# EMBRYOLOGY OF THE LITTLE & BAGG

## X-RAYED MOUSE STOCK

By T. C. CARTER
*Medical Research Council's Radiobiological Research Unit, Harwell, Berkshire, England.* *

### Introduction

Many abnormalities have been found in the descendants of the mice which C.C. Little and H. J. Bagg exposed to X-rays in 1921; they include defects of the brain, eyes, skull, kidneys, ureters, limbs, umbilical arteries, body wall and integument.

The inheritance of these defects was investigated by Little and Bagg and by several other workers between 1923 and 1936. It was found that some of the defects, involving the eyes, skull, limbs and integument, constitute a syndrome due to a single recessive gene, to which the symbol **my** was given; but there was disagreement about the inheritance of the others.

The development of the abnormalities was also investigated by Little and Bagg and by several other workers. All agreed that the defects which constitute the syndrome just mentioned are the late sequelae of superficial blebs which occur in embryos of the 12-day and later stages; but there was disagreement about the origin of the blebs themselves and of most of the other defects in the stock. By 1952, when Grüneberg reviewed the literature, thirty-eight papers had been published, but it was impossible to obtain from them a clear picture either of the extent of the syndrome or of the course of development of some of the abnormalities.

With the object of resolving the genetical conflict, a reinvestigation of the inheritance of the various defects was undertaken by Carter (1956). The conclusion reached was that all the common defects, and probably most of the rare ones as well, are parts of a variable syndrome, due primarily to the presence of **my** in the homozygous state; but that the expression of this gene is modified by the background genotype and perhaps also by non-genetic factors. In the course of the investigation further abnormalities were found and were shown to be further parts of the same syndrome.

This paper describes a reinvestigation of the morphology and development of the syndrome. The subject is much too large to permit a complete redescription; nor is one necessary, since there are many points on which there has not been any disagreement. Emphasis has therefore been placed on three aspects, namely (*a*) reinvestigation of the points on which earlier workers disagreed, (*b*) description of the embryology and morphology of those defects which have not previously been described, and (*c*) a search for developmental relationships between the various parts of the syndrome. In the event no single gene effect was identified from which the whole syndrome could be derived through what Grüneberg (1943*a*) has called a 'pedigree of causes'; but it

* Present address: Western Chicken Limited, London Road, Devizes, Wiltshire, England.

was possible to trace four subsidiary pedigrees and to demonstrate correlations, due to mechanisms as yet unidentified, between three of them.

## Material and Methods

The mouse stocks used were those described by Carter (1956). They comprised three sib-mated families, homogygous for **my** and selected for a high incidence of various abnormalities: in family A, eye defects, preaxial polydactyly and middle-digit syndactyly; in family B, pseudencephaly and eye defects; in family C, renal agenesis and preaxial polydactyly. Homozygous embryos were obtained at various ages between 8½ and 18½ days; their age- and family-distributions are summarised in table 1. Segregating litters of embryos, to provide litter-mate controls, were obtained by backcrossing +**my** males to females of the appropriate selected family (table 2).

Embryos were dissected out in saline at room temperature under a binocular microscope. Age was usually determined by the copulation plug method, occasionally from the 'external landmarks' (Grüneberg, 1943*b*). Three types of inviable embryo were recognised: 'abnormal turning', 'early dead' and 'late dead'. In 'abnormal turning' the post-umbilical part of the embryo fails to slip round the umbilicus and remains deflected to the right of the cephalic part; such embryos are found alive only between 8½ to 9½ days, when turning occurs, and 10½ or 11½ days, when they die. An 'early dead' embryo is one which died before the development of eye pigment, i.e. before 11½ days, 'late dead' thereafter. Viable embryos were examined in saline while still alive, and the presence or absence of various defects noted. These included the following: pseudencephaly, recognisable from 9½ days; blebs and polydactyly, from 12½ days; oedema of the body wall, from 13½ to 15½ days; and ectopia viscerum, from 14½ days.

With embryos of 12½ days or more the umbilical cord was usually cauterised before fixation, to prevent bleeding: it was draped over the edge of the embryo cup (the embryo remaining in saline in the cup) and a red hot stainless steel wire drawn rapidly along the edge of the cup, cutting and sealing the cord. After fixation the maximum embryonic diameter was usually measured with a travelling microscope. Embryos for serial sectioning were fixed in Bouin's fluid, cut at 10$\mu$ and stained with haematoxylin and eosin or Mallory's triple stain. Seventy-nine embryos were sectioned serially; their age distribution is shown in table 3.

Adult kidneys, ureters and bladders for serial sectioning were treated in the same way as embryos.

Juvenile and adult material for examination of the skeleton *in toto* were fixed in alcohol and stained with alizarin red S. Bones were prepared for individual examination by digestion of the soft tissues with papaine.

For differential counts of juvenile and adult hair fibre types in regions of defective coat ('saddle'), a small pinch of hair containing 150 to 200 fibres was taken between the finger and thumb and cut off near the skin with scissors; the fibres were floated in a thin layer of 70% alcohol and separated with watchmakers' forceps under a binocular microscope. Total counts of the number of hair shafts emerging per unit area of the skin were made by the method described by Slee (1957*a*); fields of 49 $mm^2$ at the eyepiece were examined at a magnification of 90. A like-sexed litter-sib was used as control, the material being treated simultaneously.

## Observations

The parts of the syndrome will be considered in five groups: defects of the central nervous system; superficial blebs in the embryo and their sequelae; defects of the body wall; morphogenetic defects of the hind limbs; and defects of the urogenital system and umbilical artery.

### (i) *Defects of the central nervous system*

(*a*) *Acrania.* Dead, bloodless, acraniate, newborn young occurred with a high frequency in family B, where selection had been exercised for this defect; they were never found in the amnion, nor were similar embryos found at dissection. They agreed with the descriptions and illustrations of acraniate young given by Little & Bagg (1923).

Table 1. *Manifestation of defects in embryos from homozygous matings, by family and embryonic age*

| Defect* | Family A | | | | | Family B | | | | | | | | | | Family C | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 13½ | 14½ | 15½ | 16½ | 18½ | 8½ | 9½ | 10½ | 11½ | 12½ | 13½ | 14½ | 15½ | 16½ | 17½ | 9½ | 10½ | 11½ | 12½ | 13½ | 16½ |
| M | 3 | 2 | 7 | 6 | 1 | 5 | 1 | — | 5 | 5 | 12 | 2 | 2 | 1 | — | 1 | — | — | 1 | 3 | 2 |
| D | — | — | — | 5 | — | — | — | — | — | — | 2 | 2 | — | — | — | — | — | — | 1 | — | — |
| T | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 3 | 1 | — | — | — |
| N | — | — | — | — | — | 14 | 3 | 16 | 8 | 5 | — | — | — | 1 | 1 | 2 | 16 | 15 | 11 | — | 2 |
| A | — | — | — | — | — | — | 1 | 8 | 10 | — | — | 1 | 1 | — | — | — | 1 | 1 | — | — | — |
| AW | — | — | — | — | — | — | — | — | — | — | 3 | 1 | 1 | — | — | — | — | — | — | — | — |
| AB | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — |
| ABW | — | — | — | — | — | — | — | — | — | — | — | 2 | — | — | — | — | — | — | — | — | — |
| ABPW | — | — | — | — | — | — | — | — | — | — | 3 | — | — | — | — | — | — | — | — | — | — |
| AE | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — |
| AEW | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — |
| AP | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | 1 | — | — |
| APW | — | — | — | — | — | — | — | — | — | — | 3 | 1 | — | — | — | — | — | — | — | — | — |
| APE | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — |
| B | 6 | 3 | 4 | 10 | — | — | — | — | 1 | 6 | 10 | 2 | 1 | 2 | 2 | — | — | — | 4 | 3 | — |
| BW | 1 | 2 | 1 | — | — | — | — | — | — | — | 5 | 5 | 2 | — | — | — | — | — | — | — | — |
| BE | — | — | 1 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| BP | 1 | 5 | 2 | 8 | 1 | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — |
| BPW | 9 | 9 | 3 | — | — | — | — | — | — | — | 2 | — | — | 3 | — | — | — | — | — | 10 | 4 |
| BPE | — | — | — | 4 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| BPEW | — | 2 | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| P | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 2 |
| PE | — | 1 | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |

* A, pseudencephaly; B, bleb, haematoma or thrombus; D, dead at 11½-day stage or later; E, ectopia viscerum; M, dead before 11½-day stage; N, normal; P, preaxial polydactyly; T, abnormal turning; W, oedema.

Table 2. *Manifestation of defects in embryos from backcross matings, by family and embryonic age*

| Defect* | Family A | | | | | Family B | | | | | | | | | | Family C | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12½ | 13½ | 14½ | 15½ | 17½ | 9½ | 10½ | 11½ | 12½ | 13½ | 14½ | 15½ | 16½ | 17½ | 18½ | 9½ | 10½ | 11½ | 12½ | 13½ | 15½ | 17½ |
| M | 4 | 7 | 2 | 1 | — | 1 | 2 | 3 | 2 | 4 | 2 | 4 | — | 1 | — | — | 2 | 1 | 1 | 1 | 1 | — |
| D | — | — | 1 | 1 | 1 | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — |
| N | 1 | 2 | 1 | 3 | 4 | 19 | 31 | 23 | 12 | 18 | 7 | 3 | 4 | 5 | 3 | 13 | 4 | 24 | 5 | 4 | — | 3 |
| A | — | — | — | 1 | — | 2 | 2 | 1 | — | 2 | — | 2 | 1 | 1 | 4 | — | 1 | 3 | — | 1 | — | — |
| AB | — | — | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — | — |
| AW | — | — | — | — | — | — | — | — | — | 1 | 4 | — | — | — | — | — | — | — | — | — | — | — |
| ABPW | — | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| APE | — | — | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| B | 1 | 5 | — | — | 2 | — | — | 1 | 3 | 7 | 2 | 8 | 2 | 1 | 4 | — | — | — | — | 3 | 1 | 5 |
| BP | — | 3 | — | — | 4 | — | — | — | — | 1 | — | — | — | — | — | — | — | — | 1 | — | — | 1 |
| BPW | — | — | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| BW | — | 4 | — | — | — | — | — | — | — | 8 | 1 | — | — | — | — | — | — | — | — | 5 | — | — |
| P | 1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |

* For key see Table 1.

Table 3. *Age distribution of embryos sectioned serially*

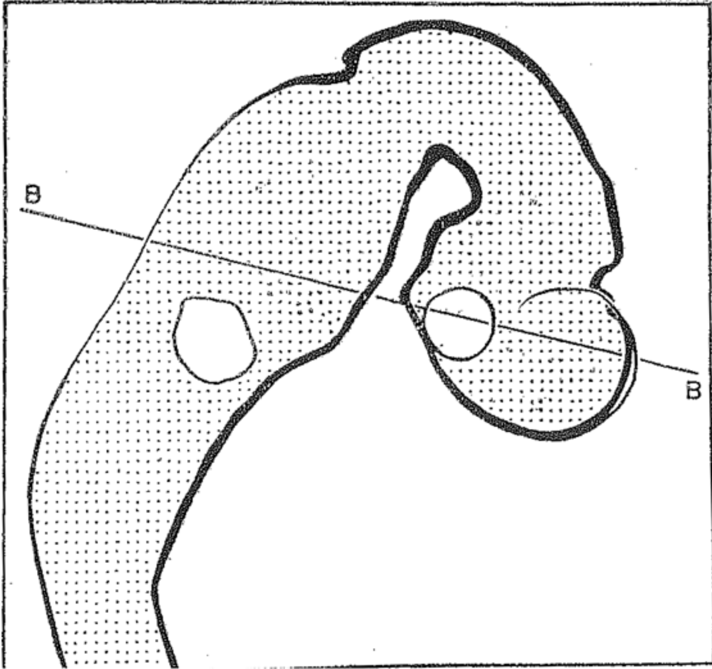
| Age of embryo (days) | 8½ | 9½ | 10½ | 11½ | 12½ | 13½ | 14½ | 16½ | Total |
|---|---|---|---|---|---|---|---|---|---|
| Number of embryos | 8 | 16 | 9 | 15 | 15 | 10 | 4 | 2 | 79 |

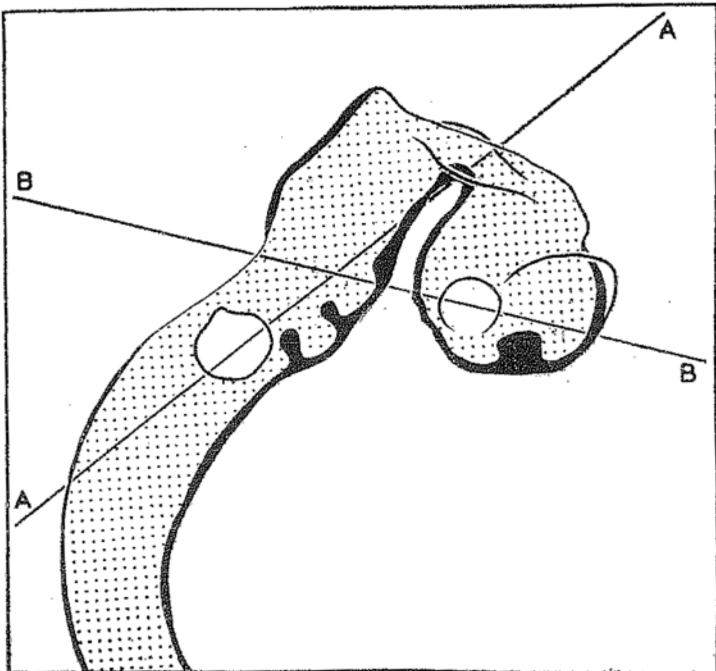
Pseudencephalic newborn young, dead or alive, also occurred in family B; they were usually still in the amnion. Pseudencephalic embryos were found at all stages from 9½ to 18½ days; from the 16½-day stage the amniotic fluid contained blood. It was concluded that the acraniate newborn young represent pseudencephalic embryos which have lost their exteriorised neural tissue, presumably at the time of removal from the amnion by the mother.

(*b*) *Pseudencephaly.* Pseudencephaly in embryos was identified *per continuitatem.* Pseudencephalic embryos of 11½ days (6 *mm*) and more agreed with the descriptions given by Bonnevie (1936). In 9½ and 10½-day embryos of about 2*mm* there was failure of the neural ridges to close in the mesencephalon or the anterior part of the myelencephalon (Text-figs. 1 and 2). With further growth the neural ridges flattened out, so that the whole dorsal surface of this part of the head consisted of exposed neural tissue (Text-fig. 3). At the same time the closed part of the neural tube, i.e., the forebrain and the rhombencephalon and posterior to it, failed to become distended, as normally occurs after closure when the tube fills with cerebrospinal fluid (Text-figs. 4 & 5). Seen from behind, the 9½- and 10½-day pseudencephalic head appeared flattened from side to side and in sections the undistended neural tube had a crumpled appearance; this became more obvious, especially in the hindbrain, in 11½-day embryos. Histological differentiation of the eyes, otic vesicles and other organs of the head appeared to be normal; any abnormality in them was of gross anatomy, following the generally distorted shape of the head.

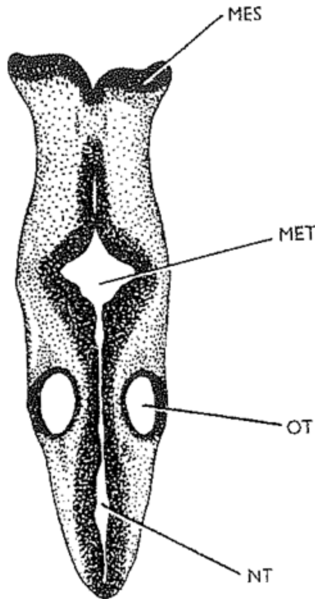
Identification of 9½- and 10½-day pseudencephalic embryos required confirmation, because in some normal embryos of this age the anterior neuropore is still open. Confirmation was obtained through observation of the size distribution of normal embryos at the time of closure. This usually occurs just after embryonic turning, i.e. at about 9½ days. Measurement of embryos from two normal stocks (*CBA* and *KLM*) showed that closure was always complete in turned embryos exceeding 1·5 *mm*. In family B, however, there were several 10½-day embryos and one turned 9½-day embryo ranging from 2·0 to 4·0 *mm* which had the neural tube still open in the head; these were identified as pseudencephalic. Table 4 shows that the incidence of pseudencephaly was constant among embryos beyond this stage.

Bonnevie (1936) reported that in normal embryos of about 8½ days the chorda was entirely separated from the floor of the brain, but that in two embryos of her stock, which she presumed to be early pseudencephalics, the chorda and neural tissue were more or less firmly joined together. The notochord was in contact with the floor of the brain in all of eight serially sectioned 8½-day family B **my my** embryos and in all of eight unturned 9½-day embryos (5 to 12 pairs of somites) from family B backcross matings.

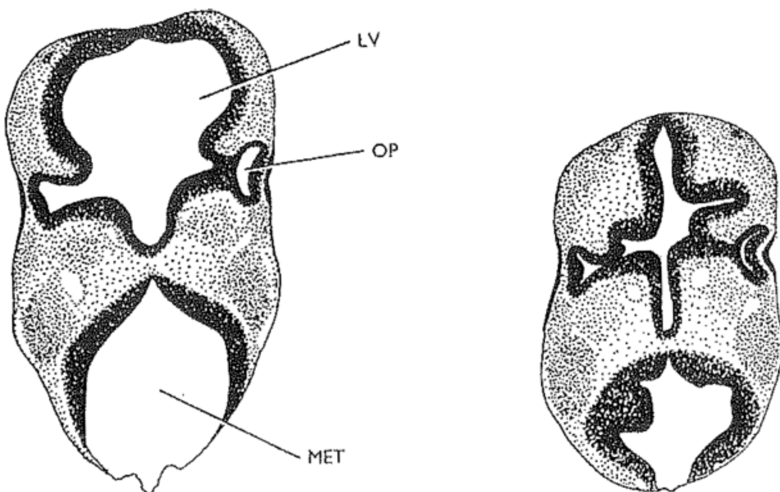



Text-figs. 1 and 2. Reconstructions from serial sections of the anterior parts of the neural tubes of normal (3·6 *mm*) and pseudencephalic (3·1 *mm*) 10½-day embryos from the same uterus. A-A, B-B, planes of sections illustrated in text-figs. 3, 4 and 5.




Text-fig. 3. Section through the mes-and metencephalon of a pseudencephalic 10½-day embryo, cut in the plane marked A-A in text-fig. 2. MES, neural tissue of mesencephalon; MET, lumen of metencephalon; NT, lumen of neural tube; OT, otic vesicle.




Text-figs. 4 and 5. Sections through the head of a normal and a pseudencephalic 10½-day embryo, cut approximately in the plane marked B-B in text-figs. 1 and 2. LV, lateral ventricle; MET, lumen of metencephalon; OP, optic cup.

Table 4. *Incidence of pseudencephaly in embryos from homozygous matings in family B. Data of Table 1*

| Embryonic age (days) | *Observed N | *Observed A | Expected N | Expected A | $\chi^2$ | Degrees of freedom | Probability. |
|---|---|---|---|---|---|---|---|
| 17½ | 3 | 1 | | | | | |
| 16½ | 7 | 0 | 10·982 | 6·018 | 1·047 | | |
| 15½ | 3 | 3 | | | | | |
| 14½ | 7 | 6 | 26·486 | 14·514 | 0·659 | | |
| 13½ | 17 | 11 | | | | | |
| 12½ | 11 | 0 | 19·380 | 10·620 | 0·056 | | |
| 11½ | 9 | 10 | | | | | |
| 10½ | 16 | 8 | 16·150 | 8·850 | 0·004 | | |
| 9½ | 0 | 1 | | | | | |
| Totals | 73 | 40 | 72·998 | 40·002 | 1·766 | 3 | 0·78 |

* Unturned embryos excluded; A, pseudencephalic; N, normal.

Where the pseudencephalic class has an expectation of 5 or fewer, the data have been lumped with those from the next younger stage for the test of significance.

### (ii) *Superficial blebs in the embryo and their sequelae*

(*a*) *Disruptive lesions of the head.* Haemorrhagic lesions and maldevelopment of the eyes, corneal opacity, maldevelopment of the eyelids, reduction of a pinna (associated with a reduced eye), shortened snout, twisted snout, malocclusion of the teeth, asymmetry of the cranium and midcerebral lesions all occurred in postnatal material of one or more of families A, B and C. They agreed with the descriptions given by Bagg & Little (1924) and by subsequent workers. Midcerebral lesions, however, have received in the past only cursory mention. They were of rare occurrence. In adult and late embryonic stages a midcerebral lesion appeared as a bump over the midline of the head between the eyes and ears, near the junction of the frontal and parietal bones. In skeletal preparations the cranium was seen to be perforated in this region (Text-fig. 6). A similar condition has been found in mice of the C57BL inbred strain by Deol & Truslove (1957) and described under the name *frontal fontanelle*.

(*b*) *Disruptive lesions of the limbs.* Clubbing, plantar-, palmar- and dorsiflexion of the extremities, with maldevelopment of the digits, sometimes accompanied by haemorrhagic lesions, were seen in postnatal material of all three families (Text-fig. 7). They agreed with the descriptions given by Bagg & Little (1924) and by later workers.

(*c*) *Disruptive lesions of the integument.* Defective skin and coat formation in the lumbar region ('saddle') occurred with a low frequency in postnatal material of all three families, and agreed with the brief description of Little & Bagg (1923). The results of differential counts of the numbers of hair types, and total counts of the number of hair shafts per unit area, in the saddles of adult **my my** mice and the corresponding

areas of normal like-sexed litter-sib controls, are summarised in tables 5 and 6. They show a total absence of guard-hairs from the saddle, a decrease in the proportion of awls, and an increase in the proportion of zigzags. Furthermore, there is no evidence of a significant change in the total density of hair shafts, so the changes are absolute as well as proportionate.

(*d*) *Curved vibrissae.* A trivial morphological change of hair type was sometimes seen in the vibrissae; these were occasionally curved, as in mice carrying a rexoid mutant. The effect was nearly always unilateral and was associated with a twisted snout. It was probably a mechanical consequence of curvature of the follicle associated with the curvature of the snout.

Table 5. *Numbers of guard-hairs (GH), awls (AW) and zigzag fibres (ZG) in samples from the saddles of four* **my my** *mice and the corresponding areas of like-sexed litter-sib controls*

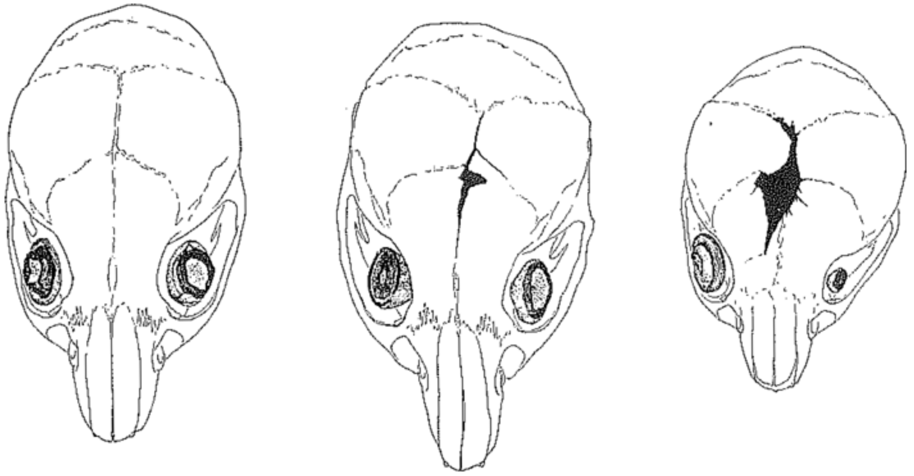
| Samples per mouse | Saddle | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|
| | GH | AW | ZG | Total | GH | AW | ZG | Total |
| 5 | 0 | 290 | 985 | 1275 | 15 | 363 | 715 | 1093 |
| 5 | 0 | 150 | 487 | 637 | 16 | 347 | 613 | 976 |
| 2 | 0 | 70 | 254 | 324 | 5 | 112 | 212 | 329 |
| 3 | 0 | 80 | 233 | 313 | 10 | 235 | 401 | 646 |
| Totals | 0 | 590 | 1959 | 2549 | 46 | 1057 | 1941 | 3044 |
| Percentage | 0·0 | 23·1 | 76·9 | 100·0 | 1·5 | 34·7 | 63·8 | 100·0 |

Table 6. *Numbers of hair shafts emerging from unit area of skin in the saddle of an* **my my** *mouse and the corresponding area of a like-sexed litter-sib control. Twenty samples counted; field* 49mm² *at the eyepiece, magnification* 90x

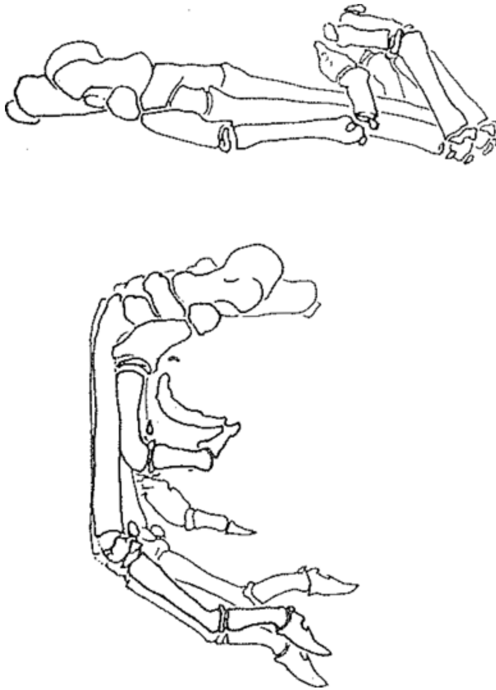
| | Mean & standard error. |
|---|---|
| Saddle | 38·4 ± 1·3 |
| Control | 36·7 ± 1·6 |

(*e*) *Parietal patches.* A transient pigmentary change was frequently seen in **my my** mice between the ages of four and eight days, when pigment is forming in the hairs and they are erupting through the skin : there was a delay of about a day in the development of pigment in the midcerebral region. These 'parietal patches' often spread down to one or other eye, or both, and when they did so there were lesions of the eyelids or eyes.

(*f*) *Pattern of pigmentation.* A permanent change of pigmentation sometimes occurred in **my my** mice carrying the mutant $\mathbf{a^t}$. In +**my** mice carrying this mutant the dorsum is black and the venter tan-coloured, the two regions being separated by a clear line of demarcation running along the sides and lower jaw of the animal. In **my my** mice the line of demarcation was often displaced towards the dorsal midline. This occurred especially where there were eye lesions or a saddle; the area of tan pigmentation then extended towards and sometimes over the lesion (Plate 1).



Text-fig. 6. Skulls of a normal and two **my my** mice, showing distortion and incomplete ossification in the fronto-parietal region.



Text-fig. 7. Bleb-type lesions on two hind feet; dorsi- and plantar flexion.

(*g*) *Clear blebs.* Clear superficial blebs were seen on 117 embryos of 6·5 to 13·5 *mm* (12½ to 15½ days) and on two of 4·9 and 5·0 *mm* (11½ days). No other worker has reported blebs on 11½-day embryos, and these two had been subjected to accidental rough treatment at dissection. This gives rise to a suspicion that their blebs may have been artifacts; they are therefore excluded from the following general description and are described and discussed later.

Table 7 summarises the distribution of the bleb sites in the 70 embryos of the homozygous families which had clear blebs. They occurred in three main regions: head, shoulders and forelimbs, rump and hind limbs. They appeared at about the same time in all three regions. Their incidence reached a maximum in embryos of 8 to 9 *mm* (13½ days), decreasing thereafter.

Within the head region the sites of the blebs did not remain constant. In embryos of 7 to 8 *mm* (12½ days) the head blebs were often small, and most commonly situated middorsally over the anterior part of the mesencephalon, filling the depression between the mesencephalon and the cerebral hemispheres. In embryos of 8 to 9 *mm* (13½ days) the blebs were usually larger and extended down one or both sides of the mesencephalon towards the eye, filling the depression posterior to the cerebral hemisphere. In embryos of 9 to 10 *mm* (13½ and 14½ days) the head blebs were usually in contact with the posterodorsal margin of the eye, but in some they did not cover the dorsal midline. In these embryos there was often a crumpling of the head ectoderm, clearly visible on the live embryo, in a region extending from the dorsal margin of the bleb to beyond the midline; this appeared to be a region which had been formerly occupied by the bleb and from which it had regressed. Purely middorsal blebs were rare in embryos of this stage. The larger lateral blebs sometimes reached the ear, and they often surrounded the eye completely. Blebs were also found from the 8 *mm* stage filling the depression between the lateral ventricles in the anterior part of the head, where the snout will subsequently form. Blebs were not found on the myelencephalon.

Hind limb blebs in embryos of 7 to 8 *mm* were often found on the mesial side of the limb bud, in the depression where the bud joins the body; in this position in the live embryo they tended to be hidden from sight, becoming apparent only in sections. Blebs also occurred at this stage on the preaxial border of the limb, immediately proximal to the apical ectodermal ridge of the footplate. In embryos of 9 *mm* and more they were found most commonly on the dorsal or plantar surface of the footplate.

A similar sequence was seen in the forelimbs.

Blebs were repeatedly seen on pseudencephalic embryos. Usually they were tibial border blebs (Plate 1), but they were also seen near the eye, though not on the exteriorised neural tissue.

The two blebby 11½-day embryos had both been squeezed by the contracting uterine muscles at dissection; this had ruptured the yolk-sac, with consequent loss of the fluid by which the gelatinous embryo of this stage is normally supported. There is always a risk of this happening when embryos of 10½ to 13½ days are dissected out, but it occurred infrequently in the present series. Records made at the time read as follows:

1. "Viable. 11½ days. Burst from all membranes at dissection but not

Table 7. *Distribution of sites of clear blebs in embryos from homozygous* **my my** *matings, according to size of embryo*

| Size of embryo (*mm*) | No. of embryos | Head * † SN | E L | ME L | MS M | ME R | E R | Forelimbs A L | SH M | A R | Hind limbs LG L | LU L | LU M | LU R | LG R | Total clear blebs | Blebs per embryo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6·0— | 1 | — | — | — | 1 | 1 | — | — | — | — | — | — | — | — | — | 2 | — |
| 7·0— | 21 | — | 2 | 2 | 12 | 10 | — | 1 | 1 | 1 | 7 | 1 | — | 1 | 6 | 44 | 2·1 |
| 8·0— | 18 | 2 | 5 | 6 | 3 | 3 | 8 | 4 | 6 | 1 | 16 | — | 2 | — | 13 | 69 | 3·8 |
| 9·0— | 16 | 4 | 2 | 6 | 1 | 8 | 4 | 1 | 2 | 2 | 9 | 2 | 2 | 2 | 8 | 53 | 3·3 |
| 10·0— | 8 | — | 2 | 4 | — | 4 | 2 | 1 | — | 1 | 2 | 2 | 1 | 2 | 1 | 22 | 2·7 |
| 11·0— | 4 | — | — | — | — | 1 | 1 | — | — | 1 | 1 | — | — | 1 | — | 5 | 1·2 |
| 12·0— | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 13·0— | 2 | — | — | — | — | — | 1 | — | — | — | — | 1 | 1 | 1 | — | 4 | — |
| Totals | 70 | 6 | 11 | 18 | 17 | 27 | 16 | 7 | 9 | 6 | 35 | 6 | 6 | 7 | 28 | 199 | 2·8 |

* A, arm; E, eye region; LG, leg; LU, lumbar region; ME, midline to eye; MS, mesencephalon; SH, shoulder; SN, snout.
† L, left; M, midline; R, right.

apparently damaged (?). Some surface blood suffusion over left hemisphere, near vein, and over mesencephalon. Ectoderm lifted over vein between left hemisphere and left side of mesencephalon where there is a depression between them, adjacent to blood suffused area. Heart beating weakly. Not yet footplates".

This embryo had a maximum diameter of 4·9 *mm.*

2. "Viable. $11\frac{1}{2}$ days. Burst from yolk-sac but not amnion and no damage believed done. Blood suffused area on both lateral ventricles and mid-cerebral region of mesencephalon. Clear bleb over vein on left of head reaching midcerebral region. Small clear tibial border bleb on right hind limb, though the hind limbs have not got footplates; this bleb fills the region between limit of apical ectodermal ridge and body wall. No other blebs".

This embryo had a maximum diameter of 5·0 *mm.*

These two provide the sole evidence for the existence of blebs in embryos of less than 6·5 *mm.*

The nature of the membranes enclosing the bleb fluid could not be determined with certainty; it was difficult to determine if there was a basement membrane in the ectoderm and, if so, on which side of the bleb it lay. The most likely interpretation is that the fluid lay immediately above the continuous, superficial layer of mesenchymal cells; in other words, the fluid lay between the mesenchyme and the basement membrane of the ectoderm, if present.

The capillaries in the mesenchyme of blebby embryos, and in particular the capillaries of the limbs, did not appear to differ in their location from those of normal embryos; nor were abnormalities seen in the distribution of the footplate marginal sinuses. These made intermittent contact with the apical ectodermal ridge in both blebby and normal embryos.

(*h*) *Haematomata and thrombi.* In many $13\frac{1}{2}$-day embryos the blebs, previously clear, became haematomata: blood cells entered the bleb fluid. From the $15\frac{1}{2}$-day stage there was also blood stasis in the underlying mesodermal tissues, as described by Plagens (1933). Distinction between such thrombi and the superficial haematomata could be made with certainty only in sectioned material; the two types have therefore been lumped together in table 8, which shows their distribution. It is seen that the number of haematomata and thrombi per embryo appears to have been lower than the mean number of clear blebs per embryo, the greater part of the difference being in the hind limb region. This implies that some border blebs are not represented subsequently by haematomata or thrombi, but that most head blebs are so represented. However, $16\frac{1}{2}$-day embryos were observed which lacked upper eyelids, and in which crumpling of the head ectoderm pointed to the presence of blebs at an earlier stage, but in which nevertheless no sign of a haematoma or thrombus was seen.

(iii) *Defects of the body wall*

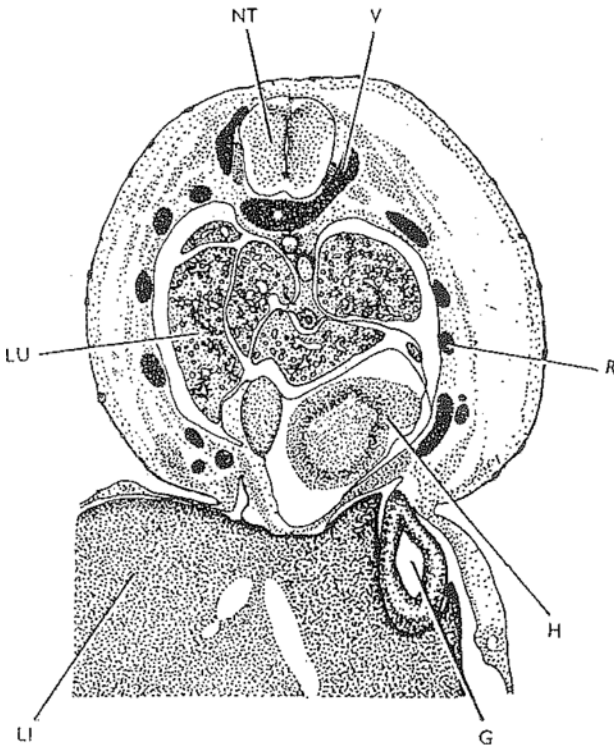
(a) *Oedema.* Mouse embryos of ages up to $12\frac{1}{2}$ days have very little histodifferentiation in the skin and body wall; seen alive in saline, they have a gelatinous appearance,

Table 8. *Distribution of sites of blood clots in embryos from homozygous* **my my** *matings, according to size of embryo*

| Size of embryo (*mm*) | No. of embryos | Head *† SN | Head E L | Head ME L | Head MS M | Head ME R | Head E R | Forelimb A L | Forelimb SH M | Forelimb A R | Hindlimb LG L | Hindlimb LU L | Hindlimb LU M | Hindlimb LU R | Hindlimb LG R | Total blood clots | Blood clots per embryo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8·0– | 1 | – | – | – | – | 1 | – | – | – | – | – | – | – | – | – | 1 | 1·0 |
| 9·0– | 4 | – | – | 2 | – | – | 1 | 1 | – | – | – | – | – | – | 1 | 5 | 1·2 |
| 10·0– | 2 | – | 1 | 1 | – | 1 | – | – | – | – | – | – | – | – | – | 3 | 1·5 |
| 11·0– | 4 | 1 | 3 | – | – | – | 1 | – | – | – | – | – | 1 | – | – | 6 | 1·5 |
| 12·0– | 5 | 1 | 1 | – | – | 1 | 3 | – | 1 | – | – | – | – | – | – | 7 | 1·4 |
| 13·0– | 8 | 2 | 4 | 1 | – | 1 | 4 | 1 | – | – | 1 | – | – | – | – | 14 | 1·7 |
| 14·0– | 4 | – | 1 | – | – | 1 | 2 | – | – | 1 | – | 2 | – | 1 | 2 | 10 | 2·5 |
| 15·0– | 6 | 3 | 4 | – | – | 1 | 1 | 1 | – | – | – | – | – | – | 1 | 11 | 1·8 |
| 16·0– | 3 | 2 | – | – | – | – | 3 | – | – | – | – | – | – | – | – | 5 | 1·7 |
| 17·0– | 2 | 2 | – | – | – | – | 2 | – | – | – | – | – | – | – | – | 4 | 2·0 |
| Totals | 39 | 11 | 14 | 4 | – | 6 | 17 | 3 | 1 | 1 | 1 | 2 | 1 | 1 | 4 | 66 | 1·7 |

*† For key see Table 7.

and it is difficult to judge if the amount of tissue fluid is normal or abnormal. Between 13½ and 15½ days histodifferentiation is occurring in the body wall, but the skin is not yet fully opaque; an abnormal amount of fluid in the mesenchyme can then be recognised, when the embryo is examined in saline, by the fact that the skin stands away from the deeper tissues and the embryo looks blown up. Oedema was detected by this method in many **my my** embryos of this age (Tables 1 and 2). From 16½ days the skin is opaque and detection of oedema becomes uncertain. In sectioned material the body wall mesenchyme of normal 13½ to 15½-day mouse embryos is seen to consist mainly of a spongy mass of stellate cells and large, intercellular spaces which in life were presumably fluid-filled. In **my my** embryos the appearance was much the same (Plate 1); oedema could be detected in the sectioned mesenchyme of some embryos which had appeared very abnormal when examined *in vivo*, but in the majority the sections looked more or less normal. The reason for this is not clear. Perhaps it was the geometrical reason that a large increment of volume may correspond with only a small increment of linear dimension; or perhaps the oedematous condition was

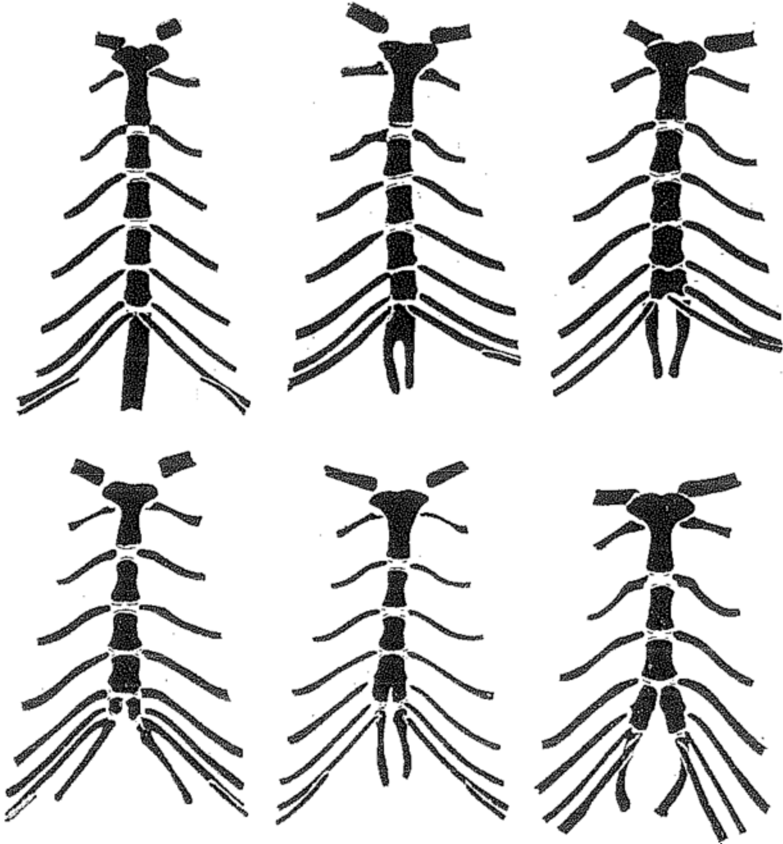



Text-fig. 8. Transverse section through the lower thorax of a 14½-day **mymy** embryo. G, ectopic gut; H, heart; LI, ectopic liver; LU, lung; NT, neural tube; R, rib; V, vertebra. Note the asymmetrical oedema; the skin is lifted far away from the thoracic basket on the left side of the embryo (right of the figure). The sternum is wide open.

partly lost during fixation, dehydration or embedding. The pia-arachnoid space appeared to be of normal dimensions in **my my** embryos (Plate 1).

(b) *Ectopia viscerum.* Normal mouse embryos of 13½ days have an umbilical hernia. By 14½ days this has largely retracted, and by 15½ days retraction is complete. In many **my my** embryos, however, the abdominal wall remained open, with the gut and liver protruding; distinction between normal and abnormal could be made on inspection at or after the 14½-day stage (Text-fig. 8). Abnormal embryos survived to term, but they were usually found dead, eviscerated and bloodless soon after birth. Evisceration presumably occurred when the mother cleaned up the newborn young, and was the cause of haemorrhage. When the belly wall defect was small, the animal sometimes survived, even though born with a partly protruding liver; the skin healed over in a day or two and only a slight hernia remained visible.

(c) *Split sternum.* Associated with ectopia viscerum was incomplete formation of the sternum and diaphragm. The number of sternebrae was reduced, and the left and right halves of the sternum were separate posteriorly (Text-fig. 9). This abnormality was already well established in embryos of 14½ days.



Text-fig. 9. Sterna of a normal young mouse (top, left) and five **my my** mice, showing splitting and reduction of the sternebrae.

Table 9. *Occurrence of tibial border blebs and polydactyly in family B embryos of 12½ days and more*

| Left hind limb | | Polydactyly − | Polydactyly + | Total |
|---|---|---|---|---|
| Border blebs | − | 55 | 4 | 59 |
| | + | 9 | 1 | 10 |
| | Total | 64 | 5 | 69 |

| Right hind limb | | Polydactyly − | Polydactyly + | Total |
|---|---|---|---|---|
| Border blebs | − | 51 | 10 | 61 |
| | + | 7 | 1 | 8 |
| | Total | 58 | 11 | 69 |

Table 10. *Occurrence of tibial border blebs and polydactyly in family C embryos of 12½ days and more*

| Left hind limb | | Polydactyly − | Polydactyly + | Total |
|---|---|---|---|---|
| Border blebs | − | 22 | 10 | 32 |
| | + | 2 | 3 | 5 |
| | Total | 24 | 13 | 37 |

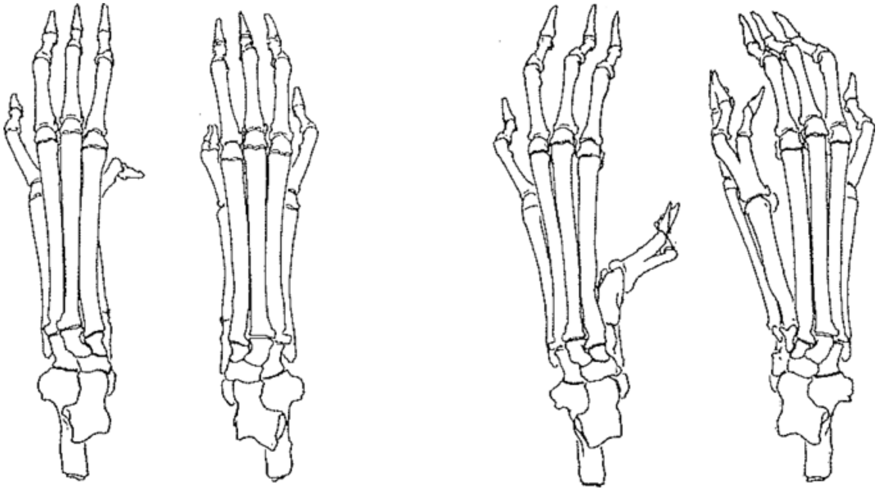
| Right hind limb | | Polydactyly − | Polydactyly + | Total |
|---|---|---|---|---|
| Border blebs | − | 20 | 13 | 33 |
| | + | 0 | 4 | 4 |
| | Total | 20 | 17 | 37 |

### (iv) *Morphogenetic defects of the hind limbs*

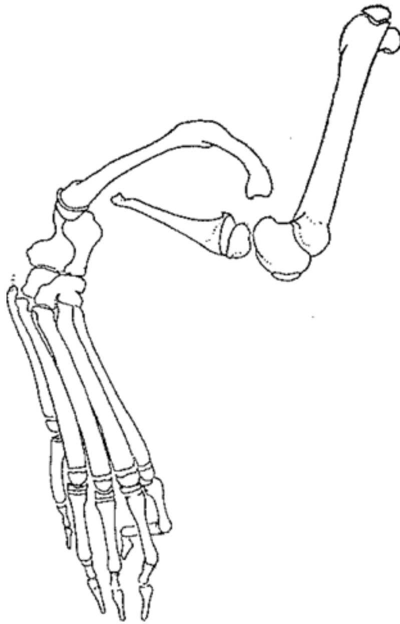
(a) *Polydactyly.* Preaxial polydactyly occurred in all three families; it had a very high incidence in family A, in which selection had been exercised for it (Text-fig. 10). As used here, the term *polydactyly* includes hyperphalangy of the hallux. It was confined to the preaxial side of the hind feet and favoured the right foot. In adult material it agreed with the descriptions of Bean (1929). In embryos it was first recognisable at the 12½-day stage as excess growth of the preaxial margin of the footplate. This was often, but not always, accompanied by a small bleb on the tibial border of the hind limb, immediately proximal to the footplate (Tables 9 and 10).

(b) *Tibial hemimelia.* Tibial hemimelia was found in one animal of family A (Text-fig. 11). The incidence of polydactyly was 100 per cent among the sibs of the hemimelic. No hemimelic embryos were seen.

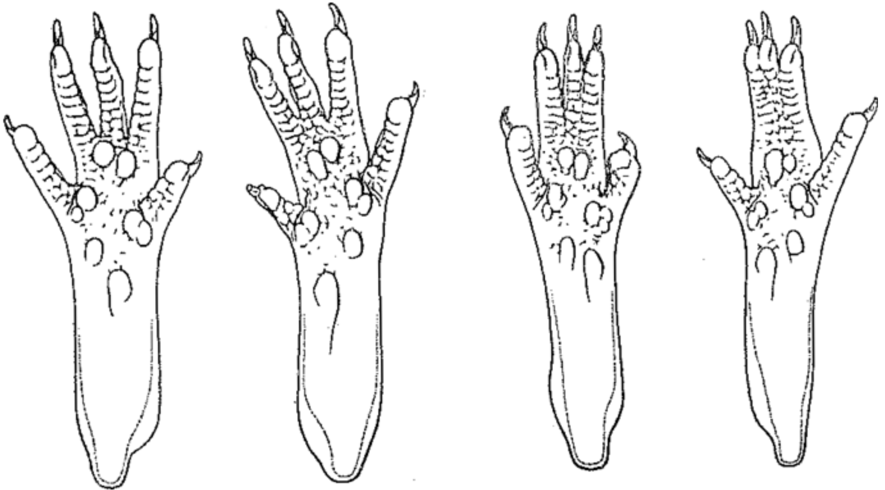
(c) *Syndactyly.* Syndactyly affecting the middle digit of the hind limbs (and, rarely, also of the forelimbs) occurred almost exclusively in family A, where its incidence responded to upward selection. Usually the third and fourth digits were affected, less commonly the second and third or all three (Text-fig. 12). With rare exceptions only the soft tissues were involved, and often there was no more than a slight webbing of the proximal phalanges; it was then difficult to recognise the condition with certainty in mice less than 14 days old. Occasionally the skeleton was involved (Text-fig. 13);



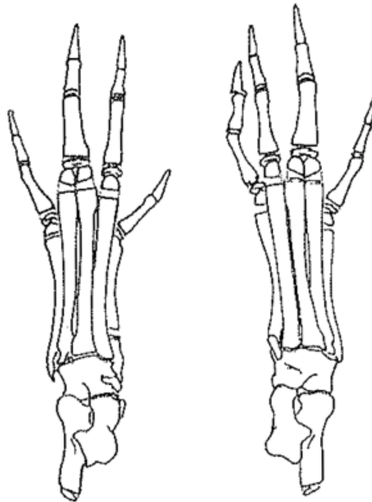
Text-fig. 10. Dorsal views of the skeletons of two pairs of hind feet. The foot on the left is normal, the others show various grades of preaxial polydactyly.



Text-fig. 11. Dorsolateral view of the skeleton of a right hind limb showing tibial hemimelia.



Text-fig. 12. Plantar views of two pairs of hind feet. The foot at the left is normal, the others show various grades of syndactyly.



Text-fig. 13. Dorsal view of the skeletons of two syndactylous hind feet; the right foot shows also hyperphalangy of the hallux.

the metatarsals remained separate, but there was fusion of the phalanges to form a single, thick digit which retained the normal morphological pattern and was functional. Syndactyly was usually accompanied by polydactyly, which had a high incidence in family A. Syndactyly was not observed in embryos. This may have been because it would have been detectable only if there had been high-grade expression, involving the skeleton, and this grade of expression was rare.

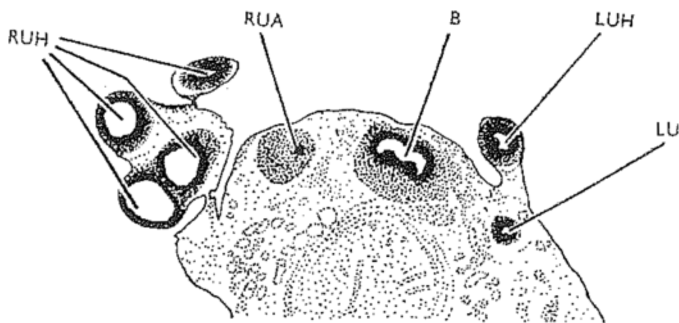
### (v) *Defects of the urogenital system and umbilical artery*

(a) *Renal agenesis and renal hypoplasia.* Renal agenesis occurred with a high incidence in family C, in which selection for it had been exercised. It occurred also in the other two families, but with a lower incidence. Renal agenesis in adult, juvenile and newborn material agreed with the descriptions given by Bagg (1925). Its appearance in embryos agreed in general with the descriptions given by Brown (1931), being associated with failure of the ureter to bud off from the wolffian duct or, if budded, to grow into the nephrogenic cord. In the normal mouse embryo the ureter buds off at the 11½-day stage. Among the embryos examined in serial sections the left ureter was absent from three at the 11½-day stage and one of 14½ days, the right from one of 14½ days; in two of 13½ days only a short, blind left ureter was present, without a kidney (Plate 1). In two other 13½-day embryos both ureters were present but both failed to reach the nephrogenic cord and there was no kidney induction; in these the nephrogenic cord ended anterior to the umbilical arterial girdle while the ureters, which originated posterior to the girdle, ended inside it. Unilateral double ureter was seen in three embryos, two of 12½ days and one of 14½ days; in one the supernumerary ureter ended blind, in another the normal ureter did so, and in the third both reached the nephrogenic cord and induced small kidneys. A small spherical kidney, of about half the normal size, was sometimes seen in postnatal material at autopsy, especially in family B. One which was sectioned proved to be histologically normal; it had a rather pronounced papilla, extending well down the ureter.

(b) *Hydronephrosis.* Hydronephrosis occurred with a low incidence in postnatal material of family A and in two mice of family C; it was not seen in family B. Experimental puncture showed that there was no occlusion of the ureter above the point where it entered the wall of the bladder; the ureters and bladders of three animals were therefore sectioned at this level. In two of them one ureter was normal, the other abnormally narrow and apparently atretic a short distance from the opening into the bladder; above this level the abnormal ureters were hydropic, and one of them contorted. In the third animal there were two ureters each side (Text-fig. 14). On the right they entered the bladder by a short common duct which was narrow and apparently atretic; above this level they separated, one ending blindly and the other becoming hydropic and grossly contorted. On the left they remained separate throughout; one was hydropic and ended blindly outside the bladder, the other apparently normal. Hydronephrosis was not seen in any embryos.

(c) *Umbilical asymmetry.* The umbilical arteries of a normal mouse embryo enter their lateral (common iliac) courses at the 11½-day stage; between 11½ and 14½ days

the left and right arteries are of approximately equal size, but from 15½ days there is asymmetry, the right artery becoming the larger. In the Little & Bagg stock there was umbilical asymmetry in some 12½- and 13½-day embryos, but it was never very marked and may have been in the normal range. In two 14½-day embryos, however,




Text-fig. 14. Abnormal ureters of a 3-week old mouse sectioned transversely near their junction with the bladder. B, bladder; LU, left ureter (functional branch); LUH, left ureter (blind and hydropic branch); RUA, right ureter, atretic portion near bladder; RUH, right ureter, hydropic portion.

there was gross asymmetry of the sort described by Brown (1931). In one the right artery was almost absent, in the other the left; in each case on the contralateral side the umbilical artery was hyperplastic and the ureter and kidney missing; both embryos were pseudencephalic, oedematous and polydactylous; in one there was also very high grade ectopia viscerum.

## DISCUSSION

### (i) *Defects of the central nervous system*

Pseudencephaly was described by Bonnevie (1936), who believed it to be due to some gene, other than **my**, coincidentally present in her stock. Her material consisted of nine pseudencephalic embryos of 6 *mm* and later stages, and twenty younger embryos. The latter group comprised unspecified numbers of turned (3 to 6 *mm*) and unturned embryos; the unturned embryos were not identified as pseudencephalic *per continuitatem* and will be discussed later.

Bonnevie attributed the pseudencephaly in turned embryos to disproportionate growth of the neural tube, making it too big for the surrounding mesodermal tissues; this led to crumpling of the wall of the tube. If it occurred before closure of the anterior neuropore, it might prevent closure, most probably in the roof of the mesencephalon; if it occurred after closure, there might be subsequent rupture of the roof of the tube. The full development of pseudencephaly in later embryonic stages was considered to be a mechanical consequence of the open mesencephalon and crumpled myelencephalon. Bonnevie did not find any pseudencephalic newborn young and therefore supposed that they had died before birth; she believed death to have been

due to suppression of the heart-beat through the action of the vagus nerve, which became excessively stretched as the pseudencephaly developed.

This hypothesis is not supported by the much greater number of observations in the present investigation. Table 4 shows that in the family in which selection had been exercised for pseudencephaly, B, its incidence was constant among embryos of all stages beyond the stage of turning. Furthermore, there was little late embryonic death and newborn pseudencephalic and acraniate young were found. These observations suggest the simple hypothesis that pseudencephaly arose only through failure of primary closure of the neural tube, and that thereafter its incidence neither decreased, through death or regulation, nor increased, through abnormal development of previously normal embryos. This leaves aside the question why the neural ridges failed to close initially. Crumpling of the neural tube was observed, but only after failure of the ridges to close, and not before it; it did not appear to be the cause of failure to close, but rather a consequence of it, in that the partly open tube did not hold the cerebrospinal fluid and its wall therefore failed to become distended.

Failure of the notochord to become separated from the floor of the brain, which Bonnevie saw in two unturned 8½-day embryos and which she thought to represent an early stage of pseudencephaly, was observed in family B; it was present in all of eight homozygous 8½-day and eight unturned segregating 9½-day embryos. The incidence of pseudencephaly in homozygous family B embryos was 35 per cent (Table 4); it is therefore very unlikely either that all of these sixteen embryos were presumptive pseudencephalics or that none was. The simplest interpretation is that presumptive pseudencephalics do not differ in the relationship of notochord to neural tissue from normal embryos of this age, and that notochord and neural tissue are in contact in the normal. The illustrations accompanying Snell's (1941) description of the early development of the mouse show them in contact. This being so, the question is not why they were in contact in two of Bonnevie's embryos, but rather why they were separated in the rest.

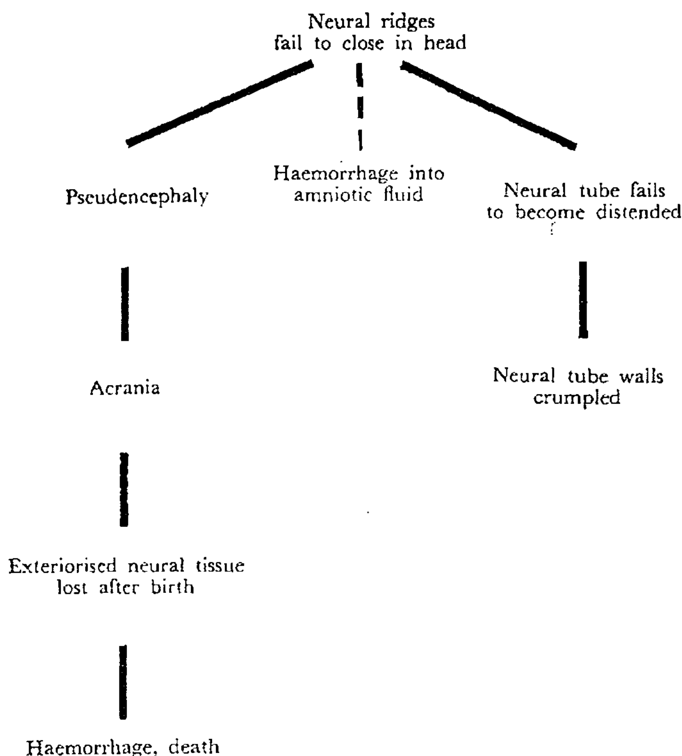
The still earlier post-implantation stages, which Bonnevie (1936) described as showing failure of the endoderm to separate from the ectoderm, followed by early death, were not reinvestigated. The data of Tables 1 and 2 are compatible with an increase in early post-implantation death among **my my** embryos; Table 11 shows that the incidence of early dead embryos was significantly higher among the inbred

Table 11. *Early death in homozygous* **my my** *and backcross matings. Data of Tables 1 and 2*

| Type of mating | Status of embryos: Early dead | All others | Totals | |
|---|---|---|---|---|
| Homozygous | 59 | 293 | 352 | |
| Backcross | 39 | 308 | 347 | $\chi_1^2 = 4.42$ |
| Totals | 98 | 601 | 699 | $P = 0.035$ |

homozygous matings (16·8 per cent) than among the backcrosses (11·2 per cent). This does not, of course, establish the cause of the early death, nor even that it was due to the gene **my**.

The observations suggest the pedigree of causes of defects of the central nervous system shown in Text-fig. 15. The origin of the blood seen in the amniotic fluid of pseudencephalic embryos aged 16½ days and more is conjectural; the connexion is therefore shown by a broken line.




Text-fig. 15. Pedigree of causes of defects of the central nervous system.

(ii) *Blebs, oedema and the body-wall defects*

The origin of the blebs has been the subject of two hypotheses, those of Plagens (1933) and of Bonnevie (1934). Plagens believed that the blebs are formed *in situ*, the bleb fluid being intercellular fluid from the mesenchyme which has collected between the mesenchyme and the ectoderm. From a study of bleb formation in the limbs he concluded that there was an excess of intercellular fluid near the bleb sites; this he attributed to excessive exudation of plasma from the walls of superficial capillaries in the mesenchyme; and this in turn he attributed to abnormal location of the capillaries, unusually near the surface. In some of his blebby embryos the marginal vein of the footplate came into intermittent contact with the apical ectoderm, whereas in his controls it was always surrounded by mesenchyme. This hypothesis of a vascular

origin for the bleb fluid found support in his observation that in 13½-day blebby embryos there was only about half as much plasma in the blood, per million erythrocytes, as in like-aged controls.

Bonnevie (1934) believed that in blebby embryos there is excessive production of cerebrospinal fluid. The excess fluid, she thought, was forced out of the neural tube through the *foramen anterius* in the roof of the myelencephalon, escaping to a position between the ectoderm and the mesenchyme. She attributed the observed distribution of the bleb sites to subsequent migration of the blebs under the action of ectoderm tension, some to the eyes and snout, some to the shoulders and forelimbs, and some to the lumbar region and hindlimbs. The hindlimb border blebs she believed to travel by way of the throat and ventral surface of the body. In support of this hypothesis she cited the work of Weed (1917), who had made an experimental study of pig embryos and shown that the pressure of cerebrospinal fluid is normally regulated in embryos of the footplate stage by leakage through the *foramen anterius*.

Observations made subsequently, both in the present investigation and elsewhere, support some parts and weigh against other parts of both hypotheses. No evidence was found of an abnormal location of capillaries in embryos of the blebby stock; and a location of the footplate marginal vein adjacent in places to the apical ectoderm, which Plagens (1933) believed to be abnormal, has been observed in normal mouse embryos of the relevant age (Carter, 1954). Apart from this, all observations in the present investigation agree with Plagens' descriptions. On the other hand, no evidence was found in support of Bonnevie's hypothesis that the blebs originate at the myelencephalon and subsequently migrate to their definitive sites. An essential feature of this hypothesis is excessive production of cerebrospinal fluid, building up pressure inside the neural tube and causing the excess fluid to be driven out through the roof of the myelencephalon. It follows that, on this hypothesis, blebs could be formed only in embryos with closed neural tubes; yet they were repeatedly seen on pseudencephalic embryos, and the evidence indicates that a pseudencephalic embryo has never had a closed tube. This alone seems to be fatal to the myelencephalic hypothesis of bleb formation; it is therefore disconcerting to find that Bonnevie (1936) illustrated and remarked on the presence of blebs on pseudencephalic embryos, but failed to realise their implication. Apart from this, Grüneberg (1955) has shown that cerebrospinal fluid leaving the neural tube through the embryonic *foramen anterius* remains inside the meninges, as in the adult: in 13-day embryos homozygous for the gene **tk,** in which formation of the vertebral arches is faulty, he found a great increase in the volume of the pia-arachnoid space. Furthermore, as he pointed out, Bonnevie had misquoted Weed (1917), who clearly stated that the embryonic cerebrospinal fluid stays inside the meninges.

As the blebs lie between ectoderm and mesenchyme, there are only three routes by which fluid could reach them: (*a*) from the mesenchyme, (*b*) by lateral migration from an adjacent position between ectoderm and mesenchyme, and (*c*) through the ectoderm. Plagens favoured route (*a*), Bonnevie (*b*). Bonnevie's hypothesis is now excluded, but there still remains (*c*) as a possible alternative: the bleb fluid might be

amniotic fluid absorbed through the ectoderm. However, evidence on this question may be provided by the two 11½-day blebby embryos. There are two possible interpretations of these: first, that it was a matter of chance that in both of them two rare events coincided, viz. rupture of the membranes at dissection and bleb formation at 11½ days; second, that there was a causal connexion, and that the uterine squeezing which ruptured the membranes also caused the blebs to appear. In retrospect the latter seems the more likely explanation. If so, the blebs must have appeared almost instantaneously, since they were observed as soon as the embryos were transferred to saline and examined. Now it is easy to visualise how excess pressure on some part of an embryo might cause excess fluid already present in the spongy mesenchyme to squirt out at some other point, lifting the ectoderm away from the mesenchyme and so forming a bleb; but it is not apparent how it could cause a sudden sucking-in of amniotic fluid through the ectoderm, and yet leave the ectoderm unpunctured so that it subsequently maintains the distended bleb. The evidence of these two embryos therefore suggests that there may be an excess of fluid in the mesenchyme of 11½- and 12½-day **my my** embryos, and that it usually escapes to form a bleb at 12½ days, but that it may escape earlier if the embryo is squeezed. This hypothesis receives support from the observation that many **my my** embryos are grossly oedematous by 13½ days. I am therefore inclined to think that the mesenchymal intercellular fluid is the most probable source of the bleb fluid.

Though Bonnevie's postulate of a myelencephalic origin for the blebs must be rejected, her postulate of bleb migration found some support in the present investigation, though only in the limited sense of localised migration. A typical head bleb is middorsal when first it appears, filling a depression in the surface of the head between the cerebral hemispheres and the mesencephalon; as it grows, it extends along the depression in the side of the head, posterior to the cerebral hemisphere, until it reaches the depression surrounding the eye; thereafter it regresses, starting at the middorsal end. Thus the centre of gravity of the bleb moves from a middorsal initial position to a final position in the region of the eye. Again, a typical hind limb bleb migrates locally from an initial position on the mesial or tibial surface of the limb to a final position on the dorsal or plantar surface of the foot.

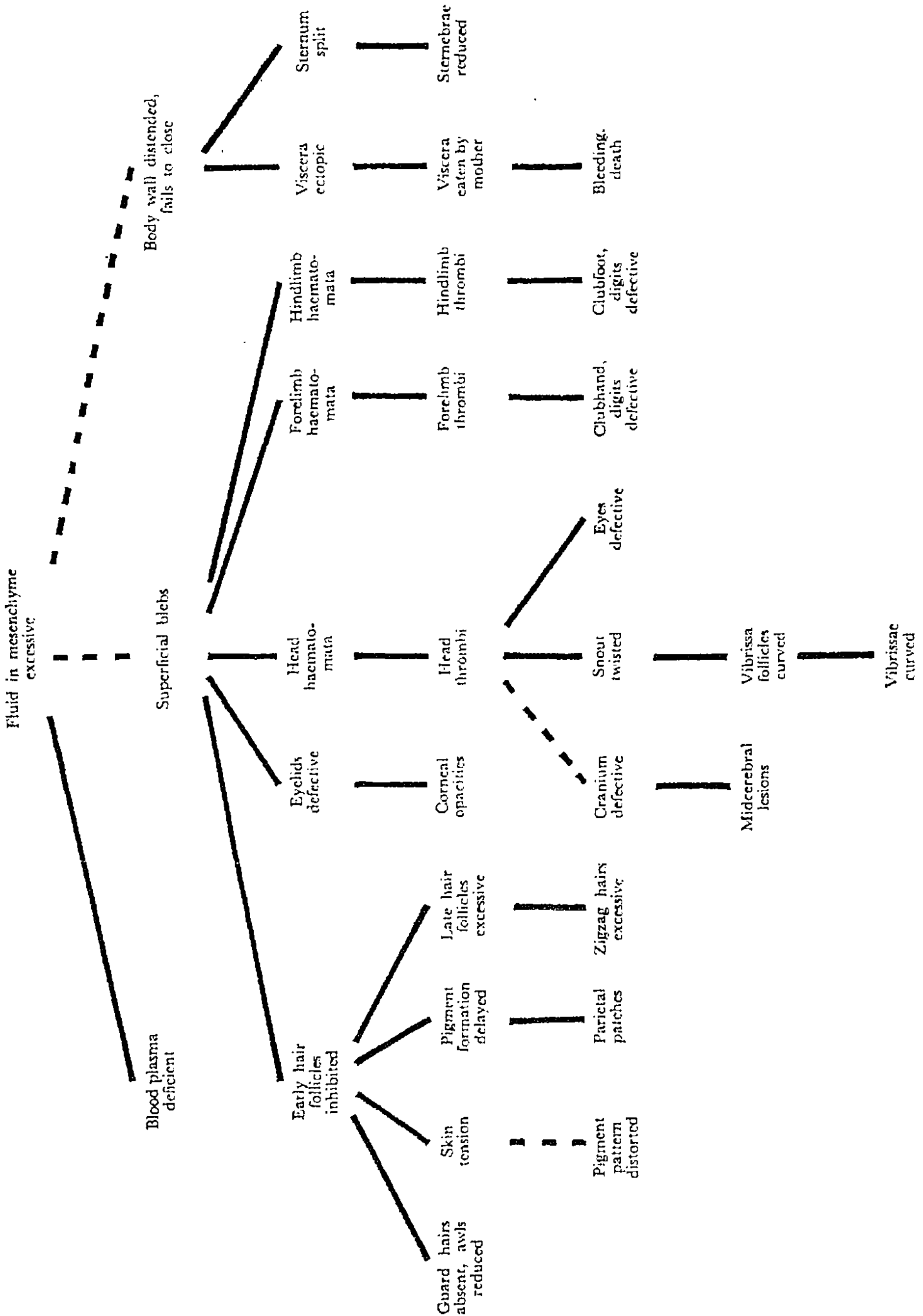
Plagens (1933) believed that the blebs and haematomata do no lasting damage, and that the disruptive lesions are entirely the result of thrombi formed in the mesodermal tissues below the blebs or haematomata. Contrary to Plagens' conclusions, however, evidence was obtained in the present investigation that some blebs may disappear without giving rise to haematomata or thrombi, but may nevertheless interfere with development of the eyelid. A similar conclusion must be drawn from the observations of the frequencies of hair types in 'saddle' regions. The work of Falconer, Fraser & King (1951) on the *crinkled* mutant and that of Slee (1957*b*) on the *ragged* mutant imply that, in normal mouse embryos, guard-hair follicles are initiated between 13½ and 17 days, awl follicles between 17 and 19 days (birth), and zigzag follicles after birth. The total absence of guard hairs from the saddle, and the reduction of awls, imply that follicle initiation or development must have been inhibited in the saddle

until some time after the 17-day stage; indeed, Plagens himself reported that no follicles were formed in the saddle up to the 18-day stage. Thus follicle initiation must have been inhibited not only by thrombi, as Plagens suggested, but also by the preceding haematomata and blebs.

The observations suggest the pedigree of causes shown in Text-fig. 16 for the bleb-induced lesions and defects of the body wall. It is supposed that the defects stem from excessive transfer of fluid to the mesenchymal intercellular spaces, starting not later than the 11½-day stage. This is presumed to lead to thickening of the blood, distension of the body wall and formation of blebs. The least firmly established item in this pedigree is the presence of oedema in 11½-day embryos. It has not been observed directly; it has been inferred from the facts that gross oedema can be observed at 13½ days, that blebs normally appear at 12½ days, and that they were seen in the two 11½- day embryos which had been squeezed. Another point of uncertainty is the origin of the midcerebral lesions: the fact that they were not seen in embryos of less than 16½ days suggests that they may be of relatively late origin, due to defective cranium formation caused by a mid-cerebral thrombus overlying a closed neural tube; but the possibility is not excluded that the cause might be weak primary closure. A third uncertain point is the mechanism of displacement of the boundary between dorsal and ventral pigmentation in black-and-tan mice. It might be due to one or other of two causes: the presence of a bleb, haematoma or thrombus in the presumptive black region might slow down dorsoventral migration of the neural crest cells; or it might cause a localised reduction in the rate of growth of the skin, followed by a compensating hypertrophy of the presumptive tan region. The absence of guard hairs from the saddle, coupled with evidence that absence of the larger types of follicle may cause skin tension (Falconer, Fraser & King, 1951) argues in favour of the second explanation.

### (iii) *Morphogenetic defects of the hind limbs*

Bagg (1929), Plagens (1933) and Bonnevie (1934) all believed that preaxial hindfoot polydactyly is due to splitting of the hallux *anlage* by a tibial border bleb; they all observed some embryos with both border blebs and polydactyly. The new observations do not disprove this hypothesis; but they establish that this could not have been the only mechanism by which polydactyly arose, since many polydactylous embryos were seen in which there was no trace of border blebs, present or past. Polydactyly was first detected, in 12½-day embryos, as excessive growth of the preaxial margin of the hind footplate; the observations therefore suggest that polydactyly in the Little & Bagg stock arose by a mechanism similar to that in the stocks studied by Chang (1939) and by Carter (1954), namely a primary error of footplate morphogenesis. In that event the data of Table 9, which show a statistical association between polydactyly and border blebs in family C, suggest either that polydactyly and border blebs have a causal element in common, or that the presence of polydactyly predisposes towards the formation of a border bleb. The observations that blebs tend to occur in places where there are depressions in the surface of the embryo, and that there is a marked



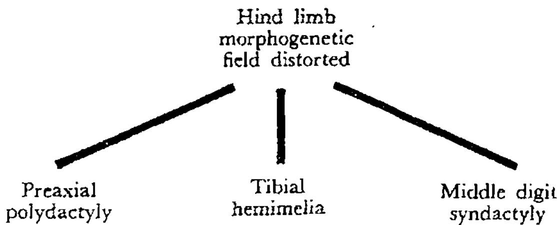

Text-fig. 16. Pedigree of causes of bleb-type defects and defects of the blood and body-wall.

reentrant angle between the footplate and leg of the 12½-day polydactylous embryo, but a very obtuse angle in the normal embryo, argue in favour of the second explanation.

No hemimelic embryos were seen, and therefore it was not possible to investigate the development of tibial hemimelia in the Little & Bagg stock. It may be significant that the one hemimelic mouse came from a family in which there had been selection for high-grade polydacty, and from a sibship which was entirely polydactylous; tibial hemimelia occurs in other strains of mice as an extreme expression of genotypes which otherwise give rise to preaxial polydactyly (Carter, 1951; Kobozieff & Pomriaskinsky-Kobozieff, 1953; Green, 1955). In these strains it is due to a primary defect of limb morphogenesis, clearly recognisable at 12½ days.

The more extreme manifestations of middle-digit syndactyly in the Little & Bagg stock resembled the defects seen in mice homozygous for the gene **sy** which are traceable to defective footplate morphogenesis at the 12½-day stage (Grüneberg, 1956). No syndactylous embryos were identified, and therefore no direct evidence was obtained about its origin. However, internal evidence, namely the presence of digital fusion at the phalangeal level while the metatarsals remain separate, suggests that it too may be traceable to defective footplate morphogenesis at the 12½-day stage.

Preaxial polydactyly, tibial hemimelia and middle-digit syndactyly are therefore probably all consequences of faulty primary morphogenesis of the hind limb. This suggests the pedigree of causes shown in Text-fig. 17.




Text-fig. 17. Pedigree of causes of the hind-limb morphogenetic defects.

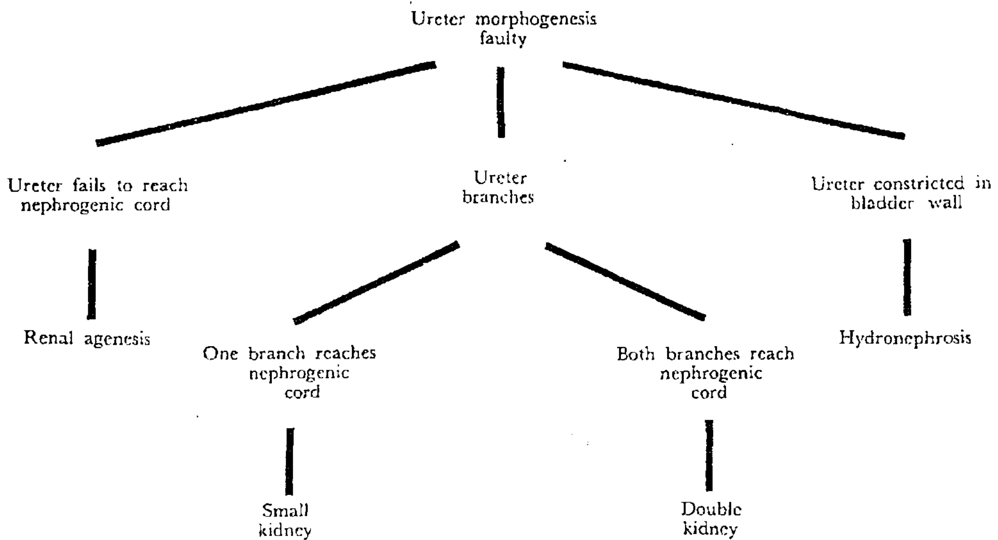
(iv) *Urogenital defects*

The urogenital defects appeared to stem from faulty development of the ureter. In some embryos it failed to reach the nephrogenic cord, thereby giving rise to renal agenesis; in others it divided into two, and one branch, or both, failed to reach the cord. This defect is reminiscent of that seen in mice carrying the *Danforth's short-tail* gene (Gluecksohn-Schoenheimer, 1945). Brown (1931), who gave detailed descriptions of the development of the urogenital abnormalities in the Little & Bagg stock, attributed renal agenesis to failure *either* of the ureter to grow into the nephrogenic cord *or* of the nephrogenic cord to reach the level of the ureteric bud. The experimental work of Grobstein (1953) has subsequently given support to this interpretation by demonstrating that the inductive relationship between these *anlagen* is mutual. The observation that in two embryos of the present series the ureters lay essentially posterior to the umbilical arterial girdle, while the nephrogenic cords ended anterior to it, suggests at

first sight additional support: but these were 13½-day embryos; at 11½ days, when the ureter first buds off from the wolffian duct, the nephrogenic cord reached the level of the ureteric bud in all the embryos examined.

Hydronephrosis appeared to be another manifestation of ureteric maldevelopment, in this instance due to abnormal narrowness and atresia of the ureter; in one animal the atretic ureter was completely separated from the bladder, presumably a secondary effect. Hydronephrosis in this stock therefore had a quite different origin from that in *luxate* mice, where it appeared to be due to occlusion of the ureter through kinks associated with gross asymmetry of the bladder and umbilical arteries (Carter, 1953). Asymmetry of the umbilical arteries was seen in the Little & Bagg stock also, but associated with contralateral ureteric agenesis; this was not seen in the *luxate* stock.

The defects of the urogenital system suggest the pedigree of causes shown in Text-fig. 18.




Text-fig. 18. Pedigree of causes of the urogenital defects.

### (v) *Relationships between the pedigrees of causes*

The four pedigrees of causes are internally consistent, but no causal connexions between them have been traced. However, the data of Table 1 show that connexions may exist.

When a stock is homozygous for a single major gene governing a variable syndrome, a statistical association between two parts of the syndrome cannot be due to segregation of the major gene; its origin must therefore be sought elsewhere. Two other mechanisms can be envisaged. First, there may be genetic heterogeneity; that is to say, there may be a modifying gene segregating which has pleiotropic effects on the two parts of the syndrome, or linked modifiers with single effects. Second, the two parts of the

syndrome may be affected, through one or more developmental mechanisms, by some heterogeneous component of the environment. Demonstration of an association between two parts of a syndrome in a homozygous stock does not, therefore, prove the existence of a common mechanism in development, i.e., a pedigree of causes in Grüneberg's (1943*a*) sense; but it does constitute valuable evidence, since the absence of an association would suggest *prima facie* the absence of a common developmental mechanism.

Families A, B and C were homozygous for **my**; and they were from six to fifteen generations brother-sister mated, so it is unlikely that segregating modifying genes played an important role in any statistical associations between parts of the syndrome. In fact there were significant associations between pseudencephaly, blebs, oedema and polydactyly; they are shown in Tables 12 to 16, which are based on the data of Table 1. Pseudencephaly belongs to the first pedigree of causes, blebs and oedema to the second and polydactyly to the third; the data therefore satisfy one condition for demonstrating the existence of a common developmental mechanism underlying the three pedigrees.

Failure to give proof positive of a unitary mechanism does not, of course, amount to proof that none exists. On the contrary, the results of experimental studies of genetically normal mouse stocks strongly suggest that there is some single developmental mechanism underlying the defects seen in the Little & Bagg stock. Of the more common defects, namely pseudencephaly, blebs, defects of the eyes and eyelids, failure of closure of the ventral thoracic wall, ectopia viscerum and polydactyly, some or all have been seen in mouse embryos which had been exposed to various developmental insults at about the 7½- and 8½-day stages. The teratological agents used included X-rays (Kaven, 1938; L. B. Russell, 1950), hypoxia (Ingalls *et al.*, 1953; Murakami, Kameyama & Kato, 1954) and injection into the pregnant mother of substances such as trypan blue (Hamburgh, 1952; Waddington & Carter, 1952; Murakami, 1952), lithium carmine, lead carbonate and phenylmercuric acetate (Murakami, *et al.*, 1954). However, the nature of the unitary mechanism, if one exists, remains for the present hidden.

(vi) *Gene nomenclature*

Disproof of Bonnevie's hypothesis of the myelencephalic origin of the blebs raises a problem of nomenclature. Little & Bagg (1923) referred to their gene as *haemorrhagic head* and used the symbol **h.** Little & McPheters (1932) renamed it *myelencephalic blebs*, with the symbol $\mathbf{m}^{bl}$. This name, but with the symbol **my,** was recommended by the Committee on Mouse Genetics Nomenclature (Dunn, Grüneberg & Snell, 1940). The symbol **h** has subsequently been used for the *histocompatibility* genes (Gorer, Lyman & Snell, 1948) and the symbol **m** for *misty* (Woolley, 1941). Much could now be said in favour of the name *blebs* and the symbol **bl.** The existence of the blebs is indisputable, whatever their origin, and they are the most regular of the gene's many manifestations; the symbol **bl** has the further feature that it combines the initial letters of the names of the two pioneer geneticists who first described the stock. This appears

to be a case warranting reconsideration by the appropriate body, namely the Committee on Mouse Genetics Nomenclature.

Table 12. *Dissociation of pseudencephaly from blebs in family B embryos aged 12½ days and more. Data of Table 1*

| | | Pseudencephaly | | | |
|---|---|---|---|---|---|
| | | − | + | Total | |
| Blebs | − | 7 | 15 | 22 | |
| | + | 41 | 6 | 47 | $\chi_1^2 = 21 \cdot 8$ |
| Total | | 48 | 21 | 69 | P = 0·000 0001 |

Table 13. *Association of pseudencephaly with oedema in family B embryos aged 13½ to 15½ days inclusive. Data of Table 1*

| | | Pseudencephaly | | | |
|---|---|---|---|---|---|
| | | − | + | Total | |
| Oedema | − | 15 | 5 | 20 | |
| | + | 12 | 15 | 27 | $\chi_1^2 = 4 \cdot 39$ |
| Total | | 27 | 20 | 47 | P = 0·035 |

Table 14. *Association of pseudencephaly with polydactyly in family B embryos aged 12½ days and more. Data of Table 1*

| | | Pseudencephaly | | | |
|---|---|---|---|---|---|
| | | − | + | Total | |
| Polydactyly | − | 43 | 12 | 55 | |
| | + | 5 | 9 | 14 | $\chi_1^2 = 9 \cdot 51$ |
| Total | | 48 | 21 | 69 | P = 0·002 |

Table 15. *Association of oedema with polydactyly in family A embryos aged 13½ to 15½ days. Data of Table 1*

| | | Oedema | | | |
|---|---|---|---|---|---|
| | | − | + | Total | |
| Polydactyly | − | 14 | 4 | 18 | |
| | + | 10 | 24 | 34 | $\chi_1^2 = 11 \cdot 08$ |
| Total | | 24 | 28 | 52 | P = 0·0009 |

Table 16. *Association of blebs with polydactyly in family C embryos aged 12½ days and more. Data of Table 1*

| | | Blebs | | | |
|---|---|---|---|---|---|
| | | − | + | Total | |
| Polydactyly | − | 13 | 7 | 20 | |
| | + | 3 | 14 | 17 | $\chi_1^2 = 8{\cdot}395$ |
| Total | | 16 | 21 | 37 | $P = 0{\cdot}004$ |

## SUMMARY

The morphology and development of the many defects in mice of the Little & Bagg X-rayed stock have been reinvestigated, in an attempt to resolve the conflicts in the findings of earlier investigators. The observation that blebs occur on pseudencephalic embryos is incompatible with Bonnevie's hypothesis that they originate as cerebrospinal fluid in the myelencephalon; other observations support Plagens' hypothesis that the blebs originate as mesenchymal intercellular fluid. No unitary gene action was found. Four pedigrees of causes were constructed covering, respectively, defects of the central nervous system, bleb-induced lesions and defects of the body wall, morphological defects of the hind limbs, and defects of the urogenital system; there were cross-correlations between defects in the first three pedigrees, but the underlying mechanisms were not identified.

Most of this work was done during 1953 and 1954 at the Institute of Animal Genetics, Edinburgh University. I am indebted to Professor C. H. Waddington, F.R.S., for the hospitality of his institute, and to Professor H. Grüneberg, F.R.S., without whose stimulation it might never have been written up for publication. I am also grateful to Mr E. D. Roberts, who drew Text-figs. 6, 7, 12 and 13; to Mr John Armstrong and Mr E. J. Lucas, who took the photographs; and to Miss Esme Mavor (now Mrs John Slee) and Miss Anne Constantine for technical assistance.

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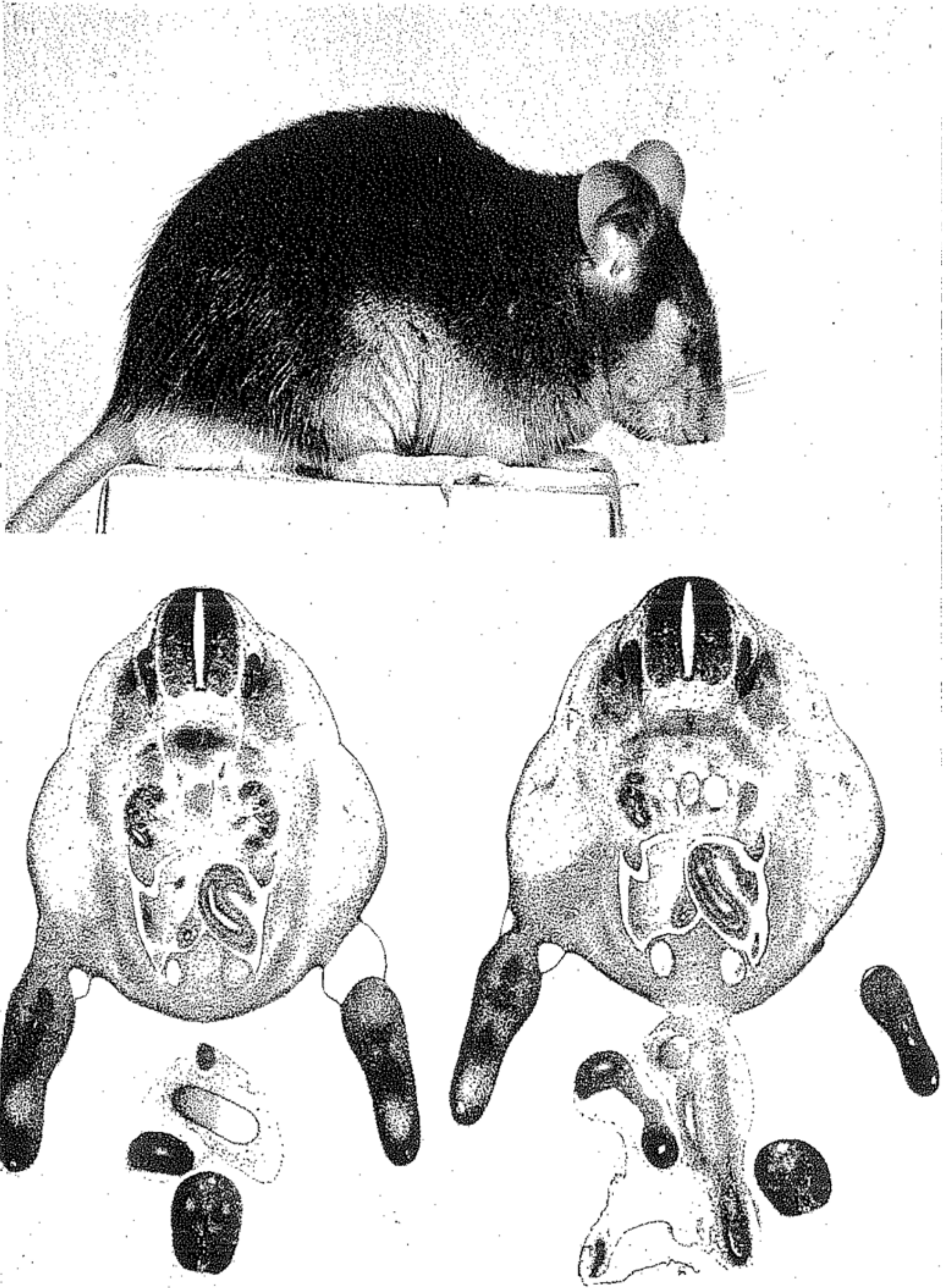

Plate 1. (1) Adult **my my** mouse lacking the right eye and showing extension of the region of ventral (tan) coloration over the orbit. (2) and (3). Transverse sections through the lumbar regions of two 13½-day **my my** embryos; the embryo on the right was pseudencephalic, that on the left not. Note the presence of limb border blebs in both. Both appeared oedematous at dissection. In the pseudencephalic embryo the left ureter (on the right side of the figure) is missing and there is no kidney induction; the nephrogenic cord constitutes a strongly condensed mass of cells.