Genetical and embryological studies of the *jt* form of syndactylism in the mouse

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1. INTRODUCTION

Several manifestations of syndactylism which affect the digits of the mouse have been described previously. Some of these are apparently the secondary effect of pleiotropic genes such as those responsible for phocomelia, *pc* (Gluecksohn-Waelsch, Hagedorn & Sisken, 1956), myelencephalic blebs, *my* (Carter, 1956) and dominant hemimelia, *Dh*, investigated by Searle (1964). Some 20 years ago Hertwig (1942) reported finding shaker mice characterized by syndactylism (*sy*) and these have been studied subsequently by Grüneberg (1956, 1962). Two additional forms of syndactylism have also been investigated extensively by Grüneberg; one (*Os*) is the expression of a dominant gene (Grüneberg, 1956, 1961) and the other (*sm*) is a recessive condition (Grüneberg, 1956, 1960). In our laboratory among the descendants of Swiss-albino mice which had been treated with the HN_2 form of nitrogen mustard, individuals have been found which are syndactyl. In the affected adult mice, two or three digits show varying degrees of fusion on one or more feet. The present paper is concerned with syndactylism as observed in this laboratory and its relation to the already known gene, *jt*.

2. MATERIALS AND METHODS

Our line of syndactyl mice was studied by means of gross and histological examination of adult and embryonic feet. Fixed specimens from animals varying in age from approximately 2 weeks to adult were studied to some extent in radiograms, but for the most part in fixed material subsequent to alizarin staining following a modification of Green's (1952) technique. Embryos were stained *in toto* with toluidine blue, originally by use of an adaptation of Williams' (1941) method but more recently by means of a modification of a techinique developed by Burdi (1965). Histological sections were cut at 7 μ or 10 μ and stained with haematoxylin and eosin. Normal feet from C57BL/6 Sfd* mice were also studied after use of the same technical procedures. In the embryological investigations, left hind-feet were compared most frequently, and the observations reported pertain to this foot unless otherwise stated. Our syndactyl mice were mated *inter se*, crossed to *jt* and *sm*

* Line of C57BL/6 maintained in the laboratory since 1955.

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syndactyl mice (from Jackson Laboratory) and outcrossed to normal C57BL/6 Sfd, and C57 Black and tan (from Dr L. M. Franks, Lincoln's Inn Fields, London), and also mated to hybrid C57BL/6-Swiss × Stanford-b-ru that carried the luxoid (*lu*) gene.

3. MORPHOLOGY IN JUVENILE AND ADULT *jt* MICE

In the type of syndactylism under consideration, digits 2, 3, and 4 are involved in a proximo-distal direction (i.e. proceeding from proximal to distal phalanges). Simultaneous involvement of all three central digits is the predominant manifestation; while the condition may be limited to digits 2 and 3, or 3 and 4, the first and fifth digits are unaffected. Generally the fusion between digits appears to affect only the skin and subjacent soft tissues while the skeletal units are rarely involved (Text-fig. 1). Varying grades of syndactylism of the central digits on both hind-feet
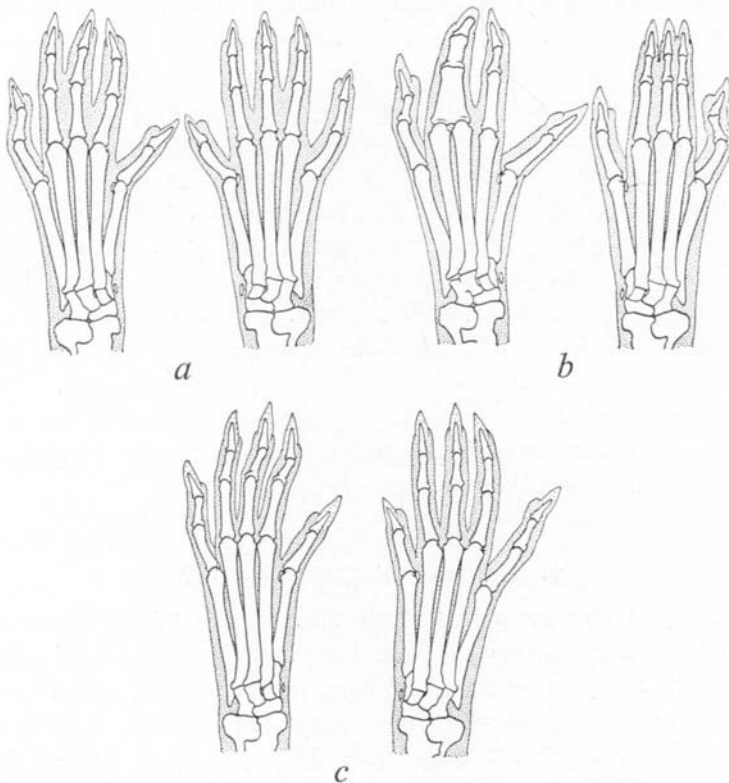
Text-fig. 1. Hind-feet from adult mice; (*a*) *jt/jt* showing soft tissue fusion; (*b*) *jt/jt* left hind-foot exhibits fusion of phalanges in digits 3 and 4, cuneiforme 3 and cubiodeum are also partially fused, right hind-foot soft tissue fusion; (*c*) normal C57BL/6. (Alizarin preparations.) Magnification approximately × 3¼.

are most frequent, while syndactylism of the digits on all four feet is the secondary form of expression (Table 1). The left feet tend to be involved more often than the right, and digits of the hind-feet are affected more frequnetly than those of the fore-feet.

Table 1. *Range of expression of syndactylism in* inter se *matings of* jt/jt *mice*

	Swiss-albino (Sw)	C57BL/6 × Sw	Totals
Both hind-feet	59	122	181
All four feet	29	40	69
Hind-feet and left fore-foot	11	23	34
Left hind-foot only	13	19	32
Hind-feet and right fore-foot	1	15	16
Right hind-foot only	2	3	5
Left hind- and left fore-feet	0	3	3
Right hind- and right fore-feet	1	1	2
Right fore-foot only	0	1	1
Right and left fore-feet	0	1	1
Both fore-feet and left hind-foot	0	1	1
Total syndactyl	116	229	345
Non-syndactyl	0	17	17
Total	116	246	362

4. GENETICAL STUDIES

Some time after the discovery of syndactylism in our laboratory we obtained a line of syndactyl or 'joined toe' (*jt*) mice from Jackson Laboratory. Crosses between our syndactyl mice and the joined toe (*jt*) stock show that the two manifestations are genetically the same. In the progeny of these crosses involving homozygotes, 65% of the 103 animals examined are syndactyl; moreover the morphology of the two forms appears to be identical. Consequently, the Stanford syndactyl animals are now referred to as *jt*; while the Jackson line has been discarded.

In spite of morphological similarities, matings between *sm* and *jt* homozygotes do not indicate that the two are the same genetically since no syndactyl young have been found among the 84 resulting offspring. Except for the occasional occurrence of preaxial polydactyly no other anomalies have been found associated with *jt* syndactylism. Neither heterozygotes nor homozygotes show any indications of reduced viability.

The penetrance of the *jt* form of syndactylism is high in *inter se* matings of the inbred Swiss-albino stock, being 100% in the 274 animals examined in this laboratory. Of the 542 offspring from the hybrid C57BL/6 × Swiss syndactyl (*jt/jt*) segregants mated *inter se*, 94% manifest syndactylism. However, following outcrosses to coloured luxoid (*lu*) in the progeny from *inter se* matings, in which both parents are syndactyl and polydactyl, only 33 of the 69 offspring are syndactyl.

Outcrosses to normal C57BL/6 mice indicate that *jt* is a recessive and probably due to a single gene (Table 2). However, the influence of genetic background is evident in that there is a low incidence of slight manifestations of syndactylism in the F_1 between normal C57BL/6 and segregant hybrid syndactyl mice, and it may also be observed that the incidence is markedly increased in the F_2 derived from normal F_1 progeny of this same cross. Recently Swiss syndactyl mice have been crossed to normal C57 Black and tan mice, and a low incidence of syndactylism is

also noted in the first generation while there is no expression of the trait in the F_1 between the inbred Swiss syndactyl and normal C57BL/6 mice.

Table 2. *Outcrosses of* jt/jt × *C57BL/6 normal* (+ +)

	Syndactyl	Normal	Total	Percentage of syndactyls
+ + × *jt/jt* (from Swiss stock)				
F_1	0	81	81	0
F_2	29	337	366	8
+ + × *jt/jt* (from C57 × Swiss cross)				
F_1	7	175	182	4
F_2	173	553	726	24

The results of linkage tests are difficult to evaluate because of variations in penetrance and the effect of genetic background on the expression of *jt*. Tests have been made for linkage with albino (*c*) and luxoid (*lu*) genes. The results of these tests (Table 3) show no evidence of linkage between *jt* and *c* or between *jt* and *lu*. However, it is evident that the penetrance of *jt* is considerably higher in the back-cross than in the intercross matings.

Table 3. *Tests for linkage of* jt *with* c, *and* lu*. *Phenotype of progeny*

Genotype of parents	Mating type	+ +	+c	jt +	jtc	Total	χ^2L	P
$\dfrac{C+}{cjt} \times \dfrac{C+}{cjt}$	IC	83	21	5	2	111	0·246	0·70–0·50
$\dfrac{C+}{cjt} \times \dfrac{cjt}{cjt}$	BC	14	20	17	14	65	1·246	0·30–0·20
		+ +	+lu	jt +	jtlu			
$\dfrac{jt+}{+lu} \times \dfrac{jt+}{+lu}$	IR	69	18	5	4	96	2·455	0·20–0·10
$\dfrac{jt+}{+lu} \times \dfrac{jtlu}{jtlu}$	BR	8	4	8	2	22	0·182	0·70–0·50

* Luxoid (*lu*) treated as a recessive, +stands for the normal allele, *c* (albinism) and *jt* (joined toes) are recessives. I = Intercross, C = Coupling, R = Repulsion, B = Backcross. χ^2L = Bailey (1961). χ^2L (χ^2_1) calculated on basis of partial manifestation of *jt* in IC and full penetrance of *jt* in BC.

5. EMBRYOLOGICAL STUDIES

Gross study of embryonic limbs and examination of specimens stained *in toto*, as well as comparison of horizontal serial sections of syndactyl and normal feet reveal differences between incipient syndactyl and normal feet as early as $11\frac{1}{2}$ days of embryological development. In comparing normal with potentially syndactyl embryonic limbs at this stage it becomes apparent that the principal difference is a loss of postaxial footplate tissue and a slight concomitant decrease in the developing footplate in the preaxial region of the incipient *jt/jt* embryo (Text-fig. 2). It also seems that slight in-foldings or corrugations of the epidermal layer primarily on
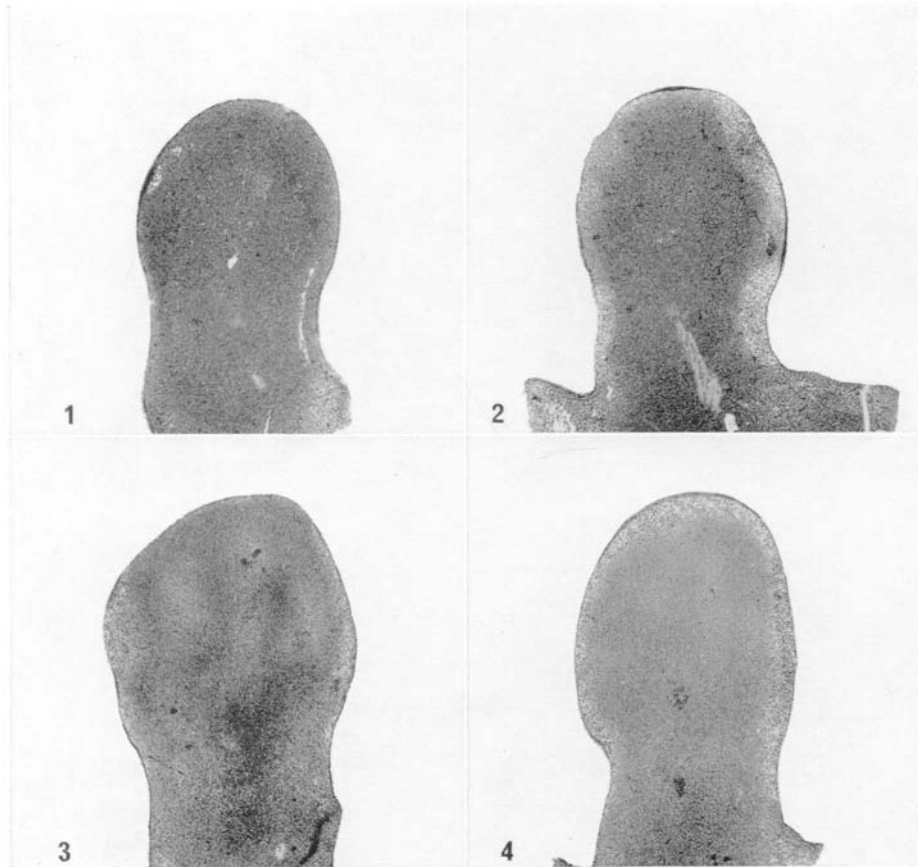
EXPLANATION OF PLATE

PLATE I

Fig. 1. Left hind-foot from 11-day C57BL/6 normal control embryo (10 μ).

Fig. 2. Left hind-foot from 11½-day *jt/jt* embryo (10 μ).

Fig. 3. Left hind-foot from 12-day C57BL/6 normal control embryo (7 μ).

Fig. 4. Left hind-foot from 12-day *jt/jt* embryo (7 μ).

Horizontal sections stained with haematoxylin and eosin, magnification ×35.
Postaxial margin is on the left.

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the postaxial border occur at about this time in some *jt/jt* embryos (Plate I, Fig. 2). The lighter peripheral zones of the footplates (Plate I, Figs. 2–4) are very likely an artifact of preparation; these are found in some sections of both normal and syndactyl specimens and are probably of no consequence in the ontogeny of the *jt* form of syndactylism.

Text-fig. 2. Gross outline of the footplate of the left hind-foot from normal C57BL/6 (left) and *jt/jt* embryos (11½ days). Magnification approximately × 20. Postaxial margin is on the left.

A comparison of histological sections of the footplates from 11½- and 12-day *jt/jt* and control embryos does not indicate overgrowth due to hyperplasia of the apical ectodermal ridge of the *jt/jt* limb. The footplates of 11½- to 13-day *jt/jt* embryos do not appear swollen or thickened in a dorsal–ventral direction; such deformation might also indicate an overgrowth of the apical ectodermal ridge. The principal effect is a narrowing of the developing footplate (Plate I, Fig. 4) due primarily to a reduction in the area between digits 4 and 5 and to some extent, a loss in the region between digits 2 and 1.

By the beginning of the thirteenth day there is a more evident decrease in the amount of tissue in the postaxial region of the footplate apparent principally in the area between prospective digits 4 and 5. Later on the thirteenth day the digits in the normal foot are spread out much like the ribs of a fan; in the incipient syndactyl foot digits 2 and 4 do not diverge as much from 3 as in the control specimens but instead develop in planes more nearly parallel (Text-fig. 3). There is less mesen-
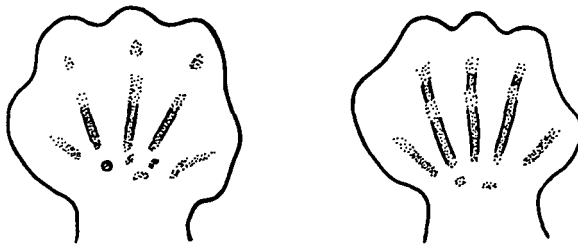
Text-fig. 3. Left hind-feet from 13-day normal C57BL/6 (left) and *jt/jt* embryos. (Toluidine blue preparations.) Magnification approximately × 14.

chymal tissue between these three digits than in the controls, in which there is a more or less equal amount of tissue between all of the five developing digits. On the fourteenth day the digits of the normal foot separate and by the fifteenth day are distinct divergent units; however, in the feet from potentially syndactyl embryos

the digits which are fused to a greater or lesser degree in the adult do not completely separate (Text-fig. 4). In normal feet on the sixteenth day of embryonic life the digits fuse superficially and are not completely separate until about the fifth day of postnatal development. Digits of syndactyl (*jt/jt*) mice apparently remain somewhat fused from earlier embryonic stages and do not become distinct anatomical elements in the juvenile mouse.

Text-fig. 4. Left hind-feet from 14½-day normal C57BL/6 (left) and two *jt/jt* embryos. (Toluidine blue preparations.) Magnification approximately × 10.

Since the left hind-foot is the one most often affected by the *jt* gene, normal and potentially syndactyl left hind-feet have been compared most frequently. Examination of right hind-feet and both fore-feet of incipient syndactyl and control embryos reveals a similar loss of footplate tissue, especially between digits 4 and 5 in the putative syndactyl embryo. Digits 3 and 4 and sometimes also 2 and 3 develop in closer proximity to one another than in the normal feet.

6. DISCUSSION

It would appear that the form of syndactylism discussed here is due to a single recessive gene (*jt*) whose expression is influenced by different genetic backgrounds. The genome of Swiss-albino mice appears to be most favorable for the expression of *jt* syndactylism while the genetic constitution of luxoid (*lu*) mice or the luxoid gene itself lowers the penetrance of the trait. In the embryonic foot of potentially syndactyl mice as early as 11½ days there is an apparent decrease in both preaxial and postaxial footplate tissue; by the thirteenth day reduction is primarily postaxial with the result that the blastemata of digits 2, 3, and 4 develop closer together and the digits tend to remain in contact to a greater or lesser extent throughout later embryonic stages and on into adult life. The embryological development is somewhat similar to that of shaker with syndactylism or *sy* (Grüneberg, 1962) in which the reduction of footplate tissue is both preaxial and postaxial.

In some respects the *jt* manifestation differs from other forms of syndactylism previously studied. There is nothing to indicate that homozygous recessives are subject to reduced viability as is the case with *sy/sy* mice (Grüneberg, 1956). Except for a low incidence of slight manifestations *jt* heterozygotes are unaffected while oligosyndactylism (*Os*) is a semidominant in which heterozygotes are regularly affected and homozygotes die before birth (Grüneberg, 1963). Unlike the *jt* homozygotes, some *sm/sm* animals also have tail abnormalities which, like the foot

anomalies, are apparently due to a systemic epidermal hyperplasia (Grüneberg, 1960).

It would seem that the development of a certain number of 'normal' digits in the foot is partly a matter of a specific amount of competent tissue being present in each of the prospective digital areas of the footplate; an excess or decrease in any one region may alter the number or morphology of the digits. We have arrived at a conclusion very similar to that of Grüneberg (1962) for it appears that the shape and amount of footplate tissue prior to and during the condensation of mesenchyme into digital elements is obviously of considerable consequence in the determination of the subsequent morphology of the foot. In this connexion one postulates that the decrease in the incidence of syndactlyism in the offspring of syndactyl–polydactyl mice mated *inter se* is possibly the result of an increase in the amount of footplate tissue owing to the morphological effect of the genetic factor which causes poly-dactylism. In several animals, e.g. the cat (Danforth, 1947), an excess of undiffer-entiated tissue is known to precede the appearance of preaxial polydactyly.

The occasional occurrence of polydactylism in the syndactyl (*jt*) inbred or hybrid stock is not easily explained. Perhaps these manifestations are the result of overall disturbances in footplate morphology, some of which may be due to the *jt* gene.

The loss of postaxial footplate tissue may be related to the slight corrugation of the epidermal layer found in some potential *jt* embryos and may result from an excess of epidermal cells in relation to the reduced volume of subjacent mesenchymal tissue in the footplate. It is obvious that the ultimate problem of what are the immediate underlying causes of the alterations in footplate morphology is as yet unsolved as is the matter of how several separate and genetically distinct hereditary factors have morphologically very similar effects.

SUMMARY

1. A form of syndactylism (*jt*) involving two or three digits on one or more feet has been found in the descendants of Swiss-albino mice treated with HN_2. The fusions are in a proximo-distal direction and usually involve only the skin and soft tissues; digits 1 and 5 are unaffected.

2. This type of syndactylism is apparently due to a single recessive gene, *jt*, whose penetrance is influenced by 'genetic background'. Apparently the identical muta-tion has occurred independently in stock maintained at Bar Harbor and Stanford. Linkage tests indicate that *jt* is not linked with *c* or *lu*.

3. Embryological studies of the developing footplate reveal a loss in both preaxial and postaxial footplate tissue, primarily the latter, prior to and during the con-densation of mesenchyme into the precartilage of the digital elements in the *jt/jt* limb.

4. The importance of the shape and amount of footplate tissue to the resultant morphology of the foot is apparent. It is evident that genes such as *jt* are responsible for changes in footplate development, but the immediate underlying factors which cause these alterations remain an unsolved problem.

REFERENCES

BAILEY, N. T. J. (1961). *Introduction to the Mathematical Theory of Genetic Linkage*. London: Oxford University Press.

BURDI, A. R. (1965). Toluidine blue–alizarin red S staining of cartilage and bone in whole-mount skeletons *in vitro*. *Stain Technol.* **40**, 45–48.

CARTER, T. C. (1956). Genetics of the Little and Bagg x-rayed mouse stock. *J. Genet.* **54**, 311–326.

DANFORTH, C. H. (1947). Morphology of the feet in polydactyl cats. *Am. J. Anat.* **80**, 143–172.

GLUECKSOHN-WAELSCH, S., HAGEDORN, D. & SISKEN, B. F. (1956). Genetics and morphology of a recessive mutation in the house mouse affecting head and limb skeleton. *J. Morph.* **99**, 465–479.

GREEN, M. C. (1952). A rapid method for clearing and staining specimens for the demonstration of bone. *Ohio J. Sci.* **52**, 31–33.

GRÜNEBERG, H. (1956). Genetical studies on the skeleton of the mouse. XVIII. Three genes for syndactylism. *J. Genet.* **54**, 113–145.

GRÜNEBERG, H. (1960). Genetical studies on the skeleton of the mouse. XXV. The development of syndactylism. *Genet. Res.* **1**, 196–213.

GRÜNEBERG, H. (1961). Genetical studies of the skeleton of the mouse. XXVII. The development of oligosyndactylism. *Genet. Res.* **2**, 33–42.

GRÜNEBERG, H. (1962). Genetical studies on the skeleton of the mouse. XXXII. The development of shaker with syndactylism. *Genet. Res.* **3**, 157–166.

GRÜNEBERG, H. (1963). *The Pathology of Development*. New York: Wiley.

HERTWIG, P. (1942). Neue Mutationen und Koppelungsgruppen bei der Hausmaus. *Z. indukt. Abstamm.- u. VererbLehre*, **80**, 220–246.

SEARLE, A. G. (1964). The genetics and morphology of two 'luxoid' mutants in the house mouse. *Genet. Res.* **5**, 171–197.

WILLIAMS, T. W. (1941). Alizarin red S and toluidine blue for differentiating adult and embryonic bone and cartilage. *Stain Technol.* **16**, 23–25.