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Genetical studies on the skeleton of the mouse

XXV. THE DEVELOPMENT OF SYNDACTYLISM

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This paper is dedicated to my teacher and friend, Professor Hans Nachtsheim, on the occasion of his seventieth birthday.

INTRODUCTION

In an earlier paper of this series* (Grüneberg, 1956), an anatomical description of the osseous and cartilaginous skeletons has been given of the three mutants syndactylism (*sm/sm*), Oligosyndactylism (*Os/+*) and shaker with syndactylism (*sy/sy*) in the mouse; in each case, the description started with 14-day embryos in which the chondrification of the limb skeleton is sufficiently advanced for the application of bulk staining techniques (methylene blue). The anatomical situation encountered was quite distinct for each of the three mutant genes: evidently they owe their existence to different mechanisms of gene action. The embryological analysis of syndactylism to be presented in this paper shows that in this condition the skeletal system is only secondarily involved.

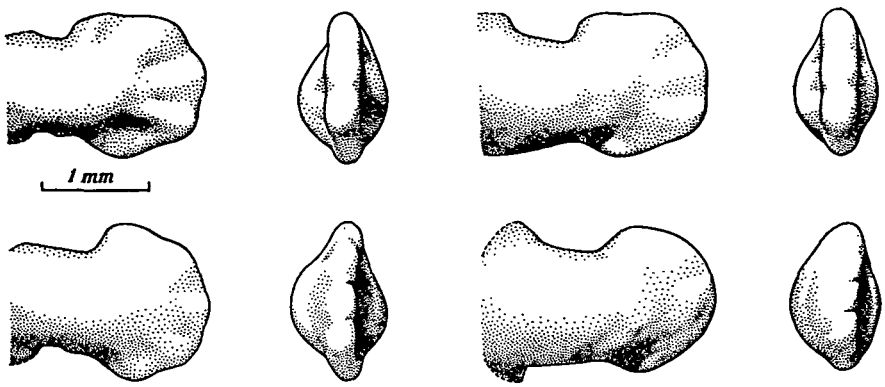
ADULT ANATOMY

As described in more detail previously (Grüneberg, 1956), the gene for syndactylism invariably affects all four feet. The third and fourth digits are always syndactylous; digit 2 is often and digit 1 occasionally involved. Digits of the fore feet are usually joined by soft tissues only, those of the hind feet generally by cartilage or bone. Fusions tend to involve all three phalanges, but not the metacarpals or metatarsals respectively. The fusions are always primary, i.e., corresponding phalanges of neighbouring digits are represented by a single cartilaginous element from the start. Secondary fusions between the os naviculare and the os cuboideum of the hind feet occurred in 8 out of 21 *sm/sm* mice and probably represent a remote gene effect; they will not be dealt with in this paper. Many *sm/sm* mice have tail kinks and occasionally a tail twist; these are confined to the distal half of the tail, with a strong preference for the third quarter (caudal vertebrae 16–21).

* The first 21 papers of this series, by the present author and by various other members and guests of this research group, have appeared in the *Journal of Genetics*, vols. 50–55, 1950–7; papers 22–24 have been published in the *Journal of Embryology and Experimental Morphology*, vol. 6, 1958.

EXTERNAL FEATURES OF SYNDACTYLOUS EMBRYOS

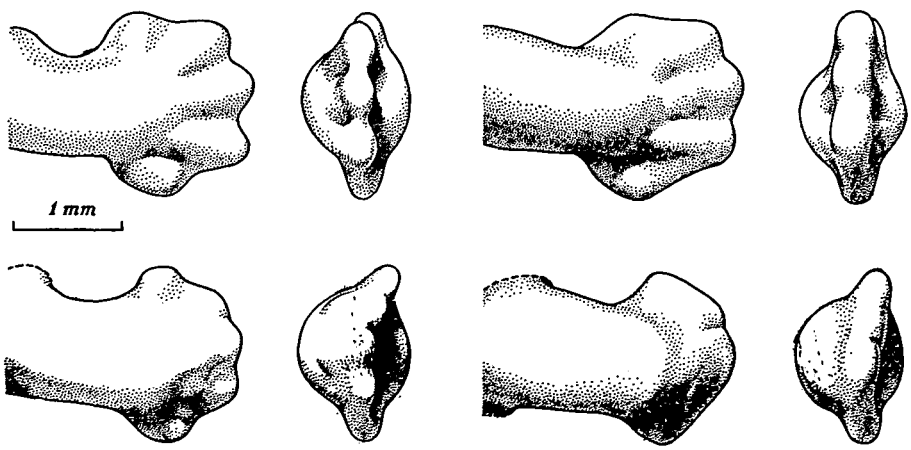
As *sm/sm* mice are fertile in both sexes, litters of embryos consisting entirely of *sm/sm* individuals can be compared with corresponding litters of normal embryos. This has been done for the younger stages where classification by external features



Text-fig. 1. The right fore and hind limbs of a normal (top row) and an *sm/sm* embryo. 12-day-old litter-mates; C.R.L. 7·8 and 8·0 mm. respectively. Camera lucida drawings.

is either uncertain or altogether impossible. From the 12-day stage onwards, classification by external inspection is easy and litter-mates from segregating litters have been compared with each other.

In litters of 10 and 11 days (mean C.R.L. 4·15 and 5·0 mm. respectively), the limb buds of *sm/sm* embryos do not appear to differ externally from those of normal embryos of comparable stages. By the 12-day stage (Text-fig. 1), a characteristic difference has made its appearance. The limb buds of *sm/sm* embryos appear bloated; the dorsal surfaces are curved more strongly than in normal embryos while



Text-fig. 2. The right fore and hind feet of a normal (top row) and an *sm/sm* embryo. 13-day-old litter-mates; C.R.L. 9·3 and 9·0 mm. respectively. Camera lucida drawings.

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the palmar and plantar surfaces are flattened. As a result of this deformation, the free edge of the foot plates is bent over in a palmar and plantar direction respectively and the length of the circumference is shortened. Less space is thus available for the middle digits to spread out.

Similar but more extreme deformations of the feet are again found in 13-day *sm/sm* embryos (Text-fig. 2). By now the palma and planta have actually become concave and the middle digits are still more crowded together as shown by the indentations of the foot margin.

In older embryos, the bloatedness of hands and feet gradually diminishes as the digits grow out, with the result that the middle and terminal phalanges of basally syndactylous digits sometimes manage to differentiate as separate entities (Grüneberg, 1956; Figs. 5-7, p. 120).

In 12- and 13-day-old *sm/sm* embryos, the tail tip is often bent over backwards in a dorsal direction. There is much variation in this respect, and it may be assumed that the more strongly affected embryos would have shown tail kinks later in life.

SECTIONED SYNDACTYLOUS EMBRYOS

The sectioned material is summarized in Table 1. Six out of sixty-eight embryos were sectioned completely; in the remainder, only the limbs (and often the tails) were sectioned. All embryos of 15–12½ days and all but one pair of 12-day embryos came from segregating matings; the normals are thus largely $+/sm$ heterozygotes. One 12-day and all 11- and 10½-day normals are F_2 embryos from a cross between

Table 1. *Normal and syndactylous embryos sectioned*

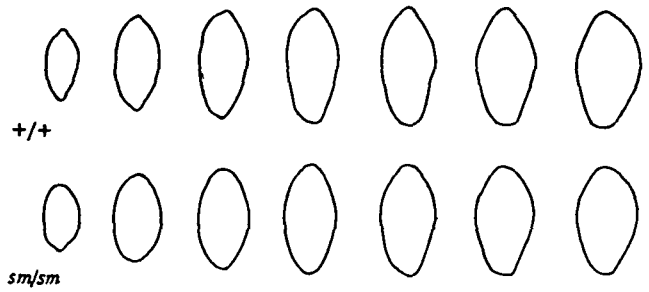
Age	Normal	*sm/sm*	Remarks
15	–	2	Palmar-plantar sections; no tails
14	2	4	1 trio palmar-plantar, 1 trio transverse sections; no tails
13	4	6	1 pair completely sectioned (transverse); 1 trio palmar-plantar and 1 trio transverse, both without tails; 1 pair transverse with tails
12½	2	2	Transverse sections, with tails
12	6	9	2 pairs completely sectioned (transverse); rest transverse sections, with tails
11	8	8	Fore limbs and tails transverse sections; hind limbs dorso-ventral sections
10½	7	8	Fore and hind limbs dorso-ventral sections; no tails
Total	29	39	

the inbred strains CBA/Gr and C57BL/Gr; they are thus $+/+$; one 12-day and all 11- and 10½-day *sm/sm* embryos come from matings of the type $sm/sm \times sm/sm$. The various abnormalities of *sm/sm* embryos will now be considered in turn.

1. *The external shape of the limbs*

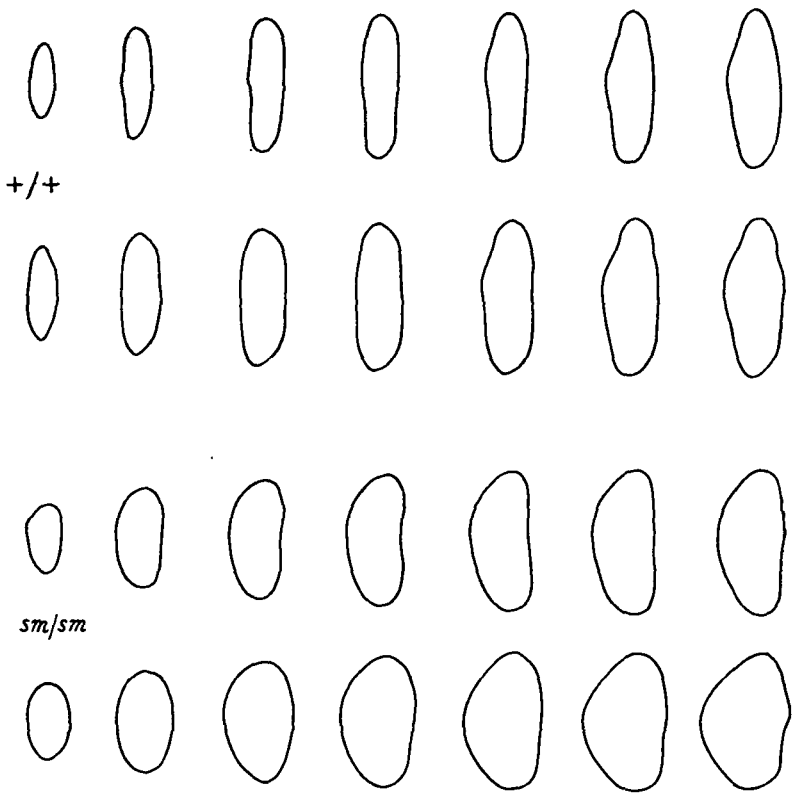
The findings are in close agreement with those from external inspection. No shape anomalies have been noticed in the limb buds of 10½-day-old *sm/sm* embryos. At that stage there is not yet any separation into foot plate and leg either in the fore

or in the hind limbs. At the 11-day stage, this separation has taken place in the fore limbs. In transverse sections of the fore limbs (Text-fig. 3), there is not yet much



Text-fig. 3. Transverse sections (7·5 μ thick) of the left fore limbs of a normal embryo (No. 1344; C.R.L. 4·9 mm; top row) and an *sm/sm* embryo (No. 1348; C.R.L. 5·1 mm; bottom row), 11 days old. In each case, the 10th, 20th . . . 70th sections as counted from the free margin are shown. Projection drawings made at magnification × 50; final magnification × 20. Same technique and magnifications in text-figs. 4–6.

difference between normal and *sm/sm* embryos; perhaps the fore limbs are a little narrower but thicker than those of the normal embryos, but the difference is at most very slight. In the hind-limb buds (which are in the stage of semicircular rudders),



Text-fig. 4. Transverse sections through the left fore and hind limbs of a normal (No. 1070; C.R.L. 7·6 mm.; top two rows) and an *sm/sm* embryo (No. 1071; C.R.L. 7·3 mm.; bottom two rows), 12 days old.

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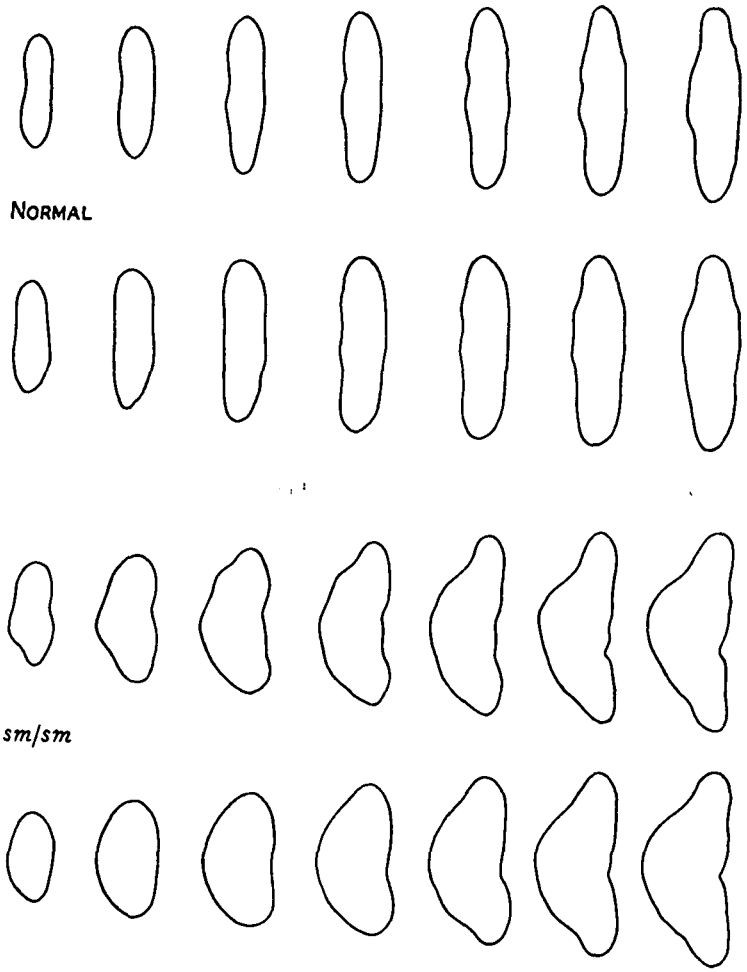
there are no obvious shape differences (Figs. 6 and 7, Plate I), though, of course, slight differences might be detectable with a more elaborate technique.

In the 12-day stage (Text-fig. 4), striking differences have developed both in the fore and in the hind limbs. The limbs are narrower from side to side but much



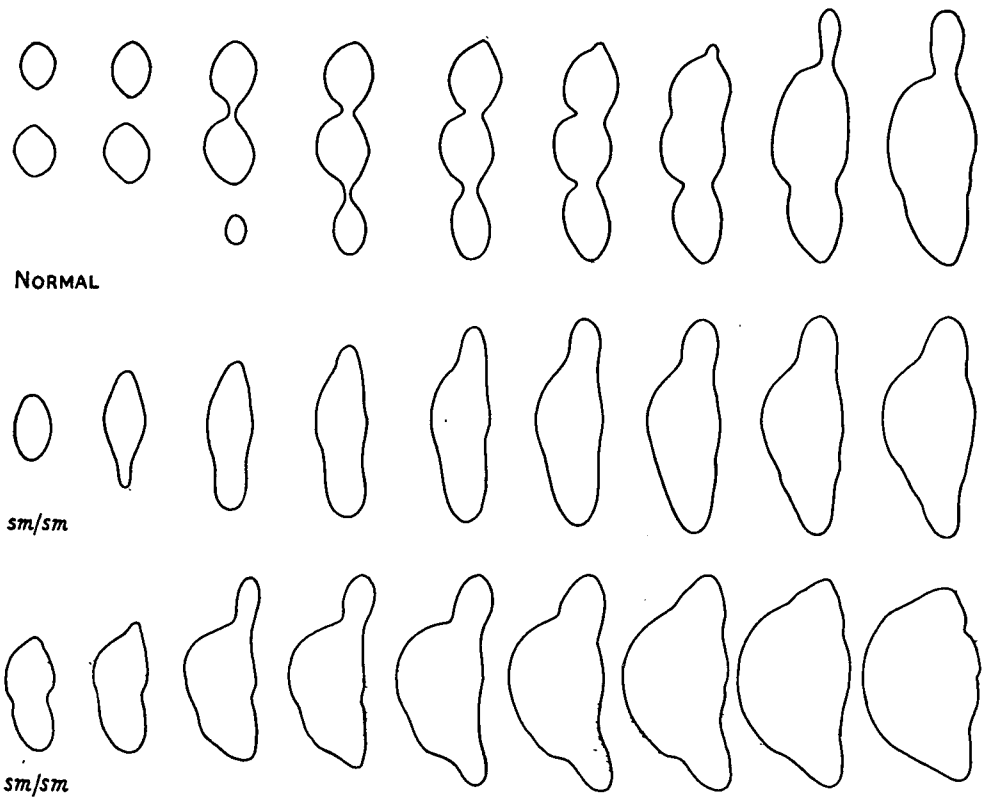
Text-fig. 5. Transverse sections through the left fore and hind limbs of a normal (No. 568; top two rows) and an *sm/sm* litter-mate (No. 569; bottom two rows), 13 days old; mean C.R.L. of litter 9·0 mm.

thicker in a dorso-ventral direction. The foot plates are blown up dorsally while the palmar and plantar surfaces are flatter than normal. The hind limbs are more strongly affected than the fore limbs.

The difference between normal and *sm/sm* limbs becomes even more marked in the 13-day stage (Text-fig. 5), with the palmar and plantar surfaces now actually concave. In the 14-day stage (Text-fig. 6), *sm/sm* embryos are more variable. In the first of the two syndactylous embryos shown, the limb is more nearly normal while in the second animal it is even more bloated than in the 13-day stage. How-

ever, as can be seen by external inspection of *sm/sm* embryos, in later stages there is a general tendency for the feet to return to more normal proportions.

Text-figs. 4–6 suggest that the foot plates of *sm/sm* embryos show a real increase in bulk and not merely an increase in thickness at the expense of width. This is



Text-fig. 6. Transverse sections through the left hind limbs of a normal (No. 571) and two *sm/sm* litter-mates (Nos. 572 and 573), 14 days old; mean C.R.L. of litter 10·3 mm.

borne out by planimetric measurements (Table 2). These show that the *sm/sm* foot plates are bulkier than those of normal embryos, and that the difference increases between the 12- and 13-day stage, at least in the fore limbs. The leg proximal to the foot plate is of about normal size. Sections show that the tissue density of the *sm/sm* foot plates is about the same as in the normal. Hence the increase in volume is not due to an oedema, but to an increase in cell number.

Table 2. *Sum of the areas of transverse sections* 10, 20, . . . 100 *in* cm^2 *in projection drawings* (× 50) *as determined by planimetry. Same animals as in Text-figs.* 4 *and* 5

	12 days		13 days	
	Fore limb	Hind limb	Fore limb	Hind limb
Normal (N)	54·1	61·1	66·4	75·3
Syndactylous (S)	62·8	78·5	95·5	98·2
S/N	1·16	1·28	1·44	1·30

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2. *Invagination of the limb epidermis*

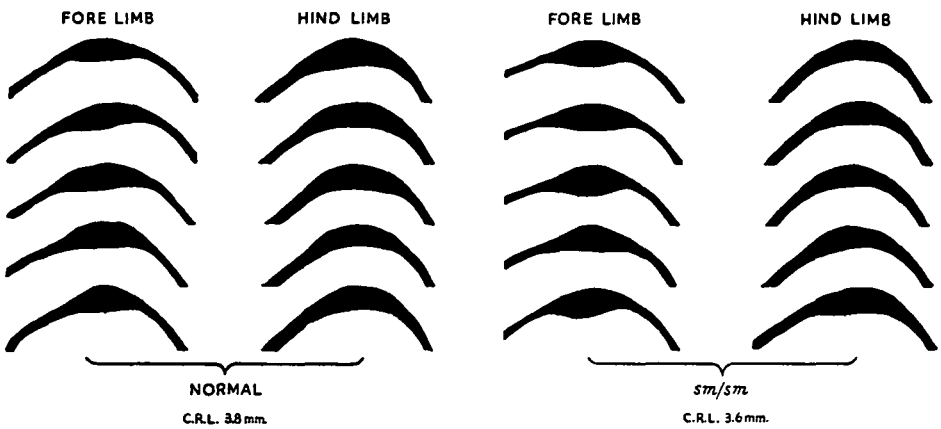
As the palmar and plantar surfaces of the foot plates become first flattened and in the end concave (i.e. from the 12-day stage onwards), folds of the epidermis of palma and planta sink into the underlying mesenchyme. Such invaginations often originate in the apical ectodermal ridge and may be visible as fine deepened lines in the intact limb (see, e.g., the end-on view of the hind limb in Text-fig. 2 above). A very slight ingrowth of this kind is shown in Fig. 10, Plate II. Sometimes such folds are not in contact with the apical ectodermal ridge, but start independently on palma or planta, like that shown in the fourth and fifth sections of Text-fig. 13 below. Commonly quite deep invaginations of epidermis may come to lie between the metacarpalia or metatarsalia, like the one shown in Fig. 11, Plate II. In transverse sections, such invaginated epidermal folds which originate in the apical ectodermal ridge are seen to reach across from the dorsal to the palmar or plantar surface respectively.

There can be no doubt that the invagination of skin-folds on the edge and on the palmar and plantar surfaces of the limbs is a mechanical consequence of the enlargement of the hands and feet which leads to an increase of curvature on the dorsal and a corresponding decrease of curvature on the opposite side. The epidermis is thus obviously passively pushed into the underlying mesenchyme by the forces which lead to the deformation of the limb.

3. *The apical ectodermal ridge*

As will be discussed in more detail below, this structure plays an important part in the growth of the avian and mammalian limb buds. It is strikingly abnormal in *sm/sm* embryos.

In embryos of a developmental age of 10 days (Text-fig. 7), the apical ectodermal



Text-fig. 7. AER of the right fore and hind limbs of a normal (No. 1370) and an *sm/sm* embryo (No. 1361), developmental age 10 days. Dorso-ventral sections through the limb buds. In each case, the middle section of the five represents, as nearly as possible, the central section through the limb bud; it is flanked above and below by the fifth and by the tenth section from the middle either way; sections 7·5 μ thick. Projection drawings made at magnification × 250; final magnification × 100.

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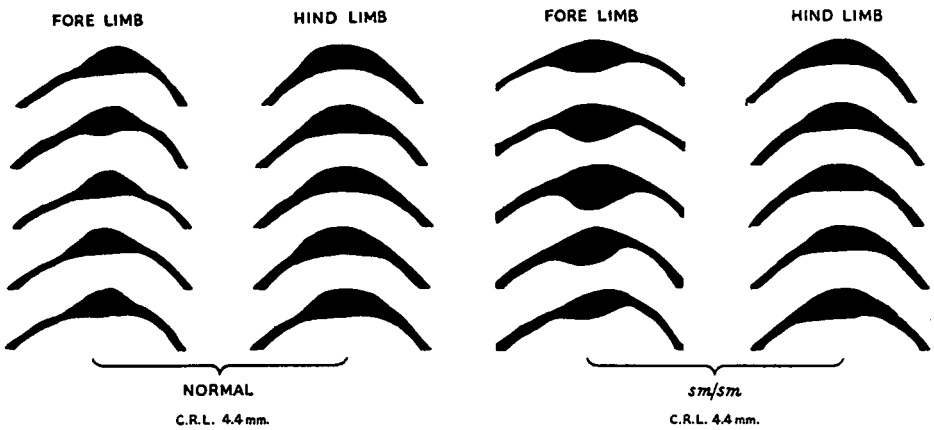
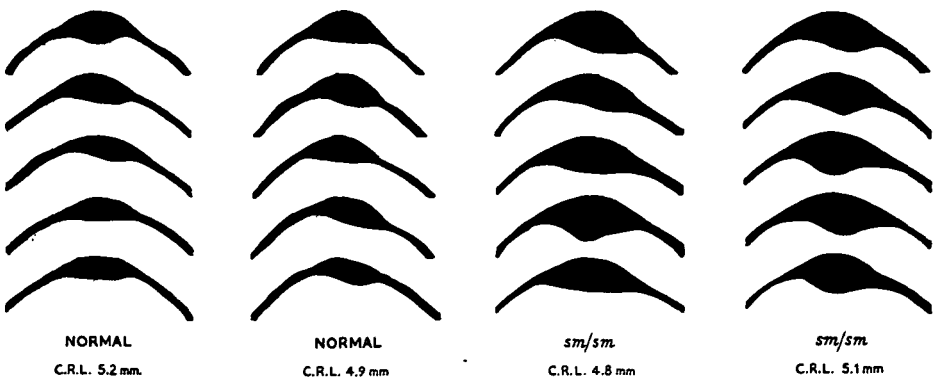
ridge (AER) of *sm/sm* embryos is still virtually normal as judged by its size. In the hind limbs there is no detectable difference. In the fore limbs, there is just a suggestion that the AER is a little thickened and projects convexly into the underlying mesenchyme; as later on this becomes a characteristic feature of *sm/sm* embryos, the



Text-fig. 8. AER of the right fore and hind limbs of a normal (No. 1364) and an *sm/sm* embryo (No. 1358), developmental age 10½ days though litter-mates of the embryos in Text-fig. 7; see there for further explanations.

small difference here encountered in the fore limbs can probably be accepted as an initial stage of the AER anomaly.

A slightly older stage is shown in Text-fig. 8 (actually, these two embryos belong to the same litters as those in Text-fig. 7, but represent a more advanced stage of

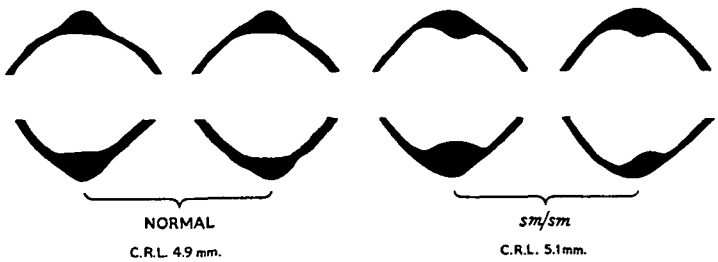


Text-fig. 9. The AER of the left hind limbs of two normal (Nos. 1343 and 1344) and of two *sm/sm* embryos (Nos. 1347 and 1348), 11 days old. For further explanations see Text-fig. 7.

development). While the AER of the hind limbs is still of approximately normal size, that of the fore limbs is now clearly thickened and projects deeply into the underlying mesenchyme. Whereas in sections the AER of the normal embryo projects nipple-like over the surface of the limb bud, that of the *sm/sm* embryo is embedded in the underlying mesenchyme rather than lying on top of it.

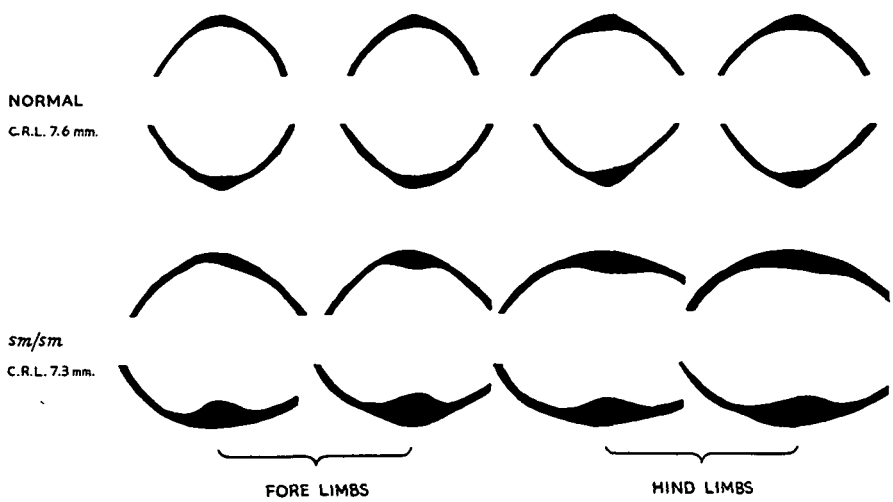
In the 11-day stage (Text-fig. 9), the AER of the hind-limb buds is also clearly thickened and much bulkier than that of the normal embryos. The difference in the AER of the fore limbs is still about as marked as it was in the $10\frac{1}{2}$-day stage (Text-fig. 10).

In normal 12-day mouse embryos the AER has become much smaller; in the fore



Text-fig. 10. The AER of the left fore limbs of a normal (No. 1344) and an *sm/sm* embryo (No. 1348), 11 days old. Transverse sections; the AER is cut twice; in each case the 30th and 40th sections as counted from the free edge of the limb have been drawn. Projection drawings made at magnification $\times 250$; final magnification $\times 100$.

limbs it has almost disappeared whereas in the hind limbs it is still present as a definite though much smaller structure; here, as generally, the fore limb is ahead in development as compared with the hind limb. In 12-day *sm/sm* embryos (Text-

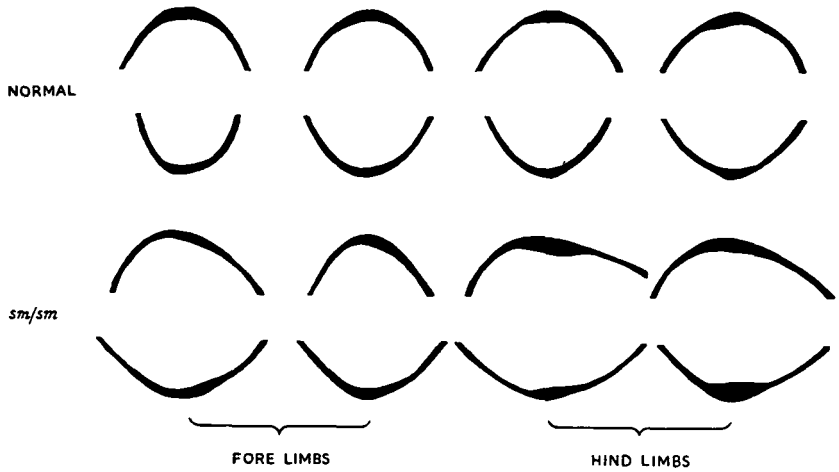


Text-fig. 11. The AER of the left fore and hind limbs of a normal (No. 1070) and an *sm/sm* embryo (No. 1071), 12 days old. Transverse sections. For further explanations see text-fig. 10.

fig. 11), the AER has also been reduced, but it still is much more massive, both in fore and hind limbs, than in the normal.

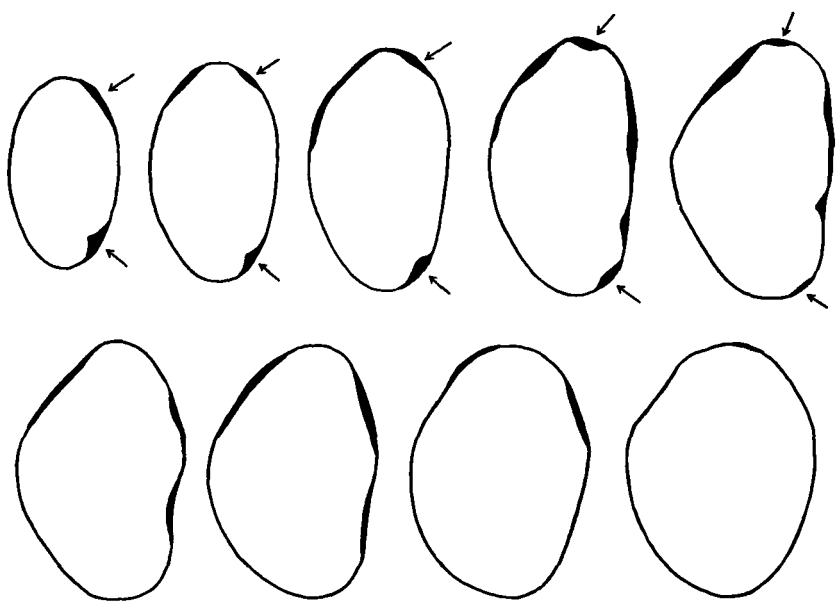
In normal 13-day mouse embryos, the AER has practically disappeared both anteriorly and posteriorly (Text-fig. 12). In *sm/sm* embryos of that age, this also

applies to the fore limbs where there is now no longer an appreciable difference. However, the AER of the hind limbs is still fairly conspicuous even at this late stage.



Text-fig. 12. The AER of the left fore and hind limbs of a normal (No. 568) and of an *sm/sm* litter mate (No. 569), 13 days old; mean C.R.L. of litter 9·0 mm. For further explanations see Text-fig. 10.

In a normal limb, the AER occupies a position almost exactly on the edge of the foot plate. In the bloated limbs of 12- and 13-day *sm/sm* embryos, the AER is regularly pulled in a palmar or plantar direction respectively (Text-fig. 13).



Text-fig. 13. Transverse sections through the left hind limb of a 12-day *sm/sm* embryo (No. 1071; C.R.L. 7·3 mm.). Sections 20, 30 . . . 100 as counted from the free edge of the limb. The AER is indicated by arrows. Projection drawings made at magnification × 80; final magnification × 40.

Microphotographs of the AER of normal and *sm/sm* embryos are given in Figs. 1–4, Plate I. No grossly pathological features have been noticed in the enlarged AER of *sm/sm* embryos in H. & E. preparations. However, there are certainly differences in structure between a normal and an *sm/sm* AER. Hence additional investigations with more refined (histochemical, etc.) techniques are desirable, and it is hoped that supplementary data will be given on a later occasion. In the meantime, as the enlargement of the AER is not merely due to an increase in cell size, but to an increase in cell number, it seems appropriate to speak of a hyperplasia of the apical ectodermal ridge.

4. *Hyperplasia of the limb epidermis*

The hyperplasia of the AER described above is not an isolated phenomenon. It is paralleled by events which take place in the epidermis of the limbs at about the same time.

The epidermis of the limbs of normal 10–12-day-old mouse embryos corresponds to Hanson's (1947) Stage B; i.e. it is two-layered with a more or less cylindrical stratum germinativum and a flat periderm whose cells are at first sparse. The normal appearance is shown in Figs. 6 and 8, Plate I. In *sm/sm* embryos, fairly sharply circumscribed areas of the limb epidermis, both on the fore and the hind limbs, become hyperplastic. The affected patches of epidermis are thickened and may ultimately be 4–5 cell layers deep in places. A typical localization is a dorsal area in the post-axial half of the limb which does not reach its free edge; it is shown, in an 11-day embryo, in Figs. 7 and 9, Plate I. A somewhat similar area is usually found on the ventral (palmar or plantar) aspect of the limb, but rather more preaxially. The distribution of hyperplastic areas on the left hind limb of a 12-day *sm/sm* embryo is shown in Text-fig. 13 above, and a microphotograph of a section through the same limb in Fig. 5, Plate I. The first signs of hyperplasia were seen in

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Explanation of Plates

All the material is fixed in Bouin's fluid, embedded by Peterfi's method, sectioned at 7·5 μ and stained with haematoxylin and eosin.

PLATE I

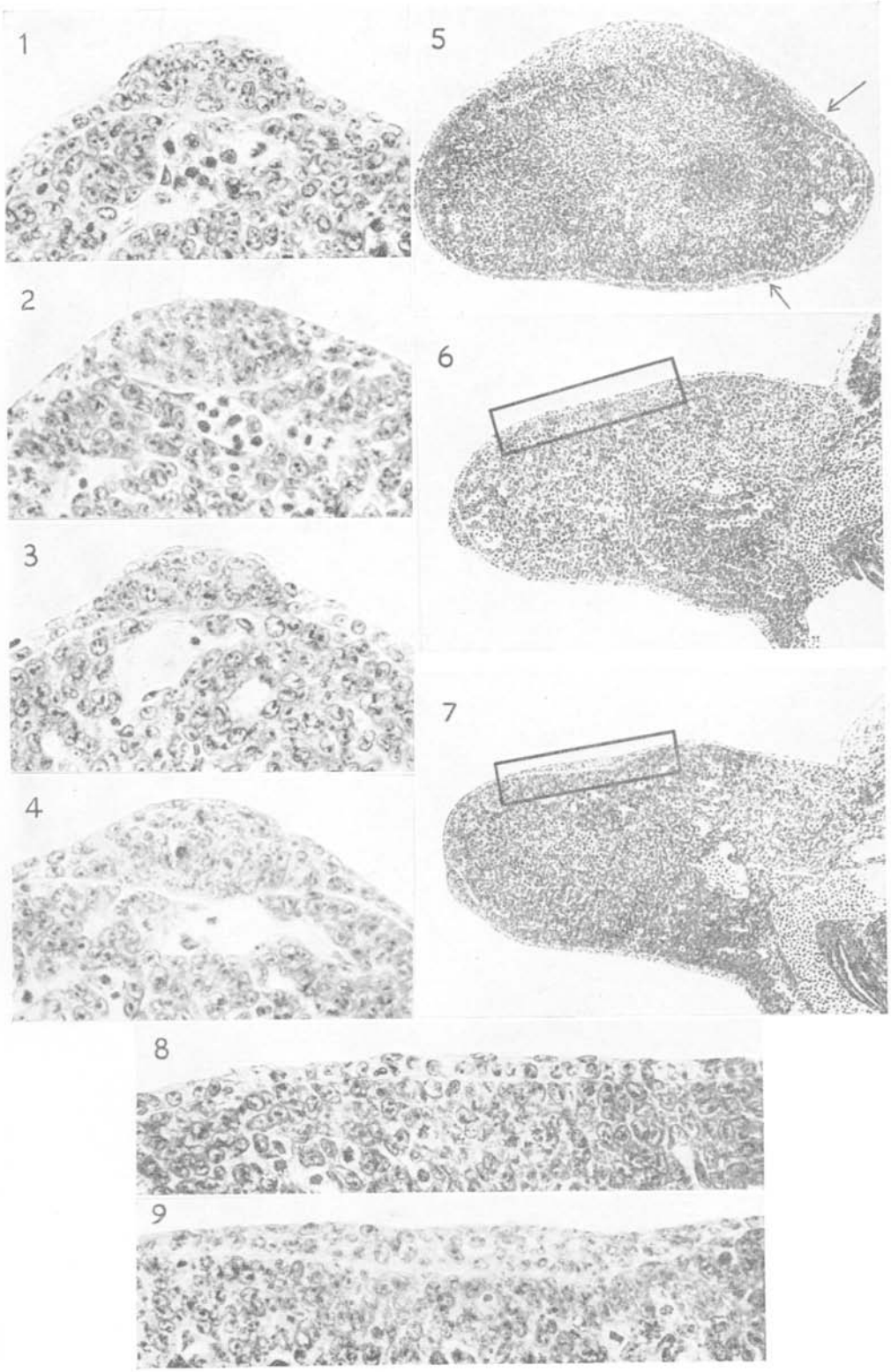
Figs. 1 and 2. The AER of the left fore limbs of a normal (No. 1364; C.R.L. 4·4 mm.) and of an *sm/sm* embryo (No. 1358; C.R.L. 4·4 mm.), 10½ days old. Dorso-ventral sections; × 335.

Figs. 3 and 4. The AER of the right hind limbs of a normal (No. 1344; C.R.L. 4·9 mm.) and of an *sm/sm* embryo (No. 1347; C.R.L. 4·8 mm.), 11 days old. Dorso-ventral sections; × 335.

Fig. 5. Transverse section through the left hind limb of an *sm/sm* embryo (No. 1071; C.R.L. 7·3 mm.; 12 days old) showing hyperplasia of the epidermis both on the dorsal and plantar surface; a trace of the AER still visible on the right. × 75.

Figs. 6 and 8. Dorso-ventral section through the right hind limb of a normal embryo (No. 1341; C.R.L. 5·1 mm.; 11 days old). Fig. 6, the whole limb at magnification × 75; Fig. 8, the boxed area at magnification × 335 to show the structure of the epidermis.

Figs. 7 and 9. Dorso-ventral section through the right hind limb of an *sm/sm* embryo (No. 1348; C.R.L. 5·1 mm.; 11 days old). Magnifications as in Figs. 6 and 8. Note hyperplasia of the epidermis in the boxed-in area.



H. GRÜNEBERG

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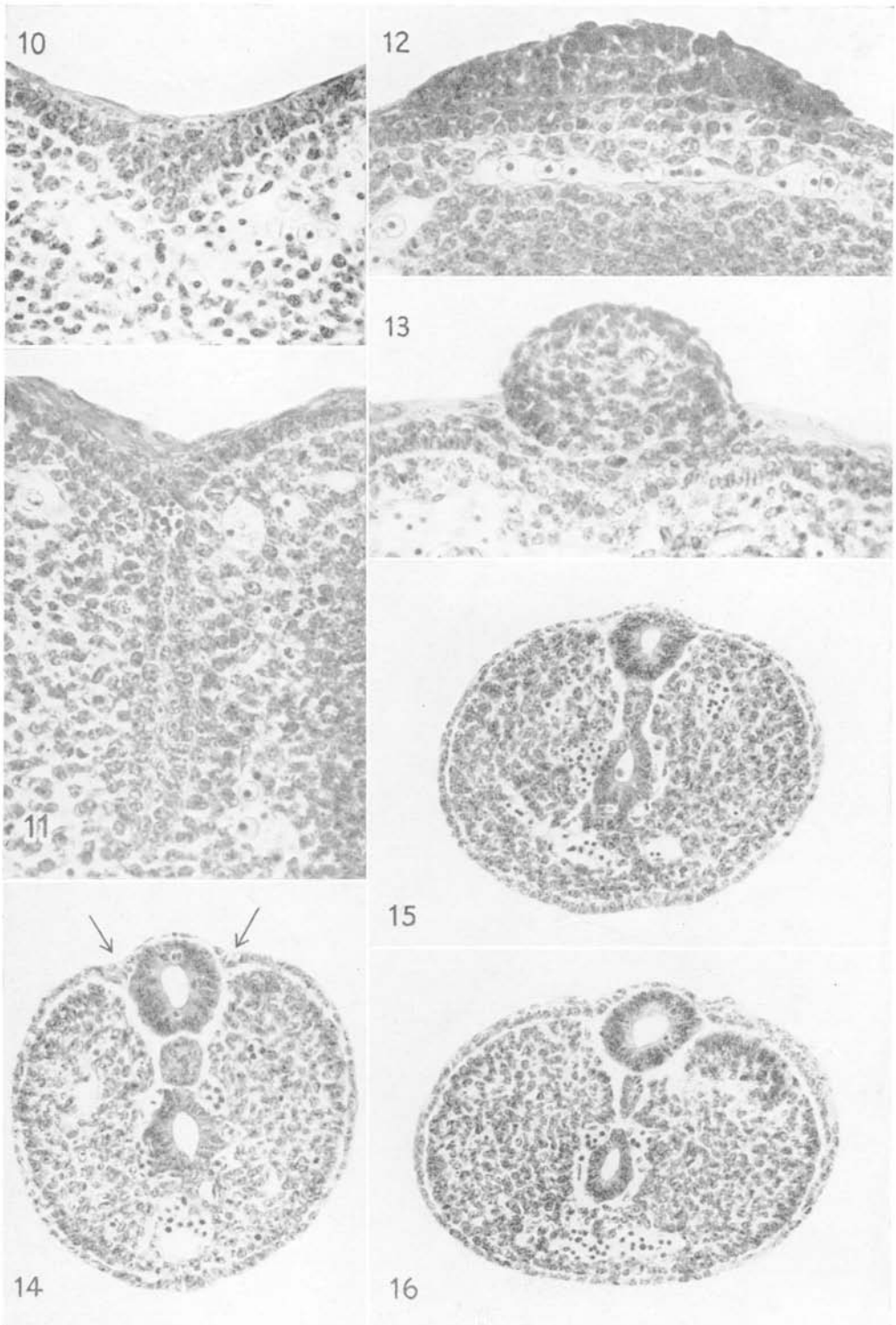

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embryos of about 4·5 mm C.R.L. (developmental age 10½ days); by the age of 11 days, the hyperplastic areas are usually fully developed. Most *sm/sm* embryos show the hyperplasia of the limb epidermis to a marked degree, but sometimes it is only slight or nearly absent. A more detailed description of the histology of this hyperplasia will have to await work with more specialized techniques.

5. *Hyperplasia of the tail epidermis*

A hyperplasia of the tail epidermis comparable to that described above for the limbs occurs in some (but not all) *sm/sm* embryos. An initial stage is shown in Fig. 14, Plate II. In this 11-day embryo, a thickening of the epidermis is present dorsally on either side of the neural tube; on top of the neural tube, the epidermis is represented by the stratum germinativum only (the lack of the periderm in this position being a normal feature of mouse embryos), and elsewhere it is also of about normal structure. The thickened area can be followed approximately from sections 32–47 (7·5 μ) as counted from the tip of the tail; the section shown is No. 39 and hence fairly distal in the tail; the ventral ectodermal ridge still being detectable in this region. At this early stage the relevant region of the tail was still straight.

A more advanced stage in a 12-day embryo is shown in Fig. 16, Plate II. The tip of the tail, for about forty-five sections, is approximately straight and cut transversely; up to section 28 (which is shown in Fig. 15, Plate II, for comparison), the epidermal structure is virtually normal; soon afterwards, a considerable thickening of the epidermis laterally and dorsally supervenes; Fig. 16 is section 42, which just grazes the last somite formed; the epidermis dorso-laterally is much thickened, and even on top of the neural tube the epidermis has become two-layered. The proximal limit of the hyperplastic area cannot be determined in this case, as on account of a sharp dorsal curvature of the tail the sections beyond No. 45 become oblique and

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PLATE II

Fig. 10. Palmar section through the right fore limb of an *sm/sm* embryo (No. 546; 13 days old) showing slight invagination of the AER into the underlying mesenchyme; externally, the ridge is covered by a cornified layer. × 335.

Fig. 11. Palmar section through the left fore limb of an *sm/sm* embryo (No. 544; 14 days old) with deep invagination of epidermis between the anlagen of two metacarpalia; parakeratosis of the top layer of the epidermis. × 335.

Fig. 12. Plantar section through the left hind limb of an *sm/sm* embryo (No. 547; 13 days old) with a large flat wart; from the top downwards wart, AER (2–3 cell layers) and mesoderm with marginal vein containing red blood cells. × 335.

Fig. 13. Plantar section through the right hind limb of an *sm/sm* embryo (No. 544; 14 days old) with a small wart flanked on either side by a thick cornified layer of epidermis. × 335.

Fig. 14. Transverse section through the tail of an *sm/sm* embryo (No. 1346; C.R.L. 4·7 mm.; 11 days old; the 39th section through the tail as counted from the tip). Beginning thickening of the epidermis on either side of the neural tube (indicated by arrows). × 190.

Figs. 15 and 16. Two transverse sections (Nos. 28 and 42 as counted from the tail tip) through the tail of an *sm/sm* embryo (No. 1071; C.R.L. 7·3 mm.; 12 days old). In Fig. 15 the epidermis is still normal, with a trace of the ventral ectodermal ridge just detectable; in Fig. 16 massive hyperplasia of the epidermis both dorsally and laterally; on the ventral circumference the ventral ectodermal ridge has disappeared. × 190.

thus unsuitable for this purpose. This is indeed the difficulty with the interpretation of many of the tails at this stage; hyperplastic areas are generally in the neighbourhood of the tail curvatures, and taken in isolation, it is often difficult or impossible to exclude the possibility that a thickening of the epidermis might be merely the result of oblique sections. However, there are enough clear cases to show that hyperplasia of the epidermis in the distal half of the tail is a genuine phenomenon in some *sm/sm* embryos though not all of them can be classified with confidence.

The tails of 10–10½-day-old embryos have not been sectioned for technical reasons. It is, however, improbable that they would have shown still earlier stages of the anomaly, if only for the fact that the tail region affected has not yet been formed at that time.

In all cases which could be clearly interpreted, the hyperplasia of the tail epidermis involved the dorsal rather than ventral aspect of the tail. If it may be assumed that hyperplastic epidermis presents some hindrance to longitudinal growth of the tail, the ventral side would be favoured in growth as compared with the dorsal, and a backwards turn or even twist might result. As the hyperplastic areas occur in the right region of the tail and indeed usually near tail curvatures, there can be little doubt that they are the mechanical cause of the tail kinks encountered later in life. The fact that they are variable and sometimes altogether absent is in agreement with the fact that more than half the syndactylous mice have quite normal tails.

A search for other hyperplastic epidermal regions has been carried out in the 12- and 13-day embryos which were sectioned completely. None have in fact been discovered. However, I am far from certain whether a more prolonged search, perhaps in material sectioned in a different plane, might not lead to the discovery of additional affected localities. It also remains possible that detailed histological studies might reveal minor anomalies in non-hyperplastic regions of the skin.

6. *Parakeratosis*

In the normal mouse embryo, the stratum corneum is formed on the sixteenth day (Hanson, 1947); as is known to every mouse embryologist, Bouin's fixative penetrates the skin readily up to the 16-day stage, but not thereafter. In *sm/sm* embryos, a premature keratinization occurs from the 13-day stage onwards. Unlike the fully mature keratin of the adult, and like some pathological keratinizations (e.g., psoriasis) in man, the keratin formed still contains some nuclei, many of them ghost-like and almost non-staining with haematoxylin. The process is thus one of parakeratosis.

Parakeratosis in *sm/sm* embryos occurs where epidermal hyperplasia has produced an excessive thickness of epithelial cells. The main locality is the apical ectodermal ridge; it is also found on hyperplastic patches of limb epidermis; whether it occurs on the tail is not known. Two typical deposits over sites of epidermal invaginations are shown in Figs. 10 and 11, Plate II; they come from 13- and 14-day stages respectively; even heavier masses are found in 15-day-old *sm/sm* embryos. Cornification occurs in patches. Very commonly it is found adjacent to the 'warts' which will be described in the next section; a clear case is shown in

Fig. 13, Plate II; similar cornified layers also flank the 'wart' in Fig. 12, Plate II, but are almost entirely outside the area included in the photograph.

The identification of keratin presents difficulties with which skin histologists are familiar. In the present case it rests on the typical position on top of the epidermis and on its histological resemblance to undoubted keratin encountered in the mouse and elsewhere; i.e. it is either structureless or shows some horizontal stratification, and it is eosinophil; most nuclei contained in it stain poorly or not at all. While these criteria make the identification very probable, more critical tests with special methods remain to be carried out.

7. '*Warts*'

From the age of 13 days onwards, sharply circumscribed roundish areas of the AER are separated from the underlying epithelium by a demarcating 'cut'. The material thus separated from the epidermis may form a fairly flat lentil-shaped mass, as in Fig. 12, Plate II, or a more roundish body which projects abruptly over the surface and then usually also indents the material beneath it, as in Fig. 13, Plate II. For want of a better word, and in spite of obvious differences, such structures may be called 'warts'. One of the differences is the fact that these bodies do not seem to cornify themselves though they are usually surrounded by horny masses, as mentioned above. Warts are present in many *sm*/*sm* embryos of 13 days or over, and a single limb may have more than one wart. The demarcated material shows dense nuclei and thus stains deeply. It may be assumed that warts will in the end be sloughed off, though the actual process has not been observed in the age range examined in this paper; no remains of them have been noticed at birth or afterwards. Obviously wart formation is one way to remove excess material from the region of the AER; however, only a very small fraction is eliminated in this fashion.

DISCUSSION

To simplify the discussion of the various effects of the *sm* gene described above, their age of onset has been summarized in Table 3. The earliest anomalies discovered

Table 3. *The age of onset of various anomalies of* sm/sm *embryos*

	Age in days					
	10	10½	11	12	13	14
Hyperplasia of AER: fore limbs	(+)	+	+	+	−	
Hyperplasia of AER: hind limbs	−	−	+	+	+	
Hyperplasia of limb epidermis	−	(+)	+	+	+	
Hyperplasia of tail epidermis			(+)	+	*	
Enlargement of the foot plates: fore limbs	−	−	(+)	+	+	+
Enlargement of the foot plates: hind limbs	−	−	−	+	+	+
Invagination of epidermis	−	−	−	+	+	+
Parakeratosis	−	−	−	−	+	+
Warts	−	−	−	−	+	+

* One out of two tails sectioned was straight and without hyperplasia, the other somewhat suspicious but not quite clear.

include the hyperplasia of the AER and of the limb epidermis which can be clearly identified at the age of 10–10½ days. Evidently, the anomaly develops when a critical stage of epidermal differentiation is reached. In the fore limbs the AER becomes hyperplastic at the age of 10 days, in the hind limbs at 11 days; the epidermis of the limbs becomes hyperplastic at 10½ days, that of the tail probably not (or not much) before 11 days. There can be little doubt that the epidermal hyperplasia is (potentially) a systemic anomaly, and that its manifestations in AER, the limb epidermis and the tail epidermis must be regarded as one and the same process. Similarly, there can be no reasonable doubt that parakeratosis and warts which first appear in the 13-day stage are consequences of the preceding epidermal hyperplasia. The *sm/sm* embryo thus suffers from a systemic disease of the skin, a situation which could scarcely have been foreseen when the adult skeletal anatomy was studied. So far as is known, this embryonic skin disease is transitory, as in the living adult *sm/sm* mouse skin and fur appear to be normal.

Turning now to the limb buds, enlargement and deformation of the foot plates is clearly present both in the fore and in the hind limbs in the 12-day stage; in the fore limbs, the very beginning of the anomaly can probably be traced back to the 11-day stage. It is immediately obvious that the deformation of the foot plates with the bending over of the margin in a palmar and plantar direction respectively is the mechanical cause for the crowding of the middle rays and thus for the ensuing syndactylism. All this happens before there are recognizable phalanges; the blastemata present at the 12-day stage probably represent no more than metacarpals and metatarsals respectively. One is used to the idea that the size and shape of our skeletal elements determines the shape of our bodies. In this case the situation is clearly reversed, as the size and shape of the phalanges is determined by that of the foot plates. There is reason to think that this is not a very exceptional situation.

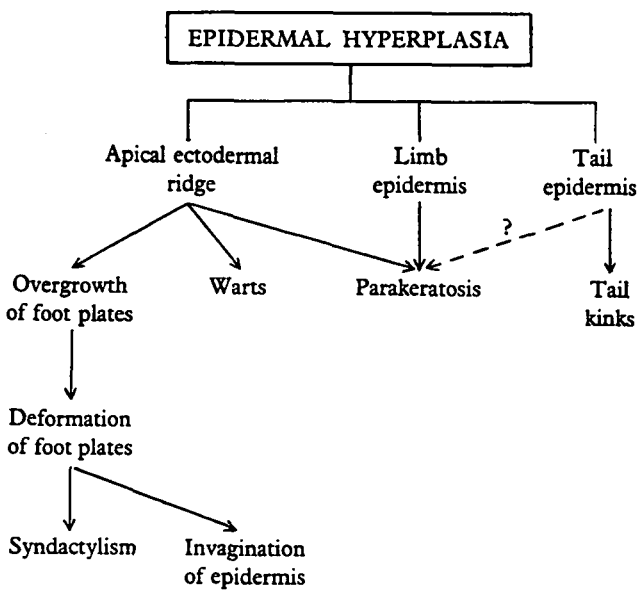
We have already discussed the origin of the epidermal invaginations; like syndactylism, these are mechanical consequences of the deformation of the foot plates.

Table 3 shows that the epidermal hyperplasia of *sm/sm* embryos can be traced back a full day beyond the earliest indications of an enlargement of the foot plates. Could the skin condition be responsible for the enlargement of the foot plates which follows it in time?

The work of Saunders (1948) in the chick embryo has shown that the AER is playing an important role in the outgrowth of the limb buds. Extirpation of the AER suppresses the formation of those parts of the limb which have not yet been formed by the apical mesodermal growth zone at the time of operation. It was also shown by Zwilling (1949) that in the wingless mutant in the chick the AER is reduced or absent. Experiments with another wingless mutant (Zwilling, 1956) with a similar regression of the AER showed that mutant AER grafted on normal mesoderm did not stimulate outgrowth; conversely, mutant mesoderm with a normal AER developed distally beyond the controls; however, after some time the AER regressed and development was not completed. From this and similar observations, Zwilling concluded that there exists a reciprocal relationship between the

mesoderm and the AER: the mesoderm grows in response to a stimulus supplied by the AER, but the AER in turn does not remain functional unless supplied with a 'maintenance factor' by the mesoderm. In the case of the wingless chicks, the fault seems to lie with the mesoderm which fails to supply the necessary maintenance factor with the result that the AER regresses and thus no longer stimulates the outgrowth of the wing bud.

There is no doubt that the AER in the chick and presumably in other organisms acts as a stimulatory organ for the growth of the underlying mesoderm. In *sm/sm* embryos, the AER is visibly hyperplastic at 10–10½ days, and about a day later enlargement of the foot plates can be demonstrated. It seems reasonable to suggest that, as reduction or absence of the AER leads to regression of the limb in wingless chickens, so hyperplasia of the AER may lead to overgrowth in the *sm/sm* mouse. The state of the AER points to a normal, but not necessarily to an increased, pro-



Text-fig. 14. The pedigree of causes of the *sm* gene.

duction of the maintenance factor: although Zwilling & Hansborough (1956) suggested that polydactylism in the chicken is due to a pre-axially more extended distribution of the maintenance factor (which acts indirectly through the AER), in other instances the AER may well be hyperactive for different reasons. In the present case, the time relations of events suggest a primary role of the AER which seems to be involved as part of a systemic tendency to epidermal hyperplasia.

If this argument is accepted, the syndactylism as well as the tail kinks of *sm/sm* mice must be regarded as secondary to an embryonic skin disease (Text-fig. 14), a relationship not hitherto described in developmental genetics.

It has sometimes been assumed that normal alleles fulfil functions in development which are similar to the phenotypic effects of the corresponding mutant genes.

For instance, if there are, say, thirty known loci which have given rise to eye colour mutations in *Drosophila*, it has been said that there must be at least thirty different steps in the elaboration of the normal eye pigments which correspond to the normal alleles of these mutants. The case under discussion demonstrates that such a conclusion may be quite misleading. The gene for *sm*, without a full embryological analysis, would presumably be regarded as a 'skeletal gene'. Yet its normal allel has obviously nothing whatever to do with the development of the skeleton.

Similarly, some writers have been tempted to deduce from an adult phenotype that such a 'skeletal gene' acts on the mesoderm. Plainly, in the present case the gene acts on the epidermis in the first instance, and its effect on skeletal structures derived from the mesoderm is secondary. However, it would be equally illegitimate to say that the *sm* gene acts on the ectoderm. It is, of course, true that the epidermis is ectodermal in origin. But so are many other structures which are not demonstrably affected by the *sm* gene. To say that *sm* affects the ectoderm would be a wanton generalization. To replace one statement by another which is manifestly less accurate is a step away from the truth.

It is widely accepted that during development genes are selectively activated at the time, and in the place, in which their services are required. The present case illustrates this principle very clearly. In normal development, keratinization of the skin happens at the 16-day stage. The hyperplastic areas of the epidermis in *sm*/*sm* embryos start to keratinize at the 13-day stage. The formation of keratin involves biochemical processes which, it must be presumed, are under the control of specific genes. In normal development, the epidermis reaches the physiological state which activates these genes at the 16-day stage, in *sm*/*sm* embryos, in certain places, some 3 days earlier. It need scarcely be pointed out that this argument would fall to the ground if the substance here tentatively identified as keratin should turn out to be something else.

So far as I know, this is the first embryological analysis of a case of inherited syndactylism. The development of various other limb anomalies which have been studied in the mouse and other animals will be discussed on a later occasion.

SUMMARY

1. The earliest manifestations of the gene for syndactylism discovered include hyperplasia of the apical ectodermal ridge of the limbs and hyperplasia of parts of the limb epidermis (10–10½-day stage). Somewhat later, a similar hyperplasia of the epidermis in the distal parts of the tail is found in some *sm*/*sm* embryos.

2. Enlargement and deformation of the foot plates in both fore and hind limbs is present in the 12-day stage; in the fore limbs it probably starts on the preceding day. The foot plates are bent over in a palmar or plantar direction respectively; the crowding together of the middle digits thus produced is the mechanical cause of syndactylism.

3. It is suggested that the enlargement of the foot plates with the ensuing deformities is due to increased stimulation of mesenchymal growth by the hyperplastic apical ectodermal ridge. On this interpretation, all the skeletal effects

(including those in the tail) are secondary to a systemic tendency to epidermal hyperplasia.

4. The hyperplastic regions of the apical ectodermal ridge and of the limb epidermis start to keratinize (parakeratosis) in the 13-day stage whereas in normal development keratinization of the skin does not start until some 3 days later.

The microscopical preparations for this paper have been made by Mrs H. Deol and by Miss H. Bartels-Walbeck and in large part by Miss Heide Schulze, who has also taken the microphotographs. Text-figs. 1 and 2 have been prepared by Mr A. J. Lee. To all of them I wish to express my appreciation. Dr W. Kocher kindly made the planimeter measurements of Table 2. The work has been supported by a grant from the Rockefeller Foundation which is gratefully acknowledged.

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Note added in proof (April 22, 1960)

Since this paper was submitted for publication, the role of the AER as a stimulatory organ for limb outgrowth has been the subject of renewed interest (Bell, E., Saunders, J. W., Jr., & Zwilling, E. Limb development in the absence of ectodermal ridge. *Nature* (*Lond.*), **184**, 1736–7, 28 Nov. 1959). It appears that in the chick embryo, limb development may proceed in the absence of the AER if the latter has been removed by methods which leave the underlying 'refractile layer of the mesoblast' intact; whether this layer is a cellular or acellular structure remains to be investigated. Pending a full publication of the facts, one may perhaps recall the mechanism of double assurance ('doppelte Sicherung') which may lead to the formation of a lens even in the absence of an eye cup. Hence though limb development may proceed in the absence of the AER, this does not necessarily mean that the AER has nothing to do with limb differentiation in the normal course of events.