GENETICAL STUDIES WITH 'VESTIGIAL TAIL' MICE

IV THE INTERACTION OF VESTIGIAL WITH BRACHYURY

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(With One Text-figure)

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Introduction

In the course of linkage tests with the recessive mutant 'vestigial tail' (**vt**) (Heston, 1951) which have been reported elsewhere (Michie, 1952, 1955*a*, *b*, *c*), opportunities arose of observing its effects when combined with other mutants affecting the tail, namely, Short-Danforth (**Sd**), undulated (**un**) and Brachyury (**T**). The effects of this last combination form the main topic of this paper.

Materials

The stocks referred to in the text are the following:

(i) 'Original vestigials', genotype **vt/vt**, **c/c**, **b/b**, **a/a**, obtained from Dr W. E. Heston via Prof. L. C. Dunn.

(ii) 'Extracted vestigials', genotype **vt/vt**, **d/d**, **b/b**, **a/a**, extracted from outcrosses of original vestigials to subline D_2 of the DBA inbred line.

(iii) 'Stock E', genotype **T/+**, **Wh/+**, of mixed origin.

(iv) '**T**/t^8 stock', genotype **T**/t^8, **a/a**, obtained from Prof. L. C. Dunn.

Results

Genetic tests of the short-tailed offspring of **+ Sd/vt +** × **vt +/vt +** backcross matings showed that **vt Sd** mice are viable in both sexes, phenotypically short-tailed, fertile in the female sex and probably fertile in the male sex (for the hypothesis of male sterility $P=0{\cdot}075$). By similar methods it was shown that **vt un** mice are viable and fertile in both sexes and phenotypically resemble non-undulated **vt** mice.

The combination of vt *with* T

Original vestigials were crossed to stock E, and F_1 mice carrying T were backcrossed to the vestigial stock. In classifying the offspring of this first series of matings two phenotypic classes only were distinguished in the records—'normal tail' and 'short or absent tail'. These numbered eight and twenty-one mice respectively. Three mice in the latter group were found to have no anal orifice. There may have been other similarly affected mice which escaped notice, since the abnormality was unexpected and was not specifically looked for. Of the three mice in which the imperforate condition was observed, two died before they were sexed. The third, a female, survived to maturity. She was

found to have a functional 'cloaca'; both urine and faeces were voided through what appeared to be the vaginal aperture. She had no tail. Although given a mate of proved fertility, she failed to become pregnant in the 5 months which elapsed between weaning and death.

It seemed likely that 'imperforate anus' was an expression of the combination of vt with T. A new series of matings were set up with this possibility in mind.

The extracted vestigial stock was crossed with the T/t^8 stock, and F_1 mice carrying T were backcrossed to the vestigials. The backcross litters were examined as soon as possible after birth, and classified for both anal and tail characters. The mice fell into three sharply distinct phenotypic classes, which were interpreted as corresponding to the genotypic classes shown in Table 1.

Table 1. *Genotypic interpretation of the three phenotypes resulting from the backcross* **T +/+ vt × + vt/+ vt**

Tail	Anus	Genotype
Normal	Normal	+/+, +/vt
Short	Normal	T/+, +/vt +/+, vt/vt
Absent	Imperforate	T/+, vt/vt

Table 2. *Classification of progenies from matings of the type* **T/+, +/vt × +/+, vt/vt**

		+/+, +/vt			{+/+, vt/vt T/+, +/vt}			T/+, vt/vt			
		Normal			Short tail			Tailless and imperforate			
Sex of heterozygous parent	Mating	♀	♂	Not sexed	♀	♂	Not sexed	♀	♂	Not sexed	Total
♀	578	1	—	—	5	2	—	1	—	—	9
♀	581	—	—	1	4	2	3	1	1	1	13
♀	585	—	3	—	—	3	—	1	—	—	7
♀	596	—	2	—	4	—	—	—	—	—	6
♀	597	2	1	—	2	1	3	—	—	—	9
Total		3	6	1	15	8	6	3	1	1	44
♂	579	2	—	—	2	6	—	—	—	—	10
♂	580	1	—	—	2	2	—	—	1	—	6
♂	583	—	—	—	4	1	—	—	—	—	5
♂	584	2	—	—	—	1	—	—	—	—	3
♂	587	1	2	—	4*	3	—	3	1	3	17
♂	600	—	2	—	2	2	—	—	1	—	7
Total		6	4	—	14	15	—	3	3	3	48
Grand total		9	10	1	29	23	6	6	4	4	92
Observed			20			58			14		92
Expected			23			46			23		92

* Including one mouse with an anus but no tail. She was shown by breeding tests to be a phenotypic variant of T/+, +/vt.

The full classification of the backcross progenies is given in Table 2. Of the six imperforate tailless females one died aged 6 days before she could be examined for the presence of a functional cloaca, but the cloaca was functional in the remaining five. Of these, four were killed and dissected and one was allowed to live and survived to the age of 73 days. Of the four imperforate tailless males two died, one on the seventh day of life and one on the eighth, the latter with a greatly distended belly. The remaining two were killed and dissected.

Description of T/+, vt/vt *females*

In all four females dissection confirmed that the genital and alimentary tracts opened into a common sinus before reaching the exterior, but the site of confluence was variable. The urethra, which opens from the tip of the genital papilla in normal female mice, was to a variable degree displaced towards the sinus.

Female 587.473.3 appeared ill on the 22nd day of life when she was killed. The anatomy of her pelvic viscera is diagrammatically illustrated in Fig. 1. Female 585.457.7 presented a similar picture, except that the uterine horns were confluent with the gut immediately at their junction and were distended with faeces. She was moribund when killed at the age of 19 days. In the case of female 587.473.4 the junction of the genital and alimentary

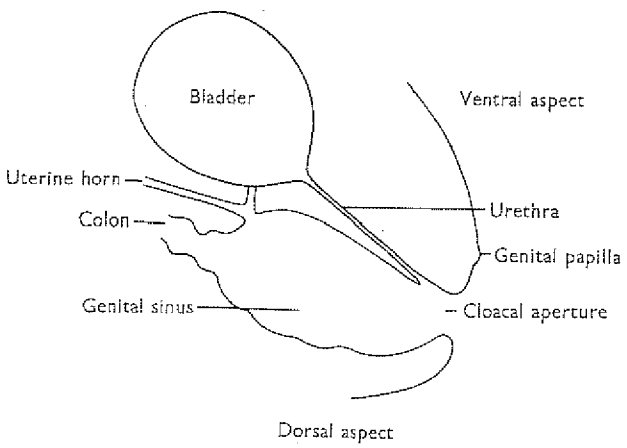

Fig. 1. Diagram of the disposition of the pelvic viscera in a vt/vt, T/+ female (no. 587.473.3).

canals could just be seen in the intact mouse. When the dorsal lip of the cloacal aperture was strongly retracted with a probe, the free edge of the septum which more proximally separated the two canals became visible. In female 578.459.9 the junction was slightly more proximal and could not quite be brought into sight by this procedure. In both these mice the urethra opened on the dorsal aspect of the genital papilla in the mid-line. Their ages when killed were 22 and 33 days respectively.

Description of T/+, vt/vt *males*

Male 587.489.1 was emaciated and moribund when killed at the age of 6 days. The gut was evidently greatly distended, since its outlines were clearly visible as raised ridges on the surface of the abdomen. Dissection revealed that the rectum ended blind soon after passing behind the bladder, and that the entire gut distal to the pylorus of the stomach was distended with a clear fluid.

Male 600.486.7 showed an identical picture on dissection. When it was killed on the seventh day of life this mouse was undersized and its belly was distended.

In neither mouse could an external urethral opening be seen with a dissecting microscope. It seems likely that the clear fluid which filled the gut in both cases was urine, passed through an urethro-rectal confluence. It is unfortunate that the post-mortem examinations of the imperforate mice were superficial and did not include chemical

analysis of the gut contents of the males. But the suggestion that it was urine is strengthened by the prevalence in other instances of the 'uro-recto-caudal syndrome' (see below) of continuity of the urinary with the alimentary tracts.

The segregation ratios

The observations summarized in Table 2 clearly represent a significant departure from the expected 1 : 2 : 1 ratio ($\chi^2_{(2)} = 7{\cdot}043$, $P < 0{\cdot}05$). Possible causes of the discrepancies are (i) linkage of **vt** with **T**, which would increase the second class above, and decrease the first and third classes below, expectation, (ii) erroneous assignment to the second genotypic class of mice which properly belonged to the third, and (iii) prenatal death of **T/+**, **vt/vt** mice, resulting in a shortage of animals in the third class.

The explanation in terms of linkage is rendered unlikely by Clark's (1934) observation of $53 \pm 5{\cdot}3\,\%$ recombination between **T** and $\mathbf{sh}_2$ (with which **vt** is closely linked (Michie, 1952, 1955*a*)).

The explanation in terms of misclassification assumes that some of the doubly mutant animals evaded at the same time both the tailless and the imperforate condition. If this were so, one would also expect some mice to show one of the two characters only. Evidence that the two manifestations of the **T/+**, **vt/vt** genotype are in fact separable has been sent to me by Prof. L. C. Dunn (1952) who performed similar crosses. He also found taillessness resulting from the interaction of the two mutants, but among forty-five tailless mice not one case of imperforate anus was observed. The numbers in the other phenotypic classes were 123 short tailed and fifty-seven normal.

Prenatal death of some of the doubly mutant mice is, on the other hand, both *a priori* likely and in agreement with the data. If we assume an estimated 54 % survival of tailless mice as compared with their non-tailless sibs, the residual $\chi^2_{(1)}$ is 2·077, $P > 0{\cdot}1$. We would expect the effect to be less pronounced in Dunn's material, where the developmental impact of the **vt**'**T** combination is milder, and this is confirmed by the higher estimated survival rate of 75 %.

Discussion

The syndrome which has been described bears strong resemblances to the effects, described by various authors, of certain other genetic combinations, all of which affect the tail.

Homozygous Short-Danforth mice resemble **vt**'**T** mice in being tailless and imperforate, and there is confluence of the alimentary tract with the urinary tract in males and with the genital tract in females (Gluecksohn-Schoenheimer, 1943). Fisher & Holt (1944), in a stock selected for long-tailed **Sd** heterozygotes, found a homozygous female with a functioning cloaca which lived for 22 days. On the other hand, **Sd** homozygotes suffer from absence or gross abnormality of the kidneys, ureters and bladder, and usually die within the first 2 days.

Dunn & Gluecksohn-Schoenheimer (1947) have described a recessive mutant (symbol **ur**), the effect of which in the homozygote is a short tail, and very occasionally an imperforate anus. But when combined with the **T**t^0 genotype it leads to imperforate anus in about 90 % of cases. Associated with the imperforate condition are a variety of deformities of the urinary, alimentary and genital systems, exhibited in differing degrees by different mice. Numbered among them are all the anomalies found in **vt**'**T** mice.

The 'uro-rectal-caudal syndrome', as Grüneberg (1952) has termed pathological constellations of this general type, has also been observed in over 50 % of mice carrying the combination of Kinky with Brachyury, and of Fused with Brachyury (Dunn & Gluecksohn-Schoenheimer, 1944).

In all the instances reviewed above, renal and ureteric abnormalities were common findings. The six **vt**/**T** mice dissected revealed no such abnormalities, but it is possible that examination of more extensive material might do so.

Thus, while the **vt**/**T** combination appears regularly to be associated with taillessness in both Dunn's and my material, the effect on the urogenital and alimentary systems is critically dependent on the action of genetic modifiers differentiating Dunn's stock from mine.

SUMMARY

The phenotypic effects of combining **Sd** and **un** with **vt** (vestigial tail) are not grossly distinguishable from the effects of **vt** alone. On the other hand, mice of the genotype **T**/+, **vt**/**vt** are tailless and, on a given genetic background, both sexes regularly show abnormalities of the urogenital and alimentary systems externally recognizable by imperforate anus.

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