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Genetical and embryological comparison of two mutations which cause foetal blebs in mice

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SUMMARY

Mutants with abnormalities which were the consequence of the formation of haematomas during gestation were designated as 'foetal haematomata' (*fh*). The *fh* homozygotes were found to be morphologically and embryologically very similar to myelencephalic bleb (*my*) mice. It was also found, however, that the *fh* and *my* mutants were separate genetic entities due to two independently segregating recessive genes.

1. INTRODUCTION

Mice showing anomalies affecting both the feet and eyes were first observed in our colony in a stock of hybrid ancestry resulting from crosses between C57BL/6Sfd and Stanford-J mice. Because the observed abnormalities were very similar to those seen in myelencephalic bleb (*my*) mice (Carter, 1959; Grüneberg, 1952) genetic studies were begun to determine whether or not this was a reappearance of the myelencephalic bleb mutation. Morphological and embryological investigations into the nature and cause of the anomalies were also initiated. Since we found (Center, 1971) that the manifestation of the characteristic morphology involved the formation of haematomas during gestation, we designated the mutation as 'foetal haematomata' and gave it the genetic symbol of *fh*.

2. MATERIALS AND METHODS

Mice of the myelencephalic bleb (*my*) stock and C57BL/6Sfd strain were originally obtained from the Jackson Laboratory. The luxoid (*lu*) mice were C57B1/6Sfd *lu* mice (Center, Hunter & Dodge, 1967) maintained in our laboratory.

Embryos were obtained from *fh* homozygotes and C57BL/6 mice. Stages were determined by timing via the utilization of vaginal plugs (day of the vaginal plug was designated as day 'zero') and the use of morphological correlation with Grüneberg's (1943) staging of mouse embryos. Subsequent to fixation in Bouin or 10% formalin solution, feet and heads removed from embryos of *fh* and C57BL/6Sfd control stocks were sectioned at 7 or 10 μm and 20 μm respectively and stained with haematoxylin and eosin. Embryonic feet were stained *in toto* with toluidine blue by means of a modification of a technique developed by Burdi (1965). Feet from postnatal specimens were stained *in toto* with alizarin red S according to modifications of Green's (1952) procedures.

3. RESULTS

(i) *Genetical Studies*

As noted above, the foetal haematomata (*fh*) anomalies were very similar in gross morphology to myelencephalic bleb (*my*) manifestations (Fig. 1). Data from crosses between *fh* and *my* mice are given in Table 1. In order to verify the genetic basis and penetrance of each of these traits, both *fh* and *my* mice were outcrossed to C57BL/6 mice. The results of these crosses are also given in Table 1.

Table 1. *Segregation of abnormal and normal young in the progeny of crosses between* my *and* fh *and outcrosses to* C57BL6(+/+) *mice*

	Progeny			
Type of mating	Normal	Eye and/or foot anomalies	Total no. of mice	Abnomal (%)
(1) *fh/fh* × *my/my*	68	0	68	0
(2) *fh/my* × *fh/my*	199	77	276	28
(3) *fh/fh* × +/+	31	0	31	0
(4) *fh/+* × *fh/+*	125	23	148	16
(5) *my/my* × +/+	10	0	10	0
(6) +/*my* × +/*my*	100	22	122	18

The data in Table 1 indicate a penetrance of 62% for *fh* and of 72% for *my* if one assumes that each of these two traits was due to a single recessive gene. If the two mutants were identical genetically even with a reduced penetrance in the F_1 resulting from crosses between *fh* and *my* homozygotes, defective individuals should have been found. If two unlinked genes were involved, there would be no affected animals in the F_1, and in the F_2, if the two genes had penetrances of 62% and 72%, one would expect to find approximately 81 defective individuals. The agreement between the observed and the expected data (on the basis of two unlinked genes) gives a χ^2 value between 0·20 and 0·10. Therefore, the idea that *fh* and *my* were two independently segregating recessive genes is completely credible. Thus, it appears that *fh* and *my* mutants were separate genetic entities.

Since 'luxoid' (Green, 1955) mice with shortened and twisted limbs were found occasionally in the *fh* stock, crosses were made to test whether *lu* and *fh* were alleles. The results of these studies are presented in Table 2. The results of the tests for allelism with luxoid were negative. If these two genes were alleles, no normal F_2 mice should result from polydactyl F_1 *inter se* matings.

(ii) *Morphological manifestations*

In the *fh* homozygotes, one or more feet and either the right or left eye were abnormal. Frequently, either the eye or foot abnormalities occurred individually, however, sometimes both the eye and foot anomalies were found in the same mouse. In the adult *fh/fh* mice, digits appeared to be reduced, flexed either

dorsally or ventrally and sometimes fused (Fig. 2). The most frequently observed abnormalities of the eye in the adult were an opaque cornea and reduction in size of the eye. Abnormalities were clearly evident in newborn homozygotes

Table 2. *Segregation of abnormal and normal young in the F_2 progeny of crosses between* fh *and* lu *mice*

Type of mating	Progeny				Total no. of mice
	Normal	*fh*	Polydactyl	Luxoid	
F_1 = normal morphology	26	4	0	0	30
F_1 = polydactyl*	7	2	8	7	24

*Luxoid (*lu*) is a semi dominant and polydactyly is the morphological expression in the heterozygote.

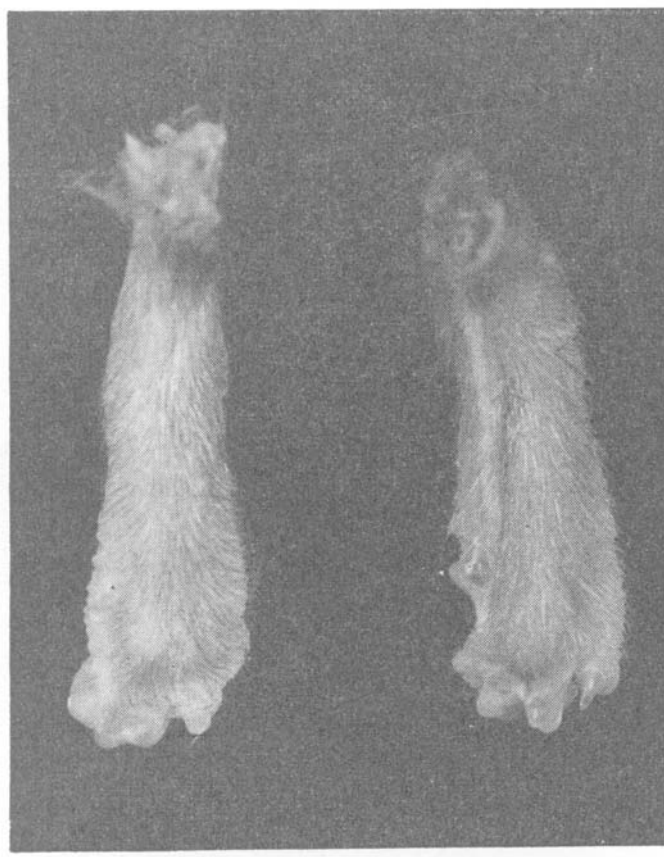

Fig. 1. Feet from adult myelencephalic bleb (left) and foetal haematomata (right) mice. (Approximately 6 × .)

(Fig. 3) as open eyelids and occasionally as a reduction in size of the eyes. Sections of one newborn with an open eyelid revealed that the eye of this mouse had an abnormally convoluted retina. In the affected feet from the newborn mice, toes

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were also flexed and sometimes haemorrhagic vesicles were visible on the dorsal surface (Fig. 3).

Study of *in toto* stained and cleared postnatal specimens revealed that the flexed digits contained shortened and distorted phalanges. While fusion of soft tissues was evident, no bony fusion was found. From comparison of newborn

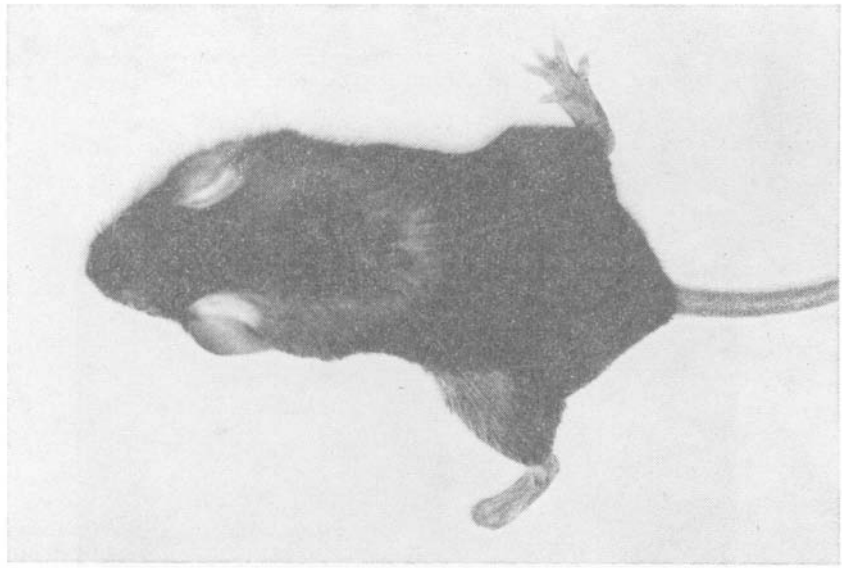

Fig. 2. Adult *fh* mouse with flexed and reduced digits on the left hind-foot and syndactyl toes on the right hind-foot.

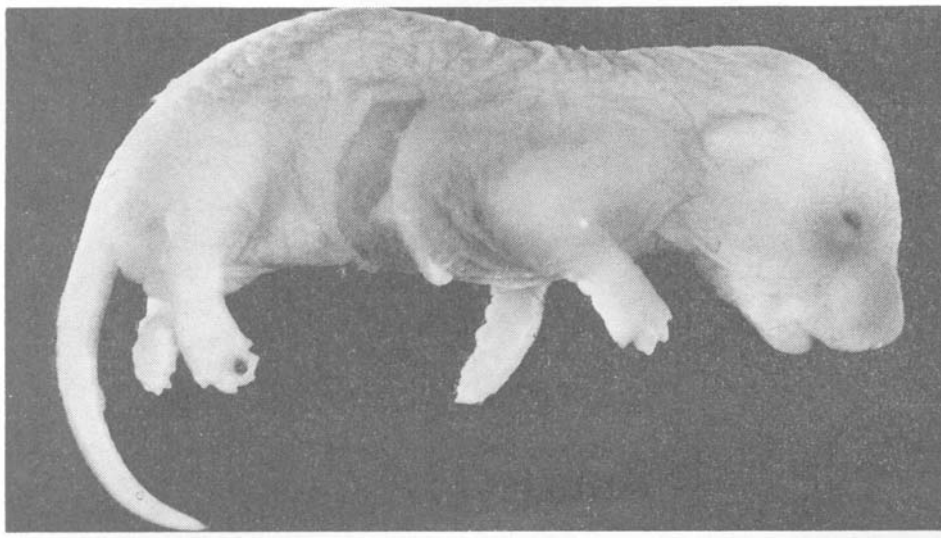

Fig. 3. Newborn *fh* mouse with an open eyelid over the right eye, and flexed and reduced digits on the hind-feet. Right hind-foot also has a small haemorrhagic region. (Approximately 4 × .)

and adult skeletons it was evident that there was a delay in ossification of the phalanges in the affected feet.

(iii) *Embryological studies*

Investigation of the embryology of the mutant *fh* mice revealed that, in some of the homozygous embryos, haematomas were frequently evident on the head and feet (Figs. 4, 9). Sections of the embryo in Fig. 4 showed that the right eye of this

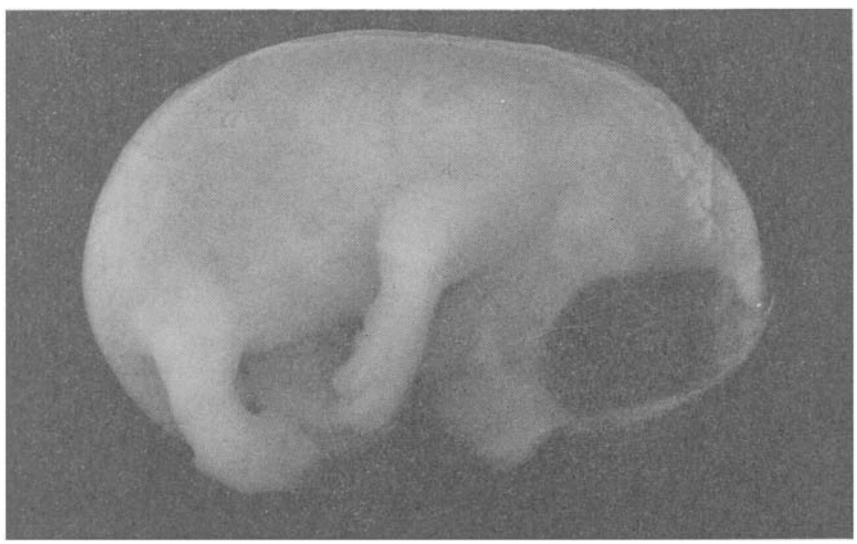

Fig. 4. A 14½-day *fh* embryo with a large area of haemorrhage over the right eye. (Approximately 7 × .)

embryo was situated deeper in the head than is normal. In addition, the posterior chamber of the eye was greatly reduced and the cornea was not developing normally (Fig. 5). Areas of slight oedema were sometimes found associated with the haemorrhagic regions in the *fh* embryos. A section of the head of a 13-day embryo with an area of oedema and haemorrhage surrounding the right eye is shown in Fig. 6. Further study revealed that the development of the lens was probably abnormal in this eye. The developing lens fibres were more irregularly arranged than those found in the lens of the normal left eye from the same embryo.

A transverse section of a still younger *fh* embryo which had an area of haemorrhage on the left side of the head is shown in Fig. 7. It appears that development of the left eye was disrupted subsequent to the optic vesicle stage. Only a slight invagination of the optic vesicle occurred and no lens or pigmented retina was evident in the left eye while the right one was completely normal. Cells within the abnormal optic cup appeared distended when they were studied at higher magnifications.

Similar interference in the normal pattern of development was evident in the arrested digits of *fh* homozygotes. Sections of an abnormal foot from a 15-day homozygote revealed a large clear vesicle and haemorrhagic regions (Fig. 8). Study

of affected feet from 12- and 13-day embryos which had been stained with toluidine blue revealed essentially normal digits distorted by the formation of blebs.

Two 14-day *fh* embryos are shown in Fig. 9 and they indicate something about the range in magnitude of the anomalies which affected the feet on *fh* mice.

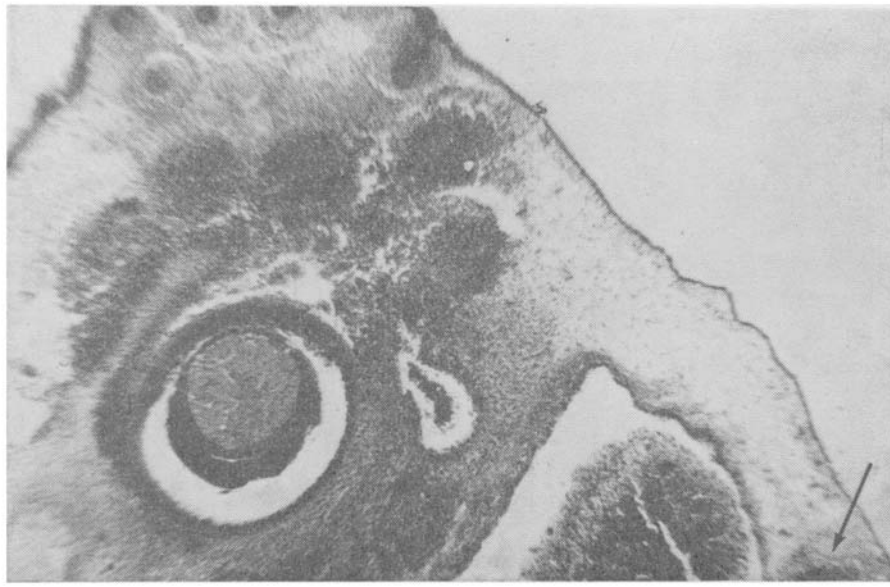

Fig. 5. Sagittal section of the head from the embryo shown in Fig. 4. Arrow indicates lateral margin of haemorrhagic region. See text for description of defective eye. (Approximately 55 × .)

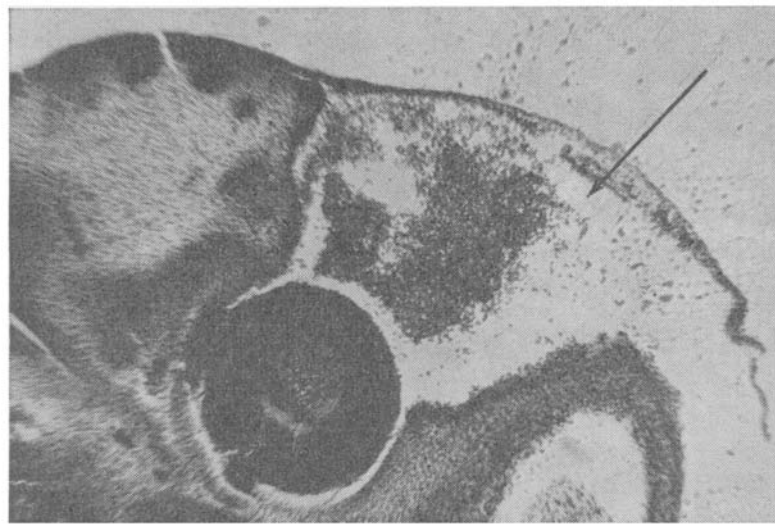

Fig. 6. Sagittal section of the head of a 13-day *fh* embryo with an area of haemorrhage and oedema surrounding the right eye. Arrow indicates haemorrhagic region above the developing eye. (Approximately 55 × .)

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A section of the right forelimb from the embryo on the left in Fig. 9 revealed that an extensive area of haemorrhage interfered with digital development (Fig. 10). Likewise, sections of the left hind-foot from a 13-day *fh* homozygote showed an extensive area of haemorrhage covering the developing digits and two areas of haemorrhage proximal to the foot (Fig. 11). It is evident that the haemorrhagic

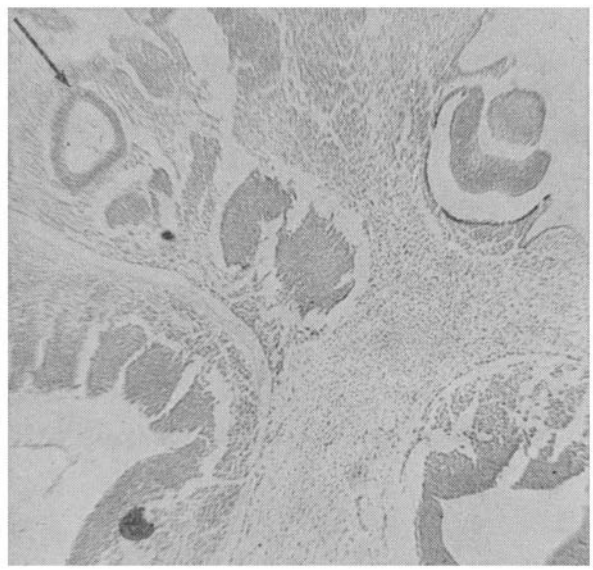

Fig. 7. Transverse section of the head of a 12-day *fh* embryo which had an area of haemorrhage on the left side of the head. Arrow indicates the defective left optic vesicle. (Approximately 55 × .)

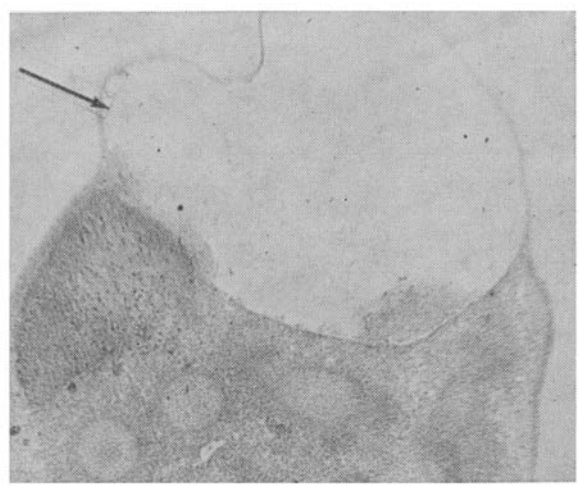

Fig. 8. Frontal section of left hind-foot from a 15-day *fh* embryo. Arrow indicates large blister in terminal region of the foot. A relatively large haemorrhagic region is visible adjacent to the blister on the left. (Approximately 55 × .)

regions were restricting the development of the digits, especially of the phalanges, in these *fh* embryos. Blood vessels were not seen feeding into the haemorrhagic vesicles which were found in mesenchymal tissues. A section of a left hind-leg from a 12-day *fh* embryo with an area of haemorrhage at the base of this leg revealed an area of mesenchymal cells that appear distended and enucleate (Fig. 12).

4. DISCUSSION

It is evident from the morphological and embryological studies that the formation of blebs which were frequently haemorrhagic interfered with the normal development of the eyes and feet in the *fh* homozygotes.

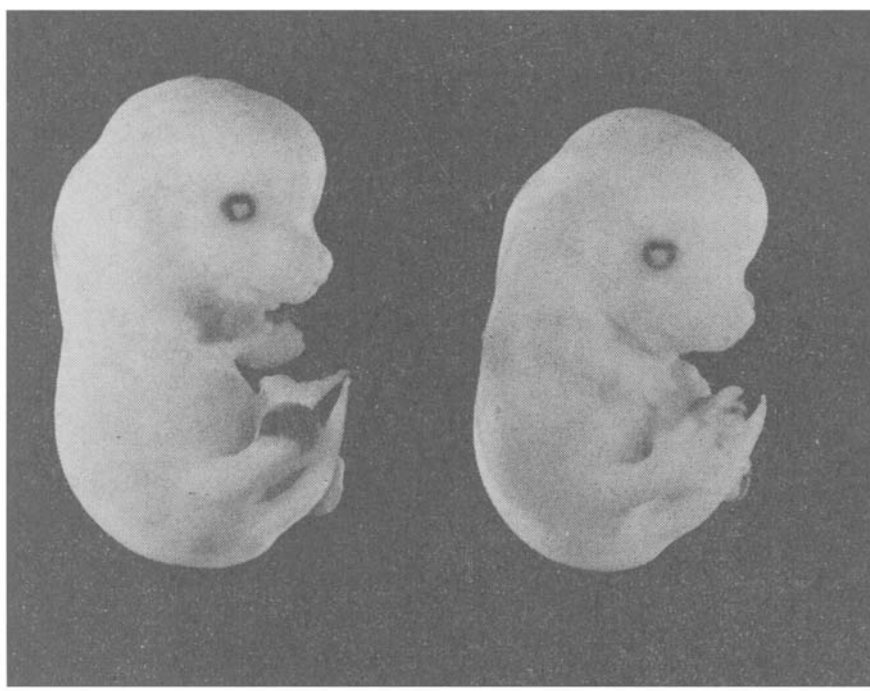

Fig. 9. Two 14-day *fh* embryos. The one on the left has four defective feet with large haemorrhagic regions. The embryo on the right shows only a small clear blister on the left hind-foot. (Approximately 6 × .)

The marked morphological and embryological resemblance of the *fh* anomalies to those found in myelencephalic bleb (*my*) mice is very apparent. Among the anomalies Carter (1959) observed in the *my* mice were 'haemorrhagic lesions and maldevelopment of the eyes, corneal opacity, maldevelopment of the eyelids' and 'maldevelopment of the digits, sometimes accompanied by haemorrhagic lesions' and, in addition, 'plantar–palmar- and dorsiflexion of the extremities'. All of these manifestations were observed in the *fh* mice. Other characteristics found in the *my* mice, such as 'defective skin and coat formation in the lumbar region' were not encountered in the *fh* stock.

Haemorrhagic regions were observed on the head or limbs in 12- to 15-day homozygous *fh* embryos. Blebs were noted in *my* embryos of $12\frac{1}{2}$–$15\frac{1}{2}$ days. It appears that these were more frequently clear blebs than was the case in the *fh* stock. As in *my* mice, the blebs in *fh* homozygotes were often found proximal to their ultimate destination in the terminal region of the limb-bud. This is apparently

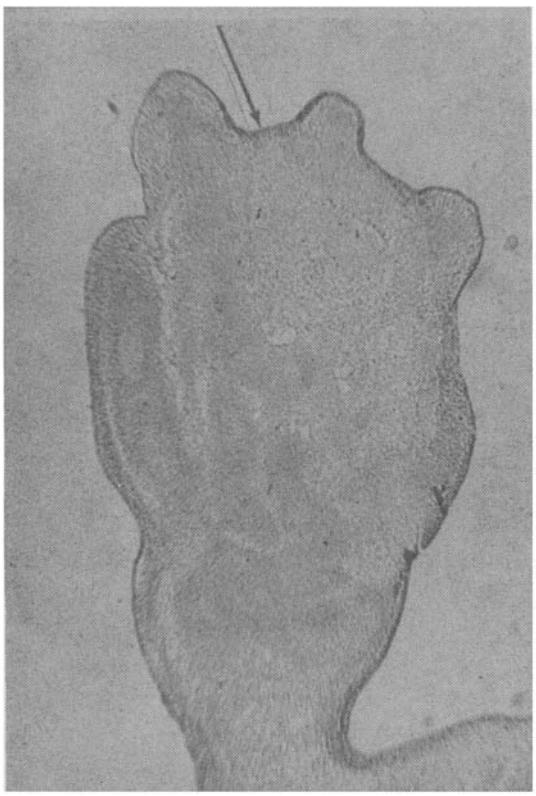

Fig. 10

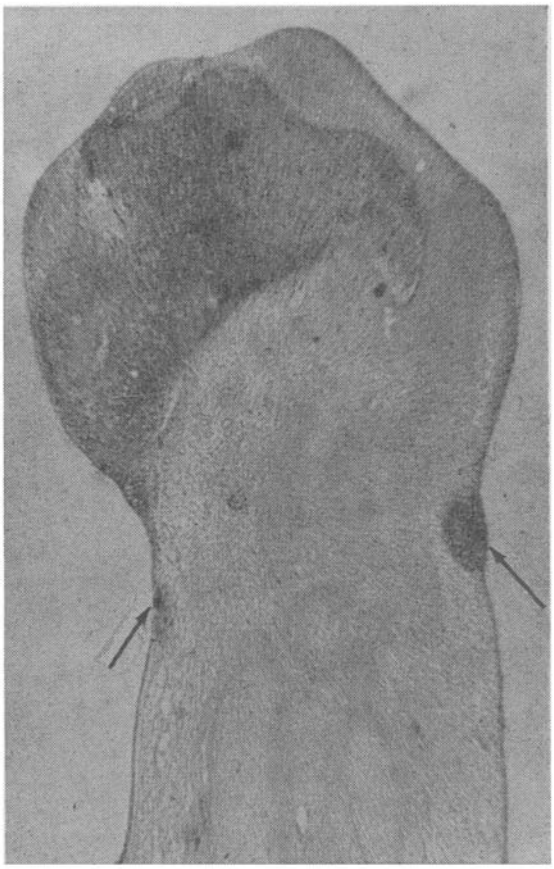

Fig. 11

Fig. 10. Frontal section of the right forelimb from the embryo on the left in Fig. 9. Arrow indicates haemorrhagic area in the region of developing digits III and IV. (Approximately 55 × .)

Fig. 11. Frontal section of the left hind-limb from a 13-day *fh* embryo showing a large area of haemorrhage over most of the developing digits. Arrows indicate two proximal haemorrhagic regions. (Approximately 55 × .)

evidence that the blebs in *fh* mice form in other regions and then migrate into tissues below the ectoderm of the limb or come to rest over the eye under the ectodermal tissues of the head.

The source and cause of the fluid-filled blebs are matters of conjecture in both *fh* and *my* stocks. Carter noted in his discussion of *my* mice that there is no evidence

to support a vascular origin for the blebs. No evidence of blood vessels feeding into the blebs was found in the sections of *fh* embryos studied. However, the distended cells found in the retina of an abnormal eye and a haemorrhagic limb of a 12-day *fh/fh* embryo indicate that Carter's explanation of *my* as the result of excess fluid in certain cells (he only cites mesenchymal cells as likely candidates) is applicable to the *fh* condition.

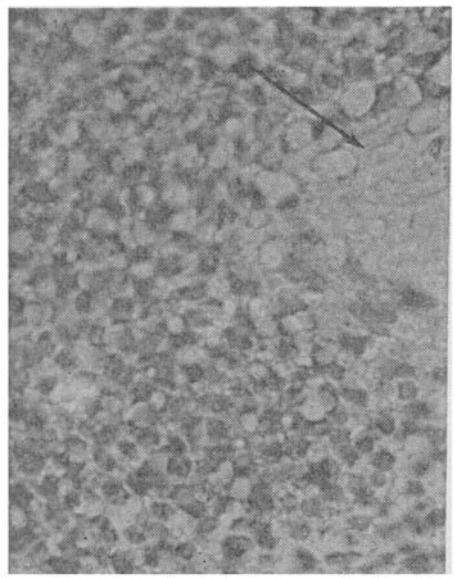

Fig. 12. Frontal section of the base of the left hind-limb of a 12-day *fh* embryo with an area of haemorrhage in this site. Arrow indicates distended and enucleate mesenchymal cells in this region. (Approximately 75×.)

Other mouse mutants characterized by oedematous regions affecting the development of the eyes and limbs are 'eye-blebs', designated *eb* (Beasley & Cruchfield, 1969) and 'oedematous' designated *oed* (Schiffman *et al.* 1975). The eye-bleb mutants are apparently characterized initially at 12 or 13 days by clear blebs (which later become haemorrhagic) in the apical regions of the limbs while in *fh* mice formation of proximal blebs in the limbs precedes or accompanies the appearance of terminal ones. The effect on the foot appears to be extreme in the *eb* mice since the foot may be entirely absent. The eyes in *eb* mice are reduced in size but no mention of open eyelids, which were frequently noted in *fh* mice, is made in the description of the *eb* mutants. Sections of haemorrhagic blebs found in *eb* mutants show small vessels opening into the blebs, which as noted above was not seen in *fh* homozygotes. Thus, the aetiology and morphological manifestations of the *fh* and *eb* mutants appear to be different.

The oedematous (*oed*) mutation is due to a lethal gene and is characterized by generalized body oedema and shiny, cracked skin. Foetal haematomata (*fh*) mutants did not manifest these characteristics. Thus it appears that there are

three separate mutants, *fh*, *eb*, and *oed* on the basis of embryological and morphological observations.

One might conclude from the above observations that *fh* and *my* mutants were identical. However, genetical studies did not indicate this identity.

The question remains concerning how and why two traits which were morphologically and embryologically very similar were not genetically identical. In mice, there have been a number of recorded cases (Grüneberg, 1963) in which separate genetic entities result in similar phenotypic manifestations. It is possible that the genes involved are responsible for different steps in a sequence of reactions leading to the phenotype. One can also hypothesize that the genetic pathways leading to a specific phenotype may be numerous and varied. Indeed, it is easy to visualize the possibility that many 'alternative' genes are necessary to increase the likelihood for normal development of a structure. Alternative genes may exist as 'backups' for damaged or defective genes in a given sequence of reactions.

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