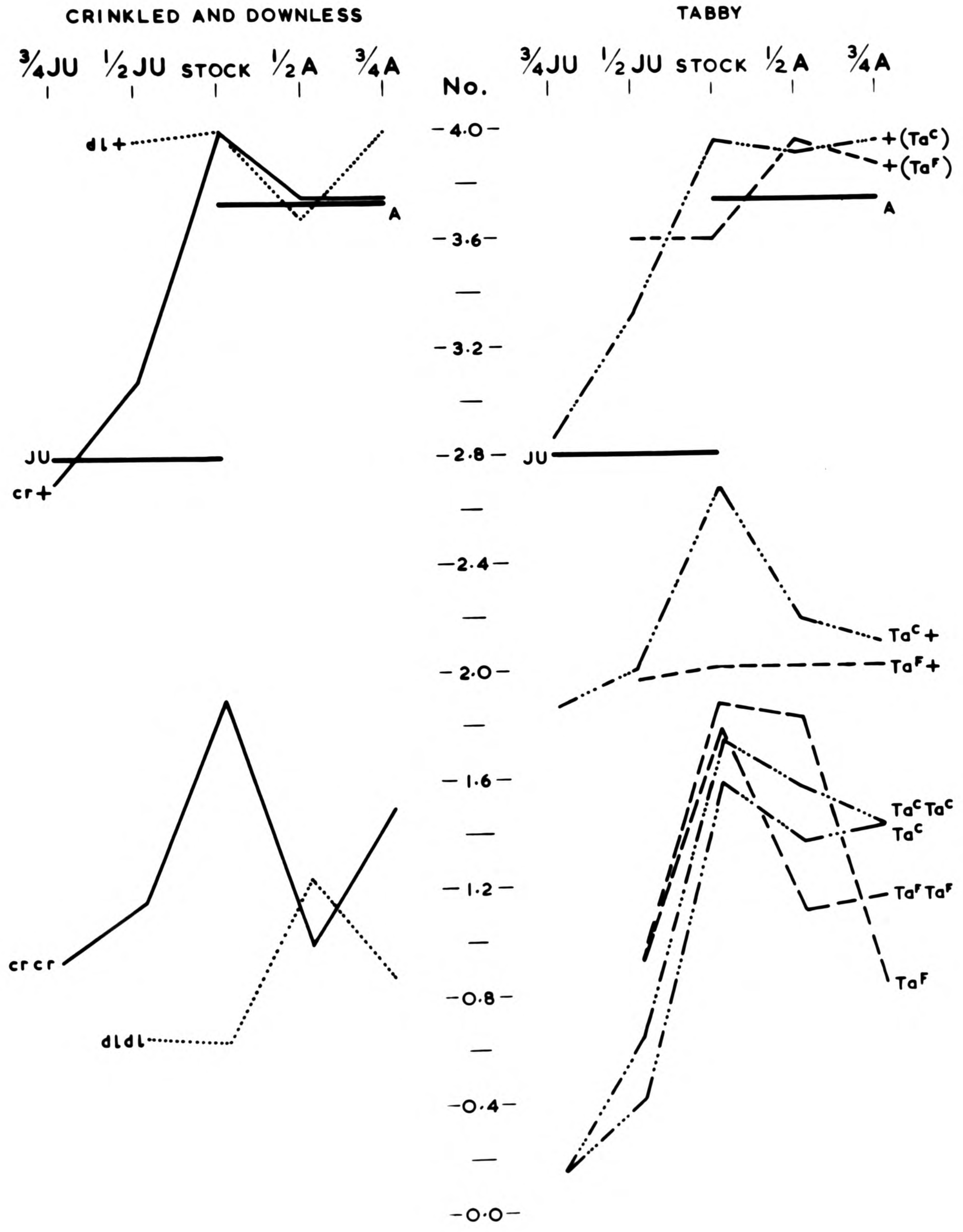


Fig. 18. Postoral Vibrissae

Crinkled and downless behaved as complete recessives. All homozygotes and hemizygotes showed a similar range of expression, though there was greater variation than in the other vibrissa groups. Tabby heterozygotes were intermediate and fairly stable around a value of 2.0 vibrissae. They were apparently less sensitive to background change than their wild type and homozygote and hemizygote litter mates.

POSTORAL VIBRISSAE

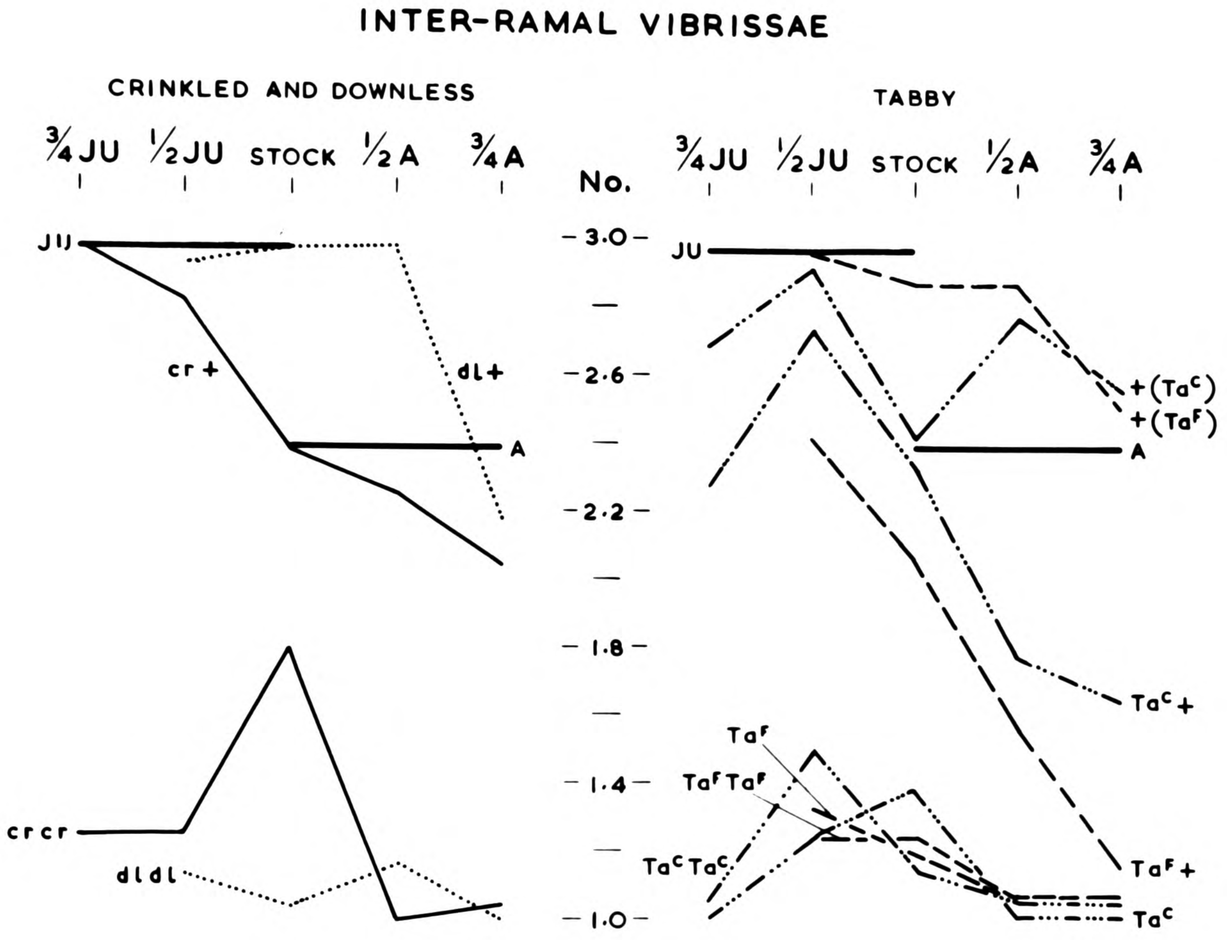


facing p. 83

Fig. 19. Inter-ramal Vibrissae

Crinkled and downless were not completely recessive. There were no marked differences amongst homozygotes and hemizygotes. Tabby heterozygotes were intermediate but varied over a very wide range. They were evidently much more sensitive to background change than their wild type and homozygote and hemizygote litter mates. This was the reverse of the situation which held for the postoral group.

The difference between groups C and D may have been associated with the different genetic behaviour of the two groups, illustrated by the A x JU crosses, and also with the fact that group C is bilateral whereas group D is single (Kindred, 1963; and 1967b).


INTER-RAMAL VIBRISSAE
CRINKLED AND DOWNLESS
TABBY
¾JU
½JU
STOCK
½A
¾A
No.
3.0
2.6
2.2
1.8
1.4
1.0
JU
cr+
dl+
A
crcr
dldl
+(Ta^c)
+(Ta^F)
Ta^c+
Ta^F
Ta^F Ta^F
Ta^c Ta^c
Ta^F+
Ta^c

facing p. 84

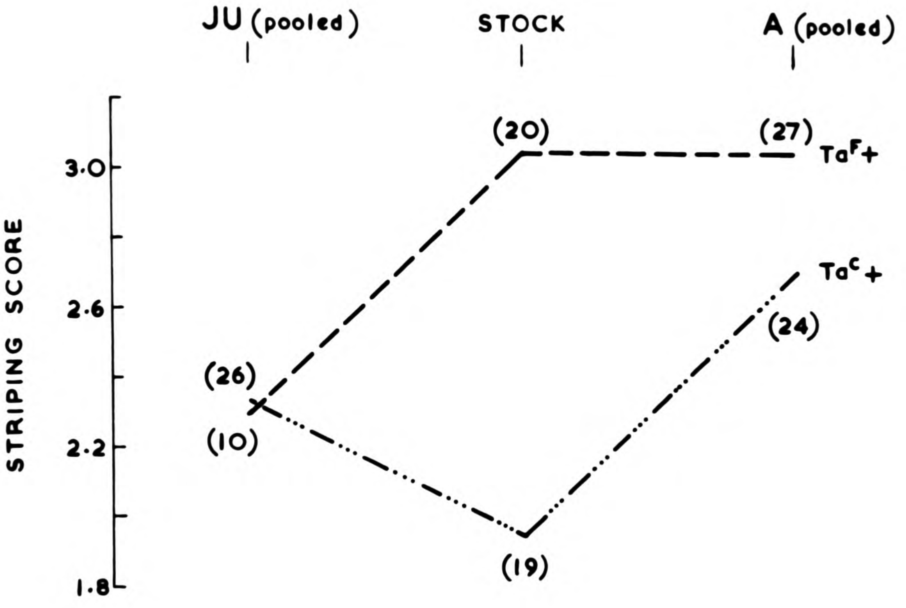
Tabby Heterozygote Striping

Unfortunately, both the inbred strains used were albino. The consequent small proportion of phenotypically agouti animals in the crosses made it necessary to pool the $\frac{1}{2}$ and $\frac{3}{4}$ groups of each background. Animals were scored on a six point scale from zero to 5. Zero was indistinguishable from wild type and 5 was the maximum intensity of striping. No zero grade animals were found. There was therefore no need to make a correction of -0.5 in the calculation of means for each group, as was the case in the analysis of the Tuck data. The mean scores are shown in Fig. 20. The numbers of animals that contributed to each mean are shown in brackets.

The total vibrissa scores of heterozygotes of the $\frac{1}{2}$ and $\frac{3}{4}$ groups of each background were similarly pooled, and with those of the stock groups were plotted against the mean striping scores. The result is shown in Fig. 21 where $r = -0.85$ ($p < 0.05$).

(iii) Summary

The results were consistent with previous work on tabby (Ta^F) and crinkled. Both types of tabby heterozygote were intermediate between the wild type and homozygous mutant levels, but the different vibrissa groups showed different degrees of dominance and differing sensitivities to changes of background genotype. By contrast, crinkled and downless were almost completely recessive, the greatest deviation from complete recessivity being shown by the inter-ramal group. There were no marked differences of homozygous expression of the genes, or between tabby homozygotes and hemizygotes.



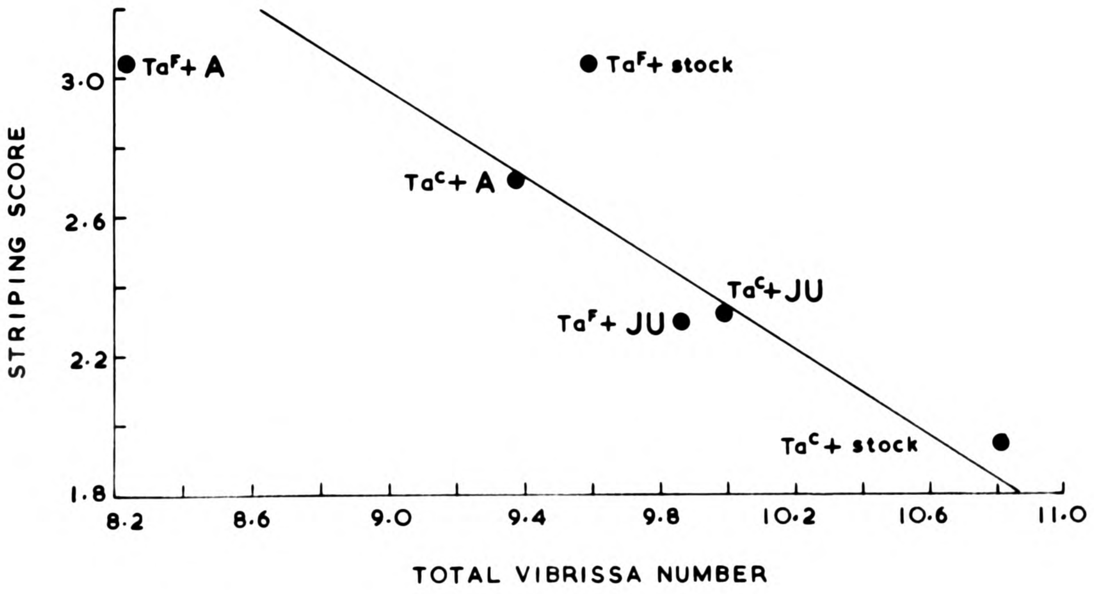


Fig. 20. (above) Variation of striping score with genetic background. The numbers of animals scored in each group are shown in brackets.

Fig. 21. (below) The relationship between striping score and total vibrissa number. $r = -0.85$ ($p < 0.05$)

Large differences which existed on the stock background were very much reduced as the background was made more common by crossing to the inbred strains. Striping and total vibrissa score in tabby heterozygotes showed a negative correlation.

4. The Dentition

(i) Scoring

Twenty-four characters within the dentition were considered. The object of the scoring method was to obtain an estimate of the mean level of abnormality of each character in each group of animals. The method combined an assessment of both penetrance and expressivity.

Table 6 lists the characters and shows the scores given to the different degrees of expression of each one. No intermediate level of expression was scored for characters 8, 9, 17, 18, 22, 23, and 24. Both sides were considered together, so that the maximum possible score was 4 per character per mouse. In each group of animals the total observed score for each character was expressed as a percentage of the total possible maximum. The effects of the mutant genes on the dentition were therefore considered in terms of percent abnormality.

Table 6

The scoring of 24 characters within the dentition.
N = normal, R = reduced, A = absent, P = present, and L = enlarged.

Character scored on each side			Score		
			0	1	2
1		Upper incisor	N	R	A
2		Lower incisor	N	R	A
3	m^1	B1	N	R	A
4		B3	N	R	A
5		1-L1 separation	N	R	A
6		L1-L2 separation	N	R	A
7		No. of roots	3	2	1
8	m^2	Rampart (Ra)	P	-	A
9		B1	N	-	L
10		B3	N	R	A
11		No. of roots	3	2	1
12	m_1	L1	N	R	A
13		B1	N	R	A
14		B2-L2 separation	N	R	A
15		B3-L3 separation	N	R	A
16		4	N	R	A
17		No. of roots	2	-	1
18	m_2	B1	P	-	A
19		B2-L2 separation	N	R	A
20		B3-L3 separation	N	R	A
21		4	N	R	A
22		No. of roots	2	-	1
23		Upper third molar	P	-	A
24		Lower third molar	P	-	A

(ii) Results

Grüneberg (1966a) has already commented on the non-random involvement of the different regions of the dentitions of tabby and crinkled heterozygotes. The present results show that the pattern of abnormalities was generally well maintained within genotypes at different levels of expression produced by different genetic backgrounds.

The results are expressed diagramatically according to the scoring system described. Rt = root.

Fig. 22. Ta^F+

There was a wide range of expression and the pattern was well maintained throughout. The stock background was intermediate, and expression was increased by crossing to the A strain, and decreased by crossing to JU.

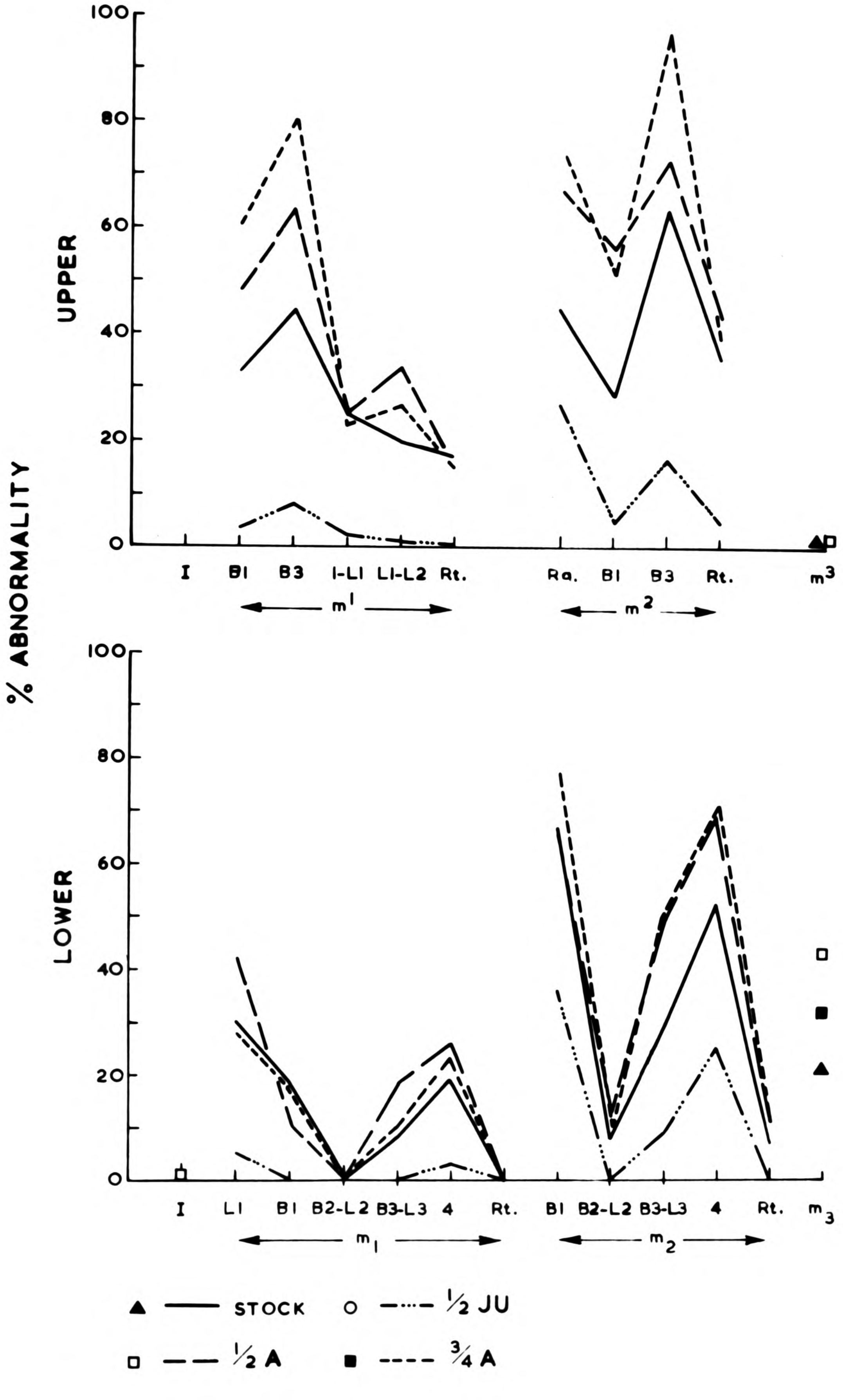


Fig. 23 Ta^c+

The range of expression here was not as great as that shown by Ta^F+. This was presumably associated with low penetrance on the stock background. Crossing to JU produced a slight increase of abnormality. Crossing to A increased it to a level almost equivalent to that shown by Ta^F+ animals on the A strain background. Thus, although the expression of Ta^c+ and Ta^F+ on their stock backgrounds was very different the difference was reduced by making genetic background more common to both.

Ta^c +

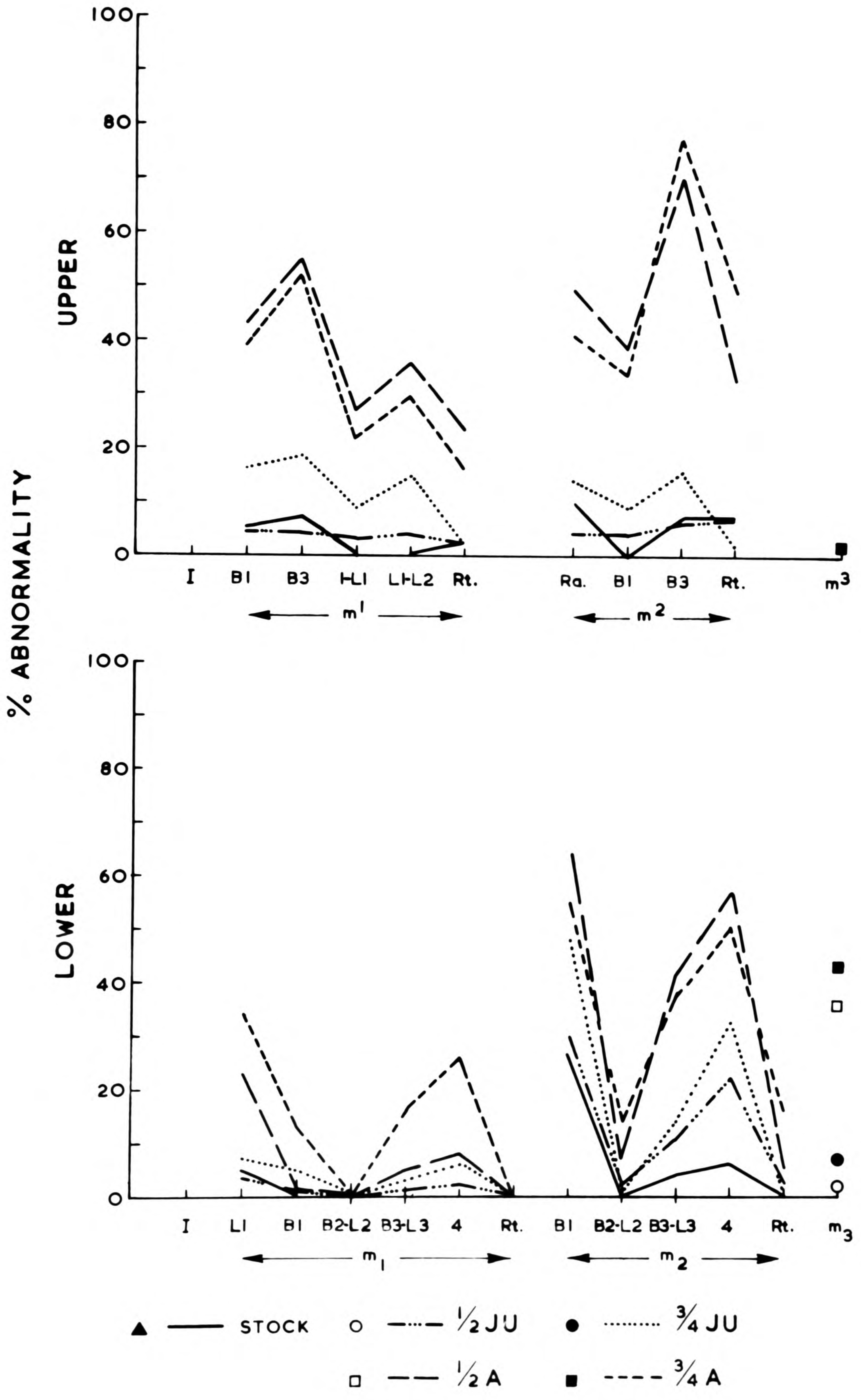

% ABNORMALITY
UPPER
100
80
60
40
20
0
I
B1
B3
I-L1
L1-L2
Rt.
Ra.
B1
B3
Rt.
m3
m1
m2
LOWER
100
80
60
40
20
0
I
L1
B1
B2-L2
B3-L3
4
Rt.
B1
B2-L2
B3-L3
4
Rt.
m3
m1
m2
STOCK
½ JU
¾ JU
½ A
¾ A

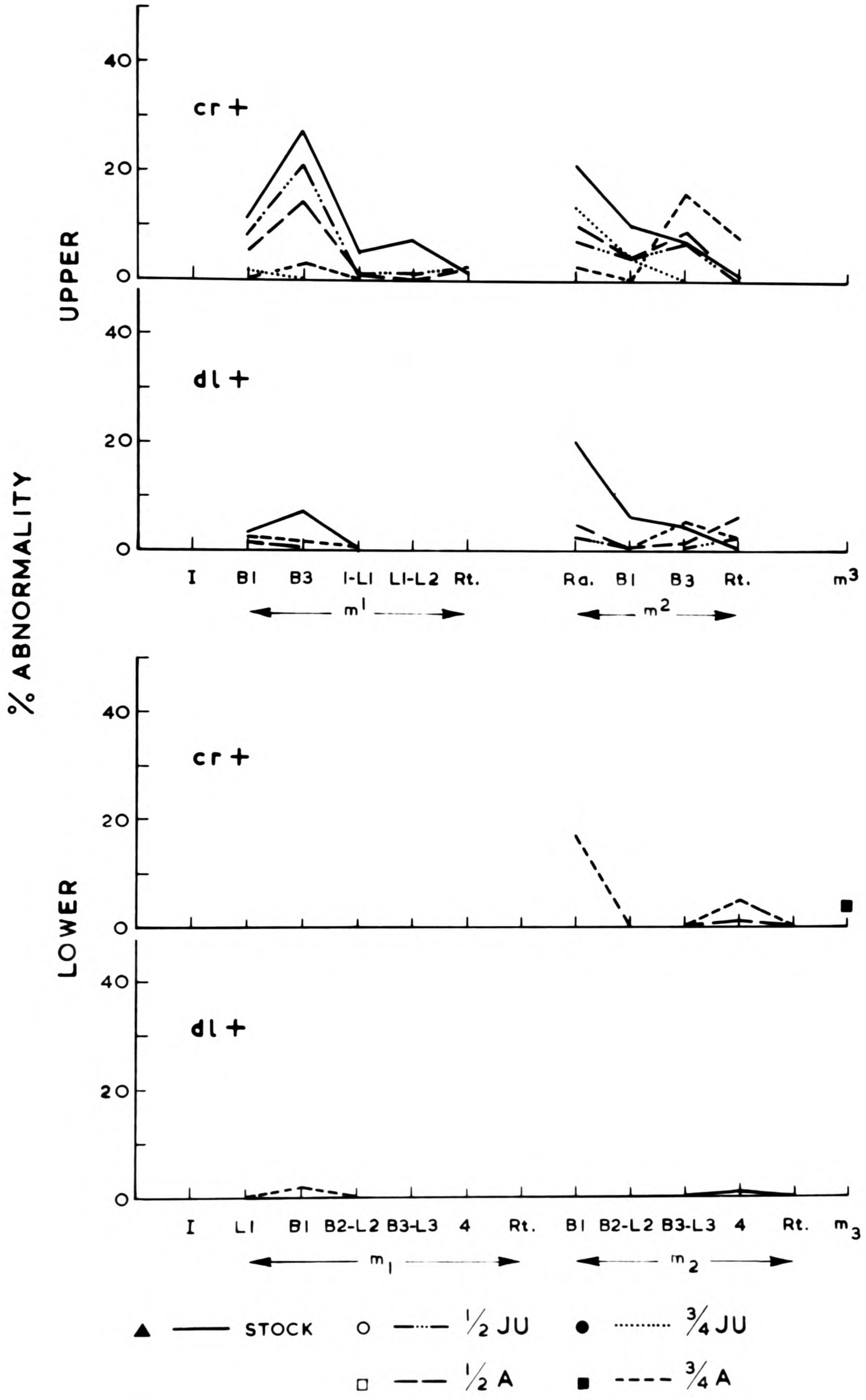
Fig. 24 cr+ and dl+

Both cr+ and dl+ animals showed relatively low penetrance of the abnormalities and little variation with changes of background. The lower jaw was hardly affected at all.

In the upper jaw the level of expression of cr+ on the stock background was higher than that of $Ta^{c}+$ on its stock background. However, whereas crossing to the A strain markedly increased the level of abnormality shown by $Ta^{c}+$, in cr+ animals abnormality was reduced (Compare with Fig. 23).

Downless behaved in a similar way to crinkled.

cr + AND dl +



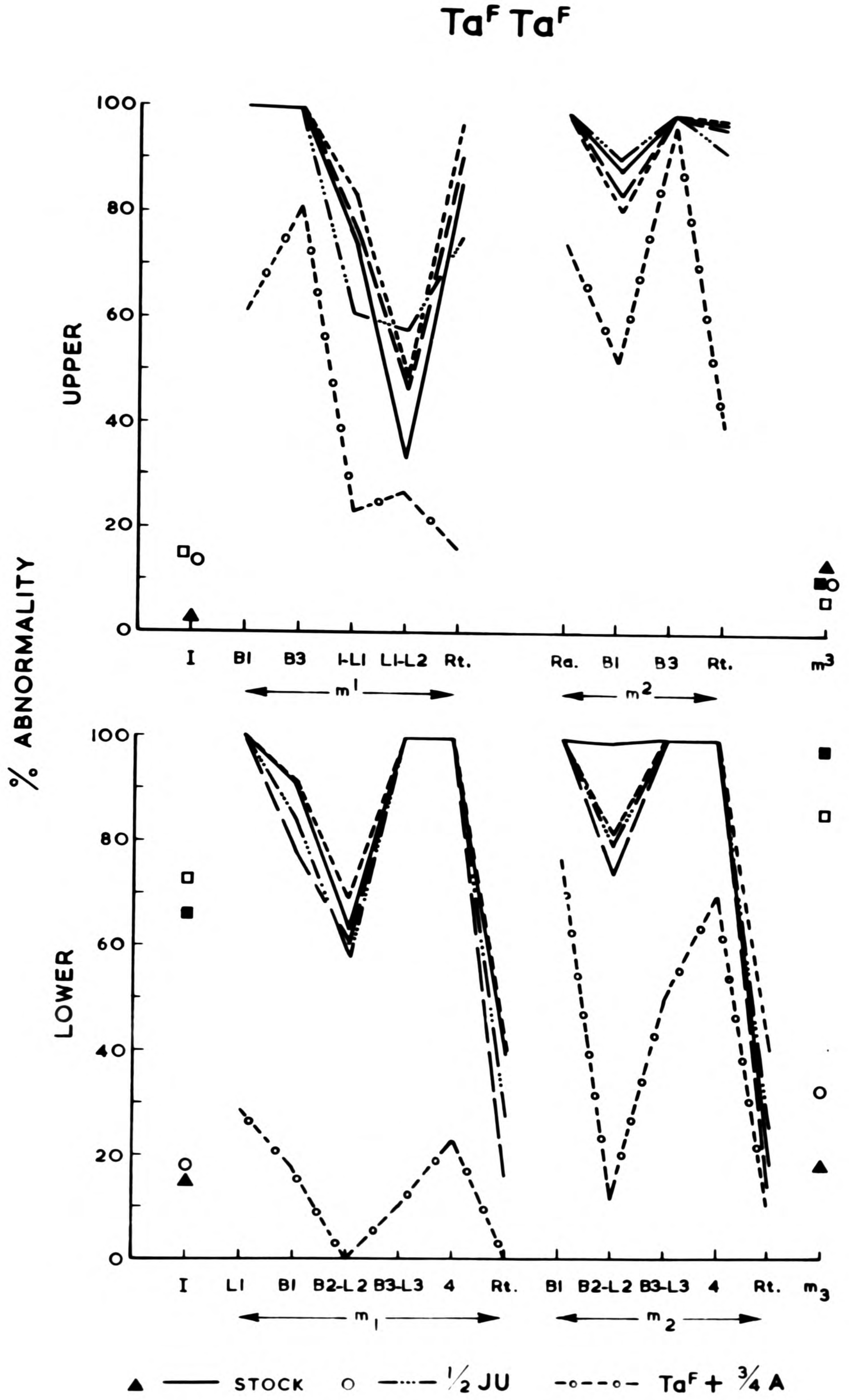
facing p. 91

Fig. 25. $Ta^F Ta^F$

The range of expression shown by the first and second molars was much less than in heterozygotes. That shown by incisors and third molars was, however, increased.

The heterozygote level on the $\frac{3}{4}$ A background is shown for the first and second molars so that the patterns and level of expression can be compared. The only inconsistency in the pattern between homozygotes and heterozygotes involves cusps L1 and L2 of m^1.

Tabby hemizygotes were similar.



$Ta^F Ta^F$
% ABNORMALITY
UPPER
LOWER
0
20
40
60
80
100
I
B1
B3
I-L1
L1-L2
Rt.
Ra.
B1
B3
Rt.
m^3
m^1
m^2
I
L1
B1
B2-L2
B3-L3
4
Rt.
B1
B2-L2
B3-L3
4
Rt.
m_3
m_1
m_2
▲ STOCK
○ 1/2 JU
Ta^F + 3/4 A
□ 1/2 A
■ 3/4 A

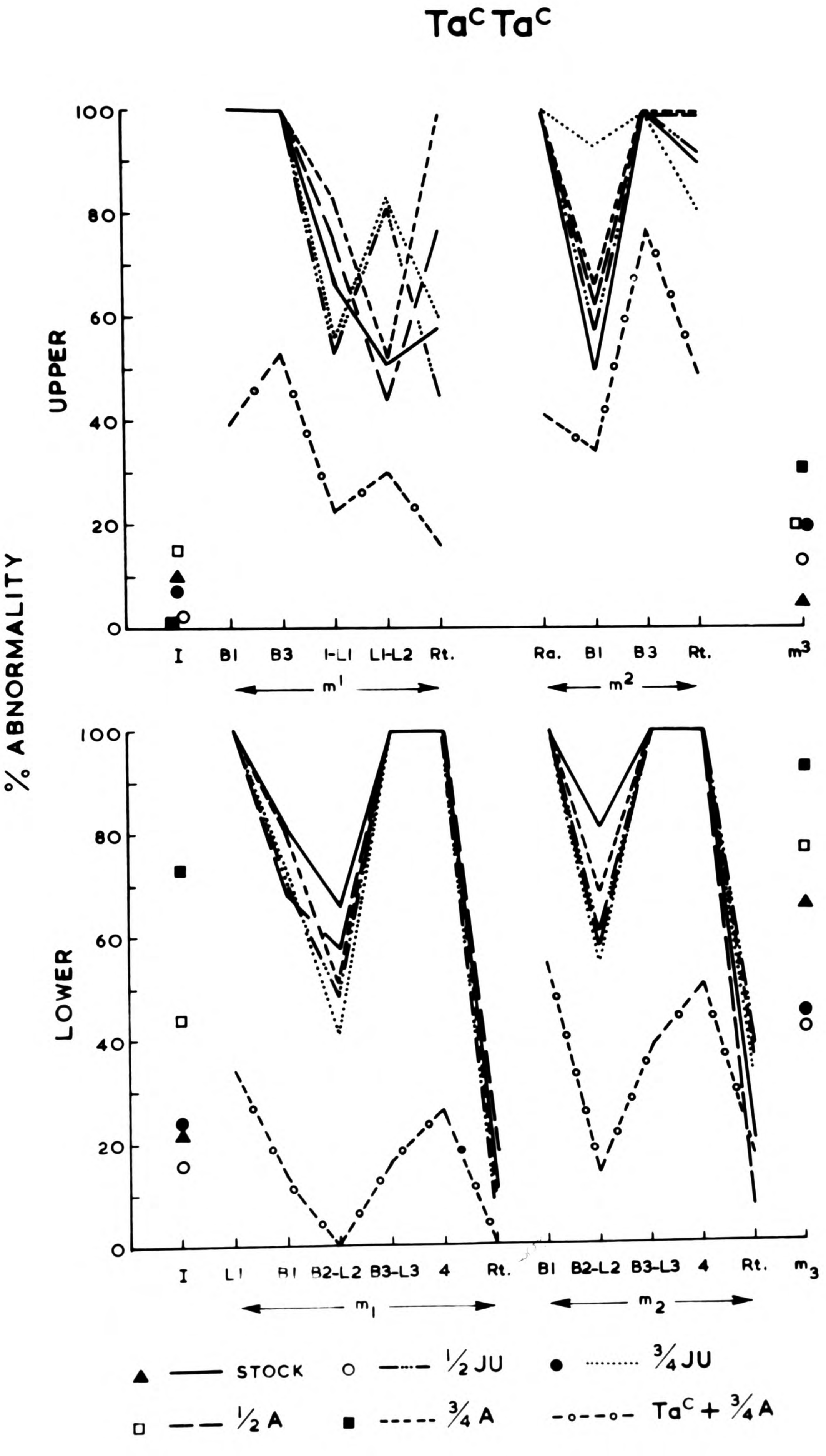
Fig. 26 Ta^cTa^c

The results present a generally similar picture to Fig. 25. There was, however, a marked difference in the pattern of abnormalities in m^1 between animals with A and JU backgrounds. (A slight difference is also detectable in Fig. 25. The regions involved are the same as those which did not conform to the heterozygote pattern).

The heterozygote level on the $\frac{3}{4}$ A background is again shown for comparison. The pattern at this level is completely consistent with that shown by homozygotes on the JU background.

Another feature is that crossing to both strains produced a general increase of abnormality in the upper molars but a general decrease in the lowers. This tendency was shown to some extent by all the genes.

Tabby hemizygotes were similar.



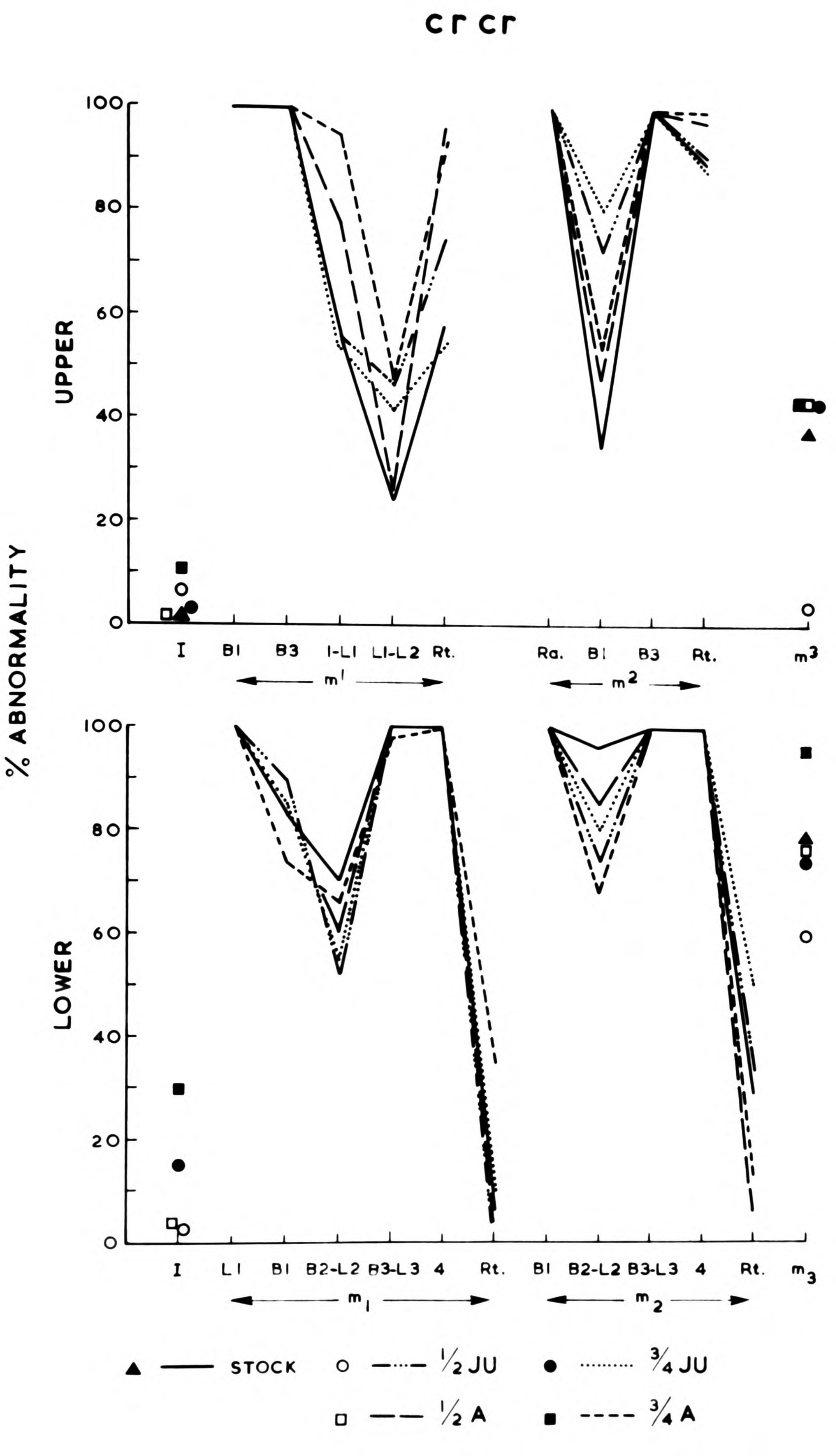
$Ta^c Ta^c$
% ABNORMALITY
UPPER
LOWER
100
80
60
40
20
0
I
B1
B3
I-L1
L1-L2
Rt.
m^1
Ra.
B1
B3
Rt.
m^2
m^3
L1
B1
B2-L2
B3-L3
4
Rt.
m_1
B1
B2-L2
B3-L3
4
Rt.
m_2
m_3
▲ STOCK
○ ½ JU
● ¾ JU
□ ½ A
■ ¾ A
Ta^c + ¾ A

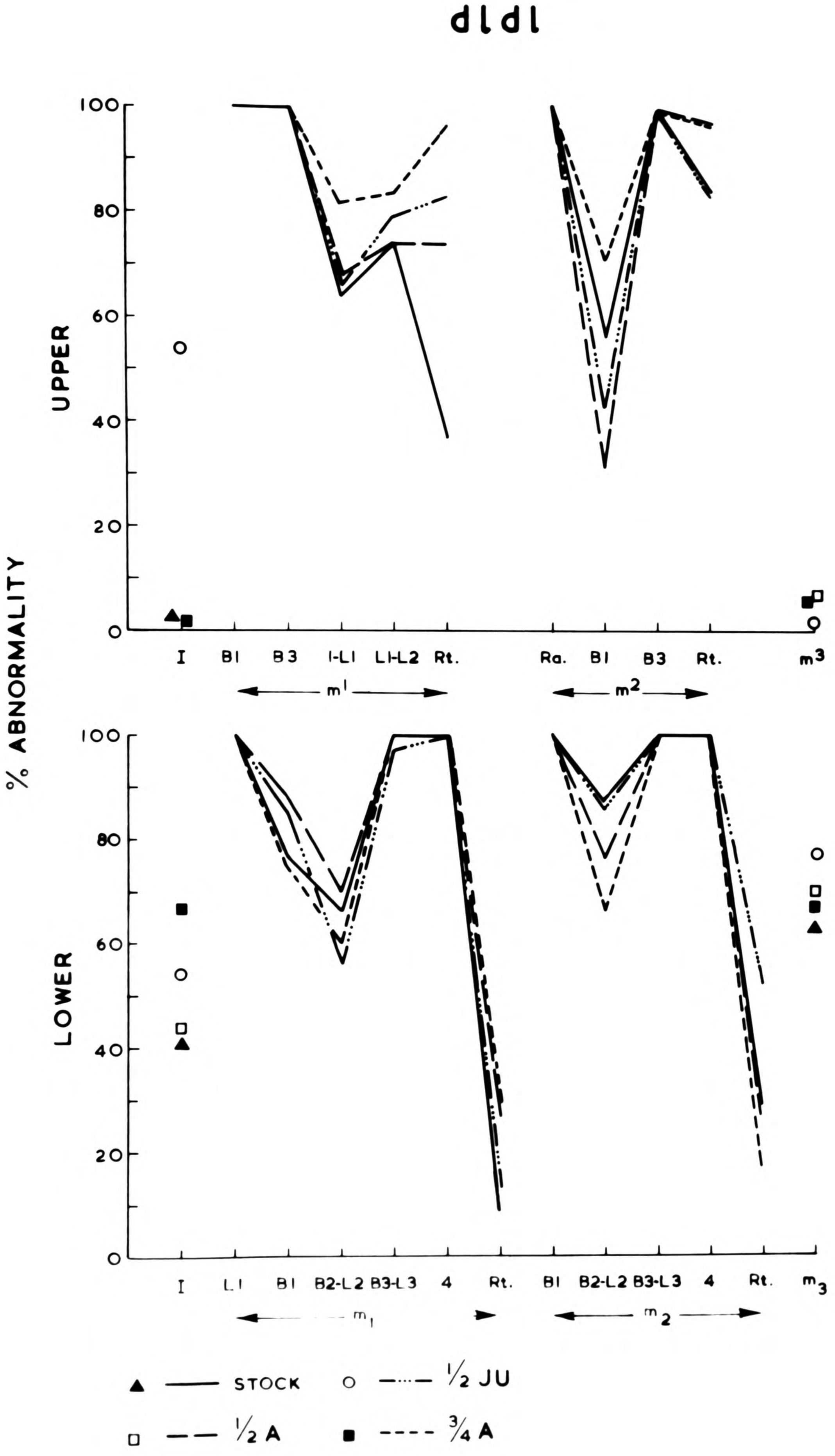
Fig. 27 crcr

The pattern here was the same as that shown by tabby homozygotes, and the levels of expression on the $\frac{3}{4}$ inbred backgrounds were similar.

Fig. 28 dldl

Downless homozygotes differed slightly from the others in respect of the pattern and intensity of abnormalities in m^1. In other respects they behaved in a similar manner to the other homozygotes.


cr cr
% ABNORMALITY
UPPER
100
80
60
40
20
0
I
B1
B3
I-L1
L1-L2
Rt.
Ra.
B1
B3
Rt.
m^3
m^1
m^2
LOWER
100
80
60
40
20
0
I
L1
B1
B2-L2
B3-L3
4
Rt.
B1
B2-L2
B3-L3
4
Rt.
m_3
m_1
m_2
STOCK
½ JU
¾ JU
½ A
¾ A



facing p. 94 (ii)

Fig. 29 Control Groups

The wild type control groups showed slight deviation from total normality. The low frequency of reduction in size of cusps and number of roots, and the absence of lower third molars in the A strain, was not unexpected. However, the occasional presence of a rampart in the + (Ta^F) and+(Ta^c) groups was surprising, as this characteristic was thought to be due specifically to the mutant genes.

The A strain, which as a background favoured the production of abnormalities in mutant animals, itself showed the highest mean incidence of abnormality amongst the control groups.

CONTROL GROUPS

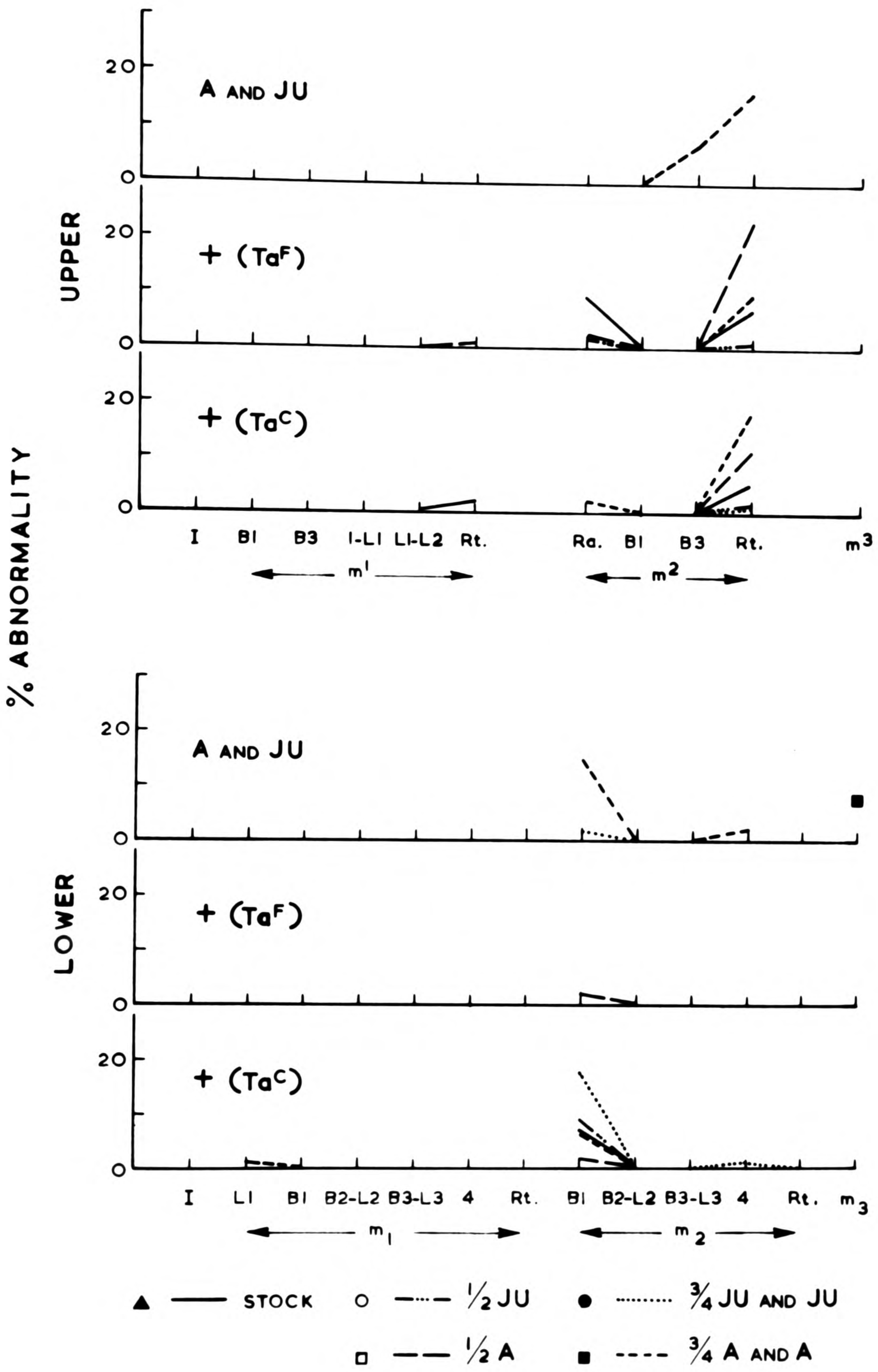
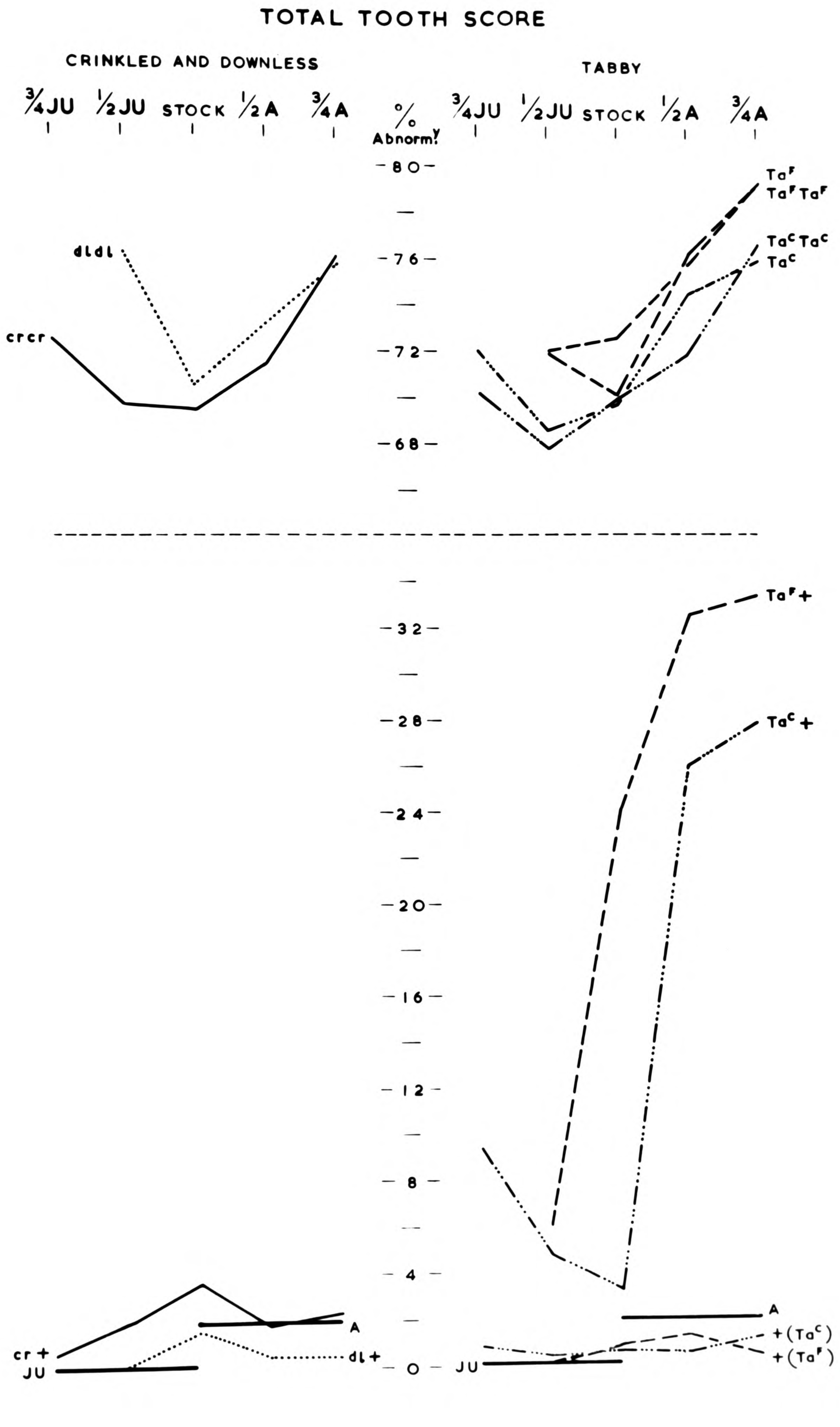


Fig. 30 Total Tooth Score

The total mean score of all 24 characters, an indication of the degree of abnormality of the dentition as a whole, is plotted here for each group in a manner similar to that used for vibrissa score. As with vibrissa score the plot demonstrates the near recessivity of crinkled and downless and the intermediate dominance of tabby. In crinkled and downless heterozygotes, and in all homozygotes and hemizygotes, expression was relatively stable. Tabby heterozygotes showed a wide range of variation with background genotype.


TOTAL TOOTH SCORE
CRINKLED AND DOWNLESS
TABBY
¾JU
½JU
STOCK
½A
¾A
% Abnorm.y
80
76
72
68
32
28
24
20
16
12
8
4
0
dldl
crcr
Ta^F
Ta^F Ta^F
Ta^c Ta^c
Ta^c
Ta^F +
Ta^c +
cr +
JU
A
dl +
+ (Ta^c)
+ (Ta^F)

(iii) Summary

The relative effects of the genes on the dentition were comparable with those on the vibrissae. Both types of tabby heterozygote behaved in a similar way and showed a wide range of variation with the background. Crinkled and downless heterozygotes varied little and were, on average, much more recessive.

Homozygotes and hemizygotes showed the same pattern of abnormalities as heterozygotes in a more extreme form. Amongst homozygotes and hemizygotes there was relatively little background variation and little, if any, difference between the genes. The only region where there was any variation in the pattern was the lingual side of the crown of m^1.

Because of the existence of a pattern which is common to homozygotes, hemizygotes and heterozygotes, the mixed features of the tabby heterozygote dentition cannot be accounted for in terms of cell specific mosaicism. A quantitative analysis of the molars of tabby heterozygotes, and a comparison of them with those of crinkled heterozygotes, led Grüneberg (1966a) to this conclusion. Grüneberg (1966b) pointed out that the involvement of the vibrissae of tabby heterozygotes also conforms to a pattern. The existence of these patterns was considered to be incompatible with the Lyon hypothesis.

5. Discussion

(i) An Explanation of the Pattern

(a) The Teeth

An attempt to explain in developmental terms the pattern of abnormalities in the crowns of first and second molars is made below.

Lower Molars

It has already been pointed out that the areas which are abnormal in tabby and crinkled teeth are generally those which develop late (Grüneberg, 1965). The order of sensitivity to the mutant genes of the various regions can be derived from the data presented in the previous section. The order in m_1, starting with the most sensitive region was: L1, 4, B3-L3, B1, B2-L2. This is almost the exact reverse of the sequence in which these regions normally develop. The only exception is that an anterior extension of the crown, which subsequently develops into L1, is present before cusp 4 appears (Gaunt, 1955). Teeth with L1 absent and cusp 4 present were found only amongst tabby heterozygotes. In homozygotes and hemizygotes both cusps were invariably absent. It seems likely that preferential suppression of the anterior end of m_1 could be the result of attempted or successful formation of a supernumerary tooth germ.

An interesting parallel to the varying grades of abnormality of m_1 was the increase in complexity of lower supernumeraries with increasing size. This is shown in Fig. 31. Up to a point the complexity of supernumerary varied as the reverse of the scale of abnormality in m_1. The sizes are roughly comparable, so a supernumerary was generally more complex for its size than m_1.

The size interaction between a developing supernumerary and m_1 has already been discussed. Fig. 31 shows that there were associated

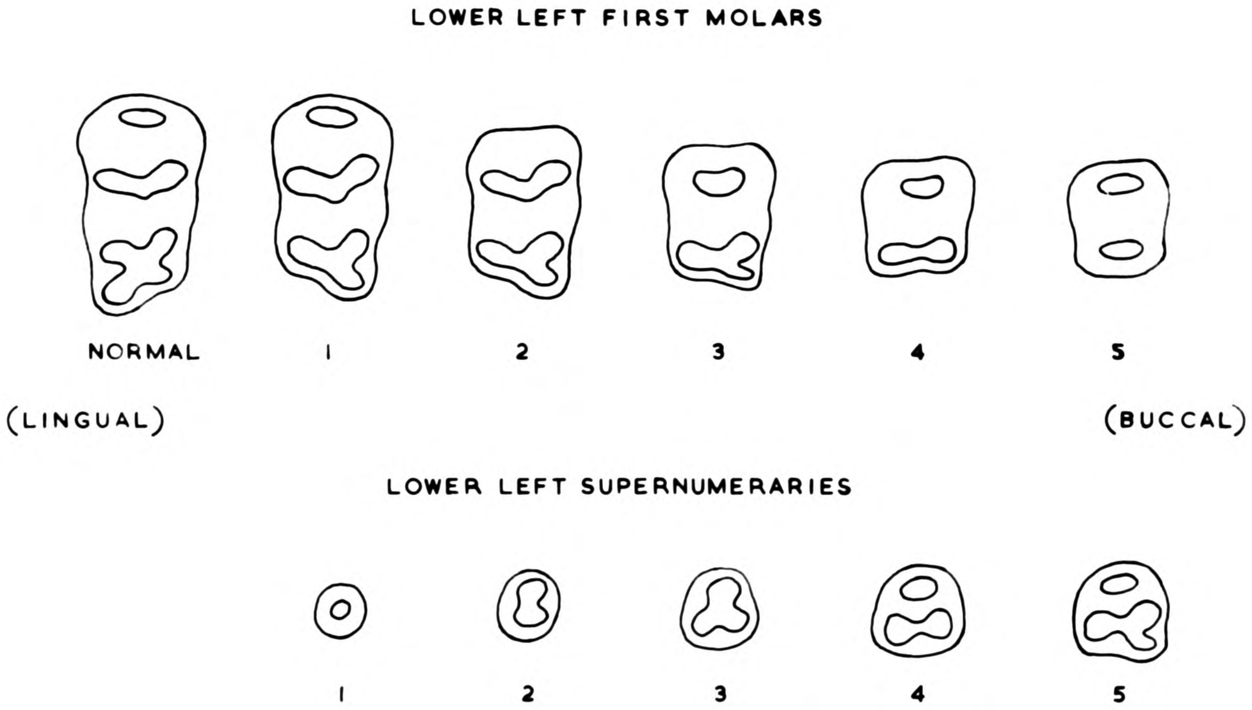


Fig. 31. Diagrams of occlusal surfaces of varying grades of lower left first molars and supernumerary teeth.

morphological changes. It is not intended, however, to convey the impression that an m_1 of a particular grade was always accompanied by a supernumerary of the same grade. All grades of m_1 were found in cases where there was no supernumerary at all. At the other extreme, there were instances where a grade 2 m_1 and a grade 5 supernumerary occurred together.

The maximum degree of abnormality of m_1 in heterozygotes was grade 4, and the most normal m_1 in homozygotes and hemizygotes was grade 2. All grades of supernumerary were found in both heterozygotes and in homozygotes and hemizygotes. It is of interest from the viewpoints of both ontogeny and phylogeny that the normal m_2 is comparable with grade 1, and the normal m_3 comparable with grade 4, of m_1 abnormality.

The order in which the various regions of m_2 were affected, starting with the most sensitive, was: B1, 4, B3-L3, B2-L2. This differs from m_1 in that B1 is the most sensitive. However, cusp B1 of m_2 is very small, and it is not difficult to understand how it could be lost first with general reduction of size and complexity of the whole crown.

Grüneberg (1966a) found that, in tabby heterozygotes, second molars were more often abnormal than the first. The relative sensitivities of the different regions of m_1 and m_2 observed in the present study are combined on the next page.

	m_1	m_2
Most sensitive:		B1
		4
		B3-L3
	L1	
	4	
	B3-L3	
	B1	
		B2-L2
Least sensitive:	B2-L2	

The difference between m_1 and m_2 can be explained in relation to the times at which they develop. The normal m_1 develops from an early to a very advanced state of morphogenesis during the follicle formation phase. The equivalent stages of development of the normal m_2 are about four days later, and therefore fall within the second period of follicle suppression. On this basis, the effects of the mutant genes on the developing m_2 would be expected to be more severe than on m_1.

Upper Molars

The relative sensitivities of the different regions of m^1 and m^2 were as follows:

	m^1	m^2
Most sensitive:		B3
	B3	Ra
	B1	
		B1
Least sensitive:	1-L1, L1-L2	

Within each tooth the order is not the exact reverse of the order of development. Referring to Gaunt (1955, 1956 and 1961), Grüneberg (1965) pointed out that B3, here shown to be the most sensitive region, develops early. The most obvious difference between normal and tabby germs at an early stage of morphogenesis is the greater bulbosity of the tabby germ. A comparison of wax reconstructions of A strain and Ta m^1 germs at 17 days showed that the tabby germ was not only considerably shorter anteroposteriorly, but also a good deal wider. A difference in the distribution of tension in the posterior part of the crown at this stage could, perhaps, prevent the formation of a sulcus which normally develops between B3 and the future cusp 3. If this were the case, what has all along been called cusp 3 of tabby upper molars would, strictly speaking, be homologous with a normal B3. The proliferation that would normally have gone to produce a sulcus between B3 and 3, and to enlarge the forming cusp 3, would then be diverted to enlarging B3 alone. B3 could then take up a fairly central position at the posterior end of the crown.

Summary

The regions of first and second molar crowns which appear later in ontogeny were generally progressively more sensitive to the effects of the mutant genes. The only major exception was the involvement of cusp B3 of the upper molars, and for this a possible explanation has been offered. Growth and differentiation of dental epithelium has been shown to be delayed in mutant animals. As mutant teeth are smaller than normal, growth must be the more severely affected of the two. The cusp pattern of a mutant molar can therefore be regarded as the result of a more or less normal but rather slow course of development which has been interrupted by the relatively premature onset of calcification.

(b) The Vibrissae

The pattern of vibrissa involvement does not appear to have a simple explanation. Developmental sequence cannot be the primary cause as the postorbital fibre, which is almost invariably absent in homozygotes and hemizygotes, develops early, whereas the central inter-ramal fibre, which is always present, develops late. In addition, the pattern is not consistent under all conditions. The relative sensitivities to reduction of groups C and D on the A strain background have been shown here to be completely reversed on the JU background.

It seems likely that local competition, as postulated by Dun (1959), may be partly responsible. Except for the postorbital site in homozygotes and hemizygotes, reduction of number in the individual vibrissa groups was usually not complete in any of the genetic situations in which they were examined. A few members of each group were generally retained in each group of animals.

It seems reasonable to suppose that the differential involvement of the vibrissae is probably dependent on a combination of developmental sequence, relative independence of genetic control, and local conditions.

(ii) Correlated Characters

It has already been shown by Dun (1959), and also in the present work, that total vibrissa number in tabby heterozygotes is negatively correlated with the degree of striping. It is now proposed to consider the response to changes of genetic background of total tooth score (Fig. 30) in relation to total vibrissa number (Fig. 15), and the incidence of lower supernumerary tooth formation (Fig. 32).

(a) Total Tooth Score and Total Vibrissa Number

Tabby heterozygotes were considered separately from all homozygotes and hemizygotes. Crinkled and downless heterozygotes were not included in the analysis. Correlation coefficients were calculated from the mean scores of each group of animals. There were therefore 9 pairs of measurements for tabby heterozygotes, and 27 pairs for homozygotes and hemizygotes.

A high negative correlation was found between total tooth score and total vibrissa number amongst tabby heterozygotes ($r = -0.83$, $p < 0.01$). Amongst homozygotes and hemizygotes, however, there was only a very low non-significant negative correlation ($r = -0.15$). This discrepancy seems to be attributable to the reducing effect of the JU background on group C vibrissae. The effect was marked in tabby homozygotes and hemizygotes, but not in heterozygotes (Fig. 18). As the JU background favoured greater normality in the teeth the vibrissa effect must have tended to blurr the inverse relationship between total tooth score and total vibrissa number. It seems likely, however, that had it been possible to examine the teeth of lines selected for total vibrissa number (Dun and Fraser, 1959), a higher negative correlation amongst hemizygotes would have been found.

(b) Total Tooth Score and Lower Supernumeraries

The criteria used for the diagnosis of supernumeraries have already been discussed. Fig. 32 shows the incidence of lower supernumeraries in the different background groups of animals, heterozygotes being separated from homozygotes and hemizygotes. The number of lower quadrants with supernumeraries was expressed as a percentage of the total for each group. In tabby heterozygotes the A strain background favoured the development of supernumerary teeth, whereas in homozygotes and hemizygotes the situation was completely reversed.

Correlation coefficients were calculated as described above. There was a high positive correlation between total tooth score and the incidence of supernumeraries amongst tabby heterozygotes ($r = +0.89$, $p < 0.01$), but a negative correlation amongst homozygotes and hemizygotes ($r = -0.45$, $p < 0.05$).

The positive correlation amongst heterozygotes is to some extent spurious in that interaction between supernumerary and m_1 occurs. The presence of a supernumerary would therefore automatically be associated with an abnormal m_1, and probably a reduced m_2 and absent m_3. However, if m_1 were not intrinsically abnormal the supernumerary would probably have never developed anyway.

In homozygotes and hemizygotes the incidence of supernumeraries was highest in the least abnormal dentitions. There does not appear to be any

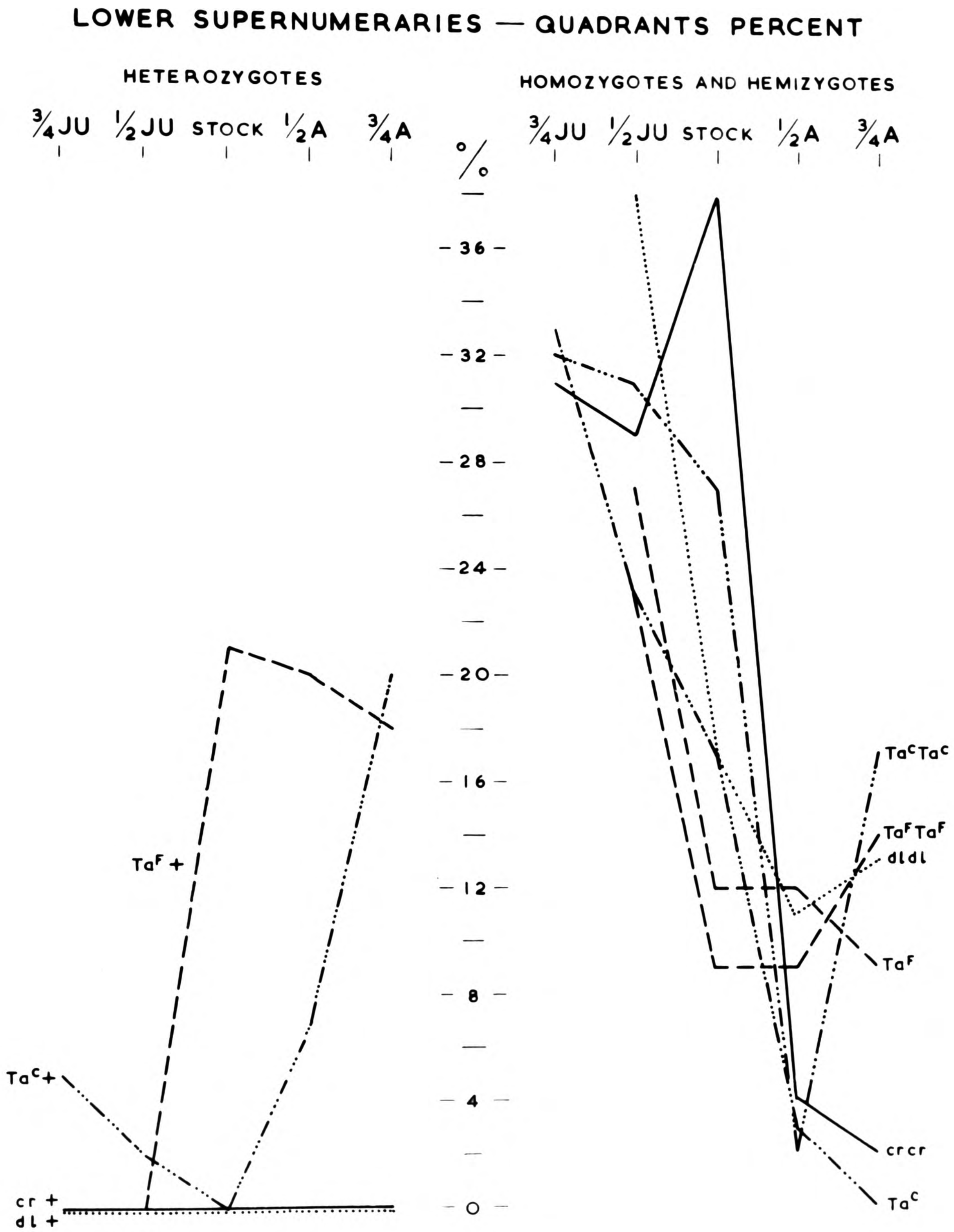


Fig. 32. The incidence of lower supernumerary teeth in different gene and background groups.

reason why this relationship should be spurious. On the contrary, an interaction such as that just mentioned would tend to obliterate it. Also, it seems improbable that wholesale misdiagnosis of supernumeraries could be responsible, as m_1 is more likely to be reduced to a point where m_2 is larger in the most abnormal dentitions. Thus, it is likely that misdiagnosis would also tend to obliterate a negative correlation. It is therefore concluded that both correlation coefficients reflect the true nature of the situation.

A model to explain these relationships is presented in Fig. 33. It is postulated that the primary effect of the mutant genes is to suppress epithelial proliferation. It has already been suggested that the differentiating cells of a tooth germ are more likely to be affected by such suppression than the less well differentiated cells of the dental lamina, and that this is the reason why supernumerary teeth develop. Using total tooth score as the criterion, suppression is at a maximum in homozygotes and hemizygotes. Within homozygotes and hemizygotes, and within heterozygotes, it varies with the genetic background, the A strain background favouring suppression and the JU background favouring normality.

It is proposed that as m_1 is progressively more suppressed, there is an accompanying increase in the tendency of the anterior extension of dental lamina to proliferate. With further increase of the suppressive influence the dental lamina itself becomes affected, the rate of increasing incidence of proliferation becomes reduced, a maximum level is reached, and a decrease towards zero commences. It follows from Fig. 33 that the hypothetical maximum incidence of supernumerary tooth formation would occur with a suppressive

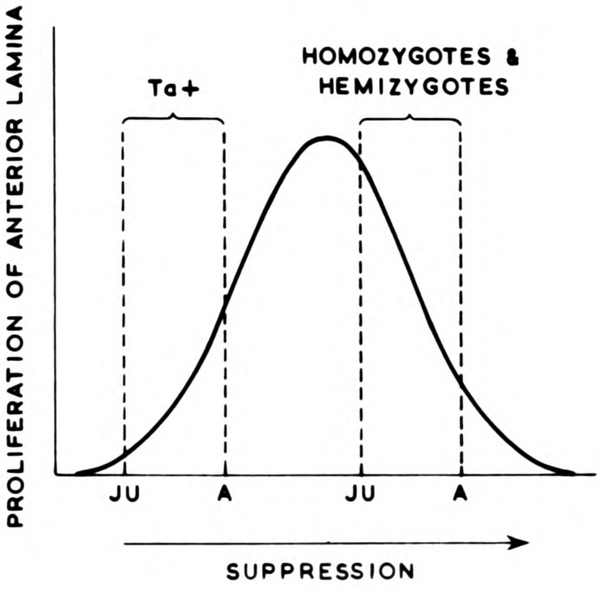


Fig. 33. A model to explain the varying incidence of supernumerary tooth formation.

influence somewhere between that in the most abnormal tabby heterozygotes and the least abnormal homozygotes and hemizygotes.

V. Gene Combinations

1. Introduction

Kindred (1967a) investigated the effects on the coat and vibrissae of combinations between tabby (Ta^F) and crinkled, the genetic backgrounds being those of the lines that had been selected for high and low total vibrissa number. Ta+. cr+ females were compared with Ta+.++ females, and Ta.cr+ males with Ta.++ males. The addition of a crinkled allele to tabby heterozygotes was found to increase total vibrissa number on the high selection line background, but not on the low line background. A similar effect was found in unselected stocks by Fraser, Nay, and Kindred (1959) but was then thought to be due to background interaction. The addition of a crinkled allele to tabby hemizygotes produced a higher total score on the low line background, but a lower score on the high line background. The effects on the coat were inconclusive. No attempts have yet been made to detect evidence of interaction in the dentition.

2. Material

Unlike the material in the main body of this study animals used here were all the progeny of homozygous mothers. The use of homozygous mothers provided an opportunity to look for differences in maternal effect between homozygous and heterozygous mothers. No such maternal effect on total vibrissa number was found when tabby homozygote and heterozygote mothers were compared (Kindred, 1961).

The following groups of animals were collected:

Group		Parents	
Genotype	Background	Mother	Father
Ta^c+	$\frac{7}{8}$ JU	Ta^cTa^c $\frac{3}{4}$JU	JU
$Ta^c+.cr+$	$\frac{3}{4}$JU	Ta^cTa^c $\frac{3}{4}$JU	crcr $\frac{3}{4}$JU
Ta^F+	$\frac{1}{2}$JU	Ta^FTa^F $\frac{1}{2}$JU	+ $\frac{1}{2}$JU
$Ta^F+.dl+$	$\frac{1}{2}$JU	Ta^FTa^F $\frac{1}{2}$JU	dldl $\frac{1}{2}$JU
Ta^cTa^c and Ta^c	stock	Ta^cTa^c stock	Ta^c stock
crcr	stock	crcr stock	crcr stock
dldl	stock	dldl stock	dldl stock
Ta^cTa^c.crcr and Ta^c.crcr	stock	Ta^cTa^c.crcr	Ta^c.crcr
Ta^cTa^c.dldl and Ta^c.dldl	stock	Ta^cTa^c.dldl	Ta^c.dldl

All groups except one were within the same range of size as those of the main experiment. The exception was the $Ta^c+.cr+$ group which, because of poor fertility, was composed of only 9 animals.

In addition to the other characters scored the number of tail hairs was counted. Absolute counts were made up to 100 hairs, but animals with a greater number were classed together as > 100.

3. Results

Groups of animals were compared in pairs by chi-square tests. The different vibrissa groups and different regions of the dentition were considered separately. Only those comparisons which showed significant differences at the 5% level or less will be considered.

(i) Maternal Effect

There were no differences between the progeny of homozygous and heterozygous mothers within each genotype group except in the case of the downless homozygotes. Downless homozygote progeny of homozygous mothers compared with those of heterozygous mothers showed a reduction in group A vibrissae ($p < .05$), an increase in abnormality of region L1-L2 of m^1 ($p < .005$), and an increase in the absence of lower third molars ($p < .01$).

(ii) Interaction

(a) Tabby (Ta^c) and crinkled

Heterozygotes

The double heterozygote group showed a number of differences from the Ta^c+ group. There was a reduction in group D vibrissae ($p < .05$); an increase in penetrance of abnormality in regions B1, B3, and 1-L1 of m^1 ($p < .005$); and an increase in the absence of upper third molars ($p < .01$).

Homozygotes

The combination group ($Ta^cTa^c.crcr$ and $Ta^c.crcr$) was compared with the parental group which showed the most extreme phenotype for each character considered.

There was a reduction in group D vibrissae compared with the

Ta^cTa^c and Ta^c group ($p < .05$); the expressivity of abnormality in region 1-Ll of m^1 was more extreme ($p < .05$); and there was an increased reduction of the root of m^1 ($p < .005$). The incidence of lower supernumeraries in the combination group (2%) was lower than in the Ta^cTa^c and Ta^c group (19%) ($p < .01$), and in the crcr group (40%).

A very striking indication of interaction was shown by the tail hairs. The frequency distributions of tail hair scores for the parental and combination groups are shown in Fig. 34.

(b) Tabby (Ta^F) and downless

There were no significant differences between the double heterozygote group and Ta^F+ animals in respect of any character. However, total vibrissa number was lower, and abnormality in the dentition was at a generally higher level, in double heterozygotes.

(c) Tabby (Ta^c) and downless

There was no evidence of interaction affecting the vibrissae. In the dentition, reduction of the root of m^1 was more extreme in the combination group than in the most abnormal parental group, Ta^cTa^c and Ta^c, ($p < .005$), and cusp Bl of m^2 was less frequently enlarged ($p < .005$).

4. Conclusions

The results are strongly suggestive of interaction between Ta^c and cr, both in the double heterozygote and double homozygote. Heterozygotes were compared with controls on a fairly standard genetic background, but the

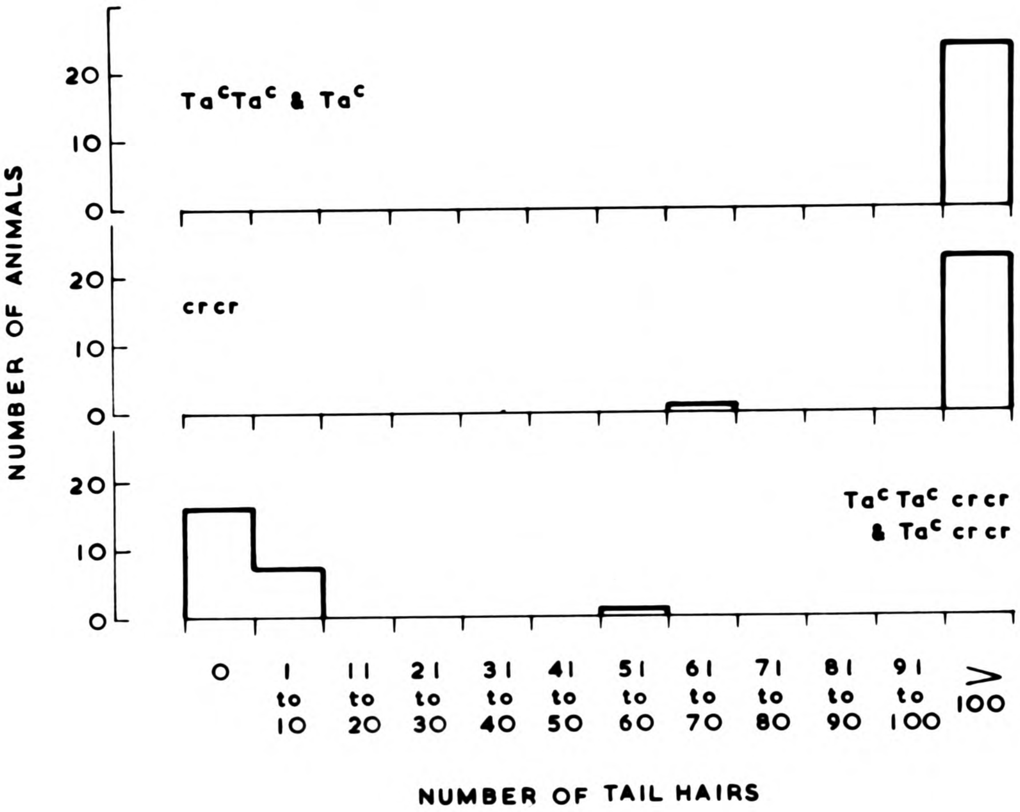


Fig. 34. The distributions of tail hair number in two parental groups and one gene combination group.

homozygote groups had their own stock backgrounds. It could be argued that the increased abnormality in the double homozygote could have been due to background interaction. However, the consistency of the results at the double heterozygote and homozygote levels makes it unlikely that background interaction could have been totally responsible. Striking evidence of interaction was shown by the tail hairs and the frequency of lower supernumeraries in double homozygotes. There were less powerful indications of interaction between Ta^c and dl at the double homozygote level, and no significant evidence of interaction between Ta^F and dl in double heterozygotes.

The indications are that interaction has served to reinforce abnormality. Although the particular gene combinations examined by Kindred (1967a) were not investigated here, the general trends of the two sets of results do not seem to be consistent. However, in Kindred's experiment the nature of the interaction varied with genetic background. Background, as well as gene differences, may therefore be responsible for the apparent incompatibility of the two sets of results.

VI. Concluding Remarks

1. The Abnormality

An attempt has been made to explain all aspects of the dental syndrome in terms of a single primary effect of the mutant genes, a suppressive influence on the growth and differentiation of dental epithelium. A subsidiary effect, a reduction in length of the tooth row, has also been suggested as a possibility. It has been postulated that the suppressive influence varies in intensity at different stages of development in parallel with the observed effects on the developing hair follicles, and that the final outcome is dependent on an interplay of suppression and interaction between the developing teeth. The explanations of the dental abnormalities that have been offered are consistent with the retarded growth and lack of differentiation of the coat. Both the dental and the hair abnormalities can therefore be considered in terms of the principle of 'unity of gene action'.

2. Differences in Local Reaction to Background Change

In general, the teeth reacted to background change in the same direction as the vibrissae, and as the coat of tabby heterozygotes. This was shown by the negative correlation of total vibrissa number with total tooth score, and of total vibrissa number with tabby heterozygote striping. However, the different groups of vibrissae, and different regions within the dentition, showed some differences in their response.

The most obvious difference within the vibrissae was the opposite response of groups C and D to the A and JU backgrounds. Within the dentition there was limited variation between backgrounds involving the lingual side of the crown of m^1 of homozygotes and hemizygotes. In addition, the general reaction in homozygotes and hemizygotes to crossing, either to A or to JU, tended towards an increase of abnormality in the upper jaw and a decrease in the lower.

It seems therefore that background modification has acted primarily at a fundamental level, swinging the whole pleiotropic pattern in the same direction, but that there are definite, although limited, indications of secondary local interactions.

3. Differences between the Genes

At the homozygote and hemizygote level there were no major differences between the genes. At the heterozygote level, however, for every character examined, crinkled and downless tended towards recessivity whereas the two tabby alleles showed variable intermediate dominance. The conclusion drawn is that the genes are qualitatively very similar, and that the quantitative differences between heterozygotes are a reflection of the difference between autosomal and sex-linkage alone.

The dominance relationships of the genes could be explained in terms of either of the two current concepts of sex-linked gene action. The two views are those of Lyon (1961 and subsequently), the 'single-active X-chromosome' hypothesis; and Grüneberg (1967), a new hypothesis involving co-dominance, both alleles of sex-linked genes being active in all cells. The results of the present investigation do not serve to throw any new light on the current controversy.

ACKNOWLEDGEMENTS

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