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Genetical studies on the skeleton of the mouse

XXIX. PUDGY

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INTRODUCTION

In previous papers of this series, ten genes of the mouse with major effects on the axial skeleton have been described. In two of them (curly-tail, *ct/ct*; Grüneberg, 1954*a*; Tail-short *Ts*/+; Deol, 1961) the involvement of the skeleton was clearly secondary. In congenital hydrocephalus (*ch/ch*; Grüneberg, 1953), the anomalies of the axial skeleton were part of a systemic effect on the membranous skeleton as a whole. In Danforth's short-tail (*Sd*/+; *Sd/Sd*; Grüneberg, 1958*a*), in Brachyury and Anury (*T*/+; *T/T*; *T/t*; Grüneberg, 1958*b*) and in Pintail (*Pt*/+; *Pt/Pt*; Berry, 1960), the axial anomalies were traceable to the notochord as the source of trouble (though in the first two of these, the notochord itself may be involved as part of a more fundamental disturbance of the primitive streak). In vestigial-tail (*vt/vt*; Grüneberg, 1957), anomalies of the distal end of the neural tube appeared simultaneously with a gross reduction of the ventral ectodermal ridge of the tail, the causal relationship between the two events remaining in doubt. In tail-kinks (*tk/tk*; Grüneberg, 1955*a*) and in undulated (*un/un*; Grüneberg, 1954*b*), the axial anomalies are the result of faulty differentiation of the sclerotomes. The only gene so far studied in this laboratory which seems to implicate segmentation as such is Bent-tail (*Bn* ♂; *Bn*/+ ♀; Grüneberg, 1955*b*) in which the distal tail vertebrae are increasingly reduced in size and, in more extreme cases, in number. However, in this case the interpretation is based on the findings in the adult skeleton and hence carries less conviction than the embryological 'eye-witness accounts' in the other mutants. The condition to be described in this paper profoundly upsets segmentation and leads to a chaotic situation of the whole vertebral column.

GENETICS

The recessive gene 'pudgy' (symbol *pu*) has possibly been induced by X-rays. It appeared in the later descendants of a ♂ derived from the 'specific locus' experiments carried out in the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A., under the direction of W. L. Russell. According to results by W. St Amand and M. B. Cupp (to be published), pudgy is located in Linkage Group I, the order of markers being *pu–p–c*^{ch}. The present investigation was carried out on the descendants of a group of animals handed over to me for study by L. B. Russell in July 1958.

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Pudgy mice can easily be identified at birth. Their tail is reduced to a small stub or all but absent, and the whole trunk region is considerably shortened. As the limbs are of about normal length, the adult pudgy mouse has a somewhat toad-like appearance. As pudgies are very poor breeders, the stock is being maintained by $+/pu \times +/pu$ matings. Twenty such matings with at least twenty classified young each produced 773 normal and 208 *pu/pu* offspring. There is thus a significant deficiency of recessives which is obviously due to reduced viability (81% at birth); there is further selective mortality in the nest and after weaning.

Embryonic material was collected by mating normal sisters of pudgies to known $+/pu$ ♂♂. The litters ranged in age from 9 to 19 days, and during the whole of this period, *pu/pu* embryos can easily be identified by their external appearance. Sixty-five litters of embryos thus obtained included 450 normal and 70 *pu/pu* individuals. On the 5:1 expectation, there is thus again a significant deficiency of recessives (70 as compared with 450/5 or 90), the prenatal viability being 70/90 or 78%, in good agreement with that found at birth. However, this good agreement may be spurious. It results from the fact that there are 33 segregating : 32 non-segregating litters instead of something much closer to a 2:1 ratio, a discrepancy presumably due to an accident of sampling. Considering only those litters which contained at least one *pu/pu* embryo, these included 216 normal and 70 *pu/pu* embryos. Allowing for the selection inherent in these data, the expected number of recessives, on a 3:1 expectation, is 78·585. The observed deficiency of recessives is thus no longer significant ($\chi^2_1 = 1{\cdot}293$), and the possibility must be considered that the viability of *pu/pu* may be normal or nearly so before birth, selective elimination of pudgies happening at birth or soon afterwards.

ADULT ANATOMY

The anatomy of pudgy has been studied in alizarin clearance preparations (21 *pu/pu* and 13 normal litter mates). In addition, papain macerations of the skulls of six *pu/pu* and of six normal litter mates have been studied.

Anomalies of an extreme kind are found from one end of the vertebral column to the other, in the ribs and in the sternum; anomalies are also often detectable in the skull in the immediate vicinity of the foramen magnum. Otherwise the skull and the appendicular skeleton, including the girdles, appear to be quite normal. A typical specimen is shown in Text-fig. 1. The general type of anomaly of the vertebral column is very uniform in all pudgies examined. But as the state of the axial skeleton can only be described as chaotic, no two pudgies agree in detail. The vertebral column is considerably shortened all along its length; it consists of a jumble of vertebrae, and fragments of vertebrae, which are irregularly fused with each other in such a complex way that it is impossible to discover in any given mouse how many elements have gone into the making of such a telescoped structure with all its irregular kyphoses and lordoses. As is to be expected, the ribs are as irregular as the vertebrae. Particularly in the caudal half of the thoracic region, the ribs tend to be bunched together, sometimes as many as six successive ones, with irregular fusions which are sometimes superficial, but often so complete

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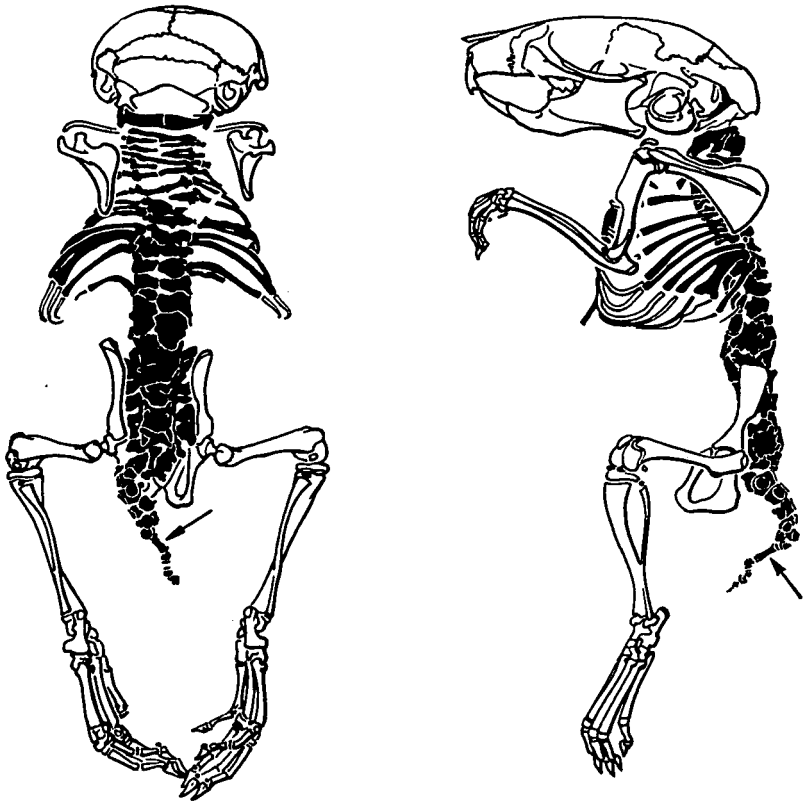
that it is difficult to decide whether a given rib is simple or composite in nature. In the sternum, fusions between adjacent sternebrae, often at an angle, are common.

More detailed study shows that vertebrae, or parts of vertebrae, often retain some of their regional specificity, such as the characteristic shape of the transverse processes in the lumbar and sacral region. Very exceptionally, a whole vertebra has escaped from the surrounding chaos, such as the caudal marked by an arrow in Text-fig. 1. Occasionally, the number of elements in a particular



Text-fig. 1. Pudgy ♀, 23 days old. Drawings based on photographs. A single virtually normal caudal vertebra is indicated by arrows.

region of the spine can be ascertained. Combining such counts from a number of pudgies, it seems probable that the jumble of ossicles and fused bony masses is, in fact, derived from the normal number of segments, or something fairly close to it, as far down as the os sacrum. In the caudal region, the number of segments represented is certainly much less than the normal average of thirty and presumably very variable.

Inevitably, adjacent soft structures like muscles, nerves and blood vessels will be involved in this tangle, but for technical reasons these have not been studied. Indeed, it is astonishing that an animal can live in such a frame at all. Considering the pain and discomfort which a single cervical rib, by pressure on the brachial

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plexus, can cause in man, I personally have no doubt that pudgy mice are enduring a martyrdom of pain throughout their lives. But as mice do not show suffering in any very obvious way, this cannot at present be proved to the satisfaction of a behaviourist, let alone a philosopher.

DEVELOPMENT

Pudgy embryos down to the 14-day stage can be recognized at a glance by their shortened tails. These are also thinner distally and are pointed at the tip; there is no evidence for any loss of material such as happens in Brachyury ($T/+$). Four litters ranging in age from 14 to 16 days and including twenty-four normal and nine pudgy embryos were stained with methylene blue and cleared in oil of wintergreen. As expected, all the chaotic anomalies of the osseous axial skeleton and of the ribs are preformed in cartilage. Perhaps it would not have been really necessary to make these preparations at all. By no conceivable mechanism could the distortions of the osseous vertebral column have arisen from a normal cartilaginous model. Nor, indeed, could so anomalous a cartilaginous axial skeleton have been formed unless its membranous predecessor was already profoundly out of order.

Normal 13-day embryos show well-defined segmentation (somites) in the distal half of their tails. The tails of 13-day pudgy embryos are not only shortened (though much less than later on), but they also show no clear segmentation distally and only indistinct segments more proximally.

Normal 12-day embryos have somites all along their tails. The tails of pudgy embryos are only slightly shortened, with no segmentation distally and only indistinct segmentation proximally.

In normal 11-day embryos, somites are clearly defined along the tail and the posterior half of the trunk, but they have become indistinct anteriorly. The tails of pudgy embryos are not yet appreciably shortened at this stage. Pudgy embryos can, however, be easily identified by irregularities of their segmentation. As in later stages, segmentation does not reach as far distally as in normal embryos. More proximally, segments are recognizable, but they tend to be somewhat irregular in shape; the intersegmental fissures are rather less well defined than in the normal and they are not always strictly parallel to each other; moreover, the width of adjacent segments is somewhat variable, so that the whole row of somites looks rather untidy. By the same token, pudgy embryos can be distinguished from their normal litter mates without any difficulty in the 10-day and in the 9-day stage. As the whole axial skeleton is similarly affected, there cannot be any doubt that by the same criteria, pudgy embryos could be identified in the 8-day stage and indeed from the onset of somite formation.

For the study of segmentation in sectioned material, two regions have been selected. These are the tail where new segments are formed in 10–12-day embryos, and the lumbo-sacral region where the same phase of development can be studied in the 9-day stage. The two regions behave alike in essentials, but the anomalies are rather more extreme in the tail which, for that reason, may be described first.

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Five pudgy embryos and five normal litter-mates ranging in age from 10 to nearly 12 days (C.R.L. 4·3–7·0 mm.) were sectioned transverse to the tail, which at this stage is usually nearly straight. The results are shown diagrammatically in Text-fig. 2 (see also Plate I). In the stages examined, the distal 500 micra or so of the tail still lack segmentation; the paraxial mesoderm forms a fairly homogeneous network of mesenchymatous cells. Part of this region is occupied by the ventral ectodermal ridge (VER) of the tail (Grüneberg, 1957) which resembles the apical ectodermal ridge of the limb buds in structure and may have a similar stimulatory function for the growth of the tail. The VER is shown in transverse sections in Figs. 1 and 4, Plate I, and its extent in proximo-distal direction in Text-fig. 2 (hatched areas). Clearly there is no significant difference between normal and pudgy embryos in this respect. The largest pudgy embryo (No. 1429) has a small VER, but at this stage the structure is in the process of disappearing so that a small difference in stage of development is a sufficient explanation.

In normal embryos, the distal unsegmented tip of the tail is followed proximally by three or four somites (Fig. 2, Plate I) in which the cells are tightly packed without any gaps, and in which the outer layer is like a columnar epithelium in arrangement. The somites are separated from each other by intersegmental fissures which cut through the whole thickness of the somite material. These fissures can be seen in several consecutive sections, and their positions in Text-figs. 2 and 3 represent, as nearly as possible, the centres of the fissures. In the normal embryos, the position of the most distal intersegmental fissure is shown as the end of the somites. Actually, some additional material in the process of forming the next somite is usually present but not shown in the diagrams as its definition is often too unsharp. For this reason, the slight delay in somite formation shown by all five pudgy embryos is probably in reality greater than appears from the diagrams, and I am inclined to regard it as a real difference. Following the three or four somites, there is, in the more cranial segments, an emigration of cells from the ventro-medial aspect of the somites towards the midline; these cells form the sclerotomes and only the outer layer of the original somites remains intact as the dermomyotome. Apart from the fact that somite formation begins a little farther from the tail tip (i.e. later), the general sequence of events is similar in the pudgy embryos, with one important exception. Although closely knit somite tissue is formed, there is a complete absence of intersegmental fissures. The tissue forms one unbroken block which is shorter than the three to four somites in the normals (see also Text-fig. 3). It is only when the somite tissue of the pudgies is cranially transformed into the loose sclerotome tissue that a belated segmentation makes its appearance in the dermomyotomes. This may start fairly promptly (as in embryo 1435) or only after considerable delay (e.g. embryo 1433), and the fissures formed may at first be only rudimentary. For the rest, both the outer shape of the somite block and the inner arrangement of the cells tends to be less regular in pudgy embryos (Fig. 5, Plate I) than in the individual somites of their normal litter-mates. Otherwise, neural tube, notochord, tail gut and the vascular apparatus of the tail seem to be normal.

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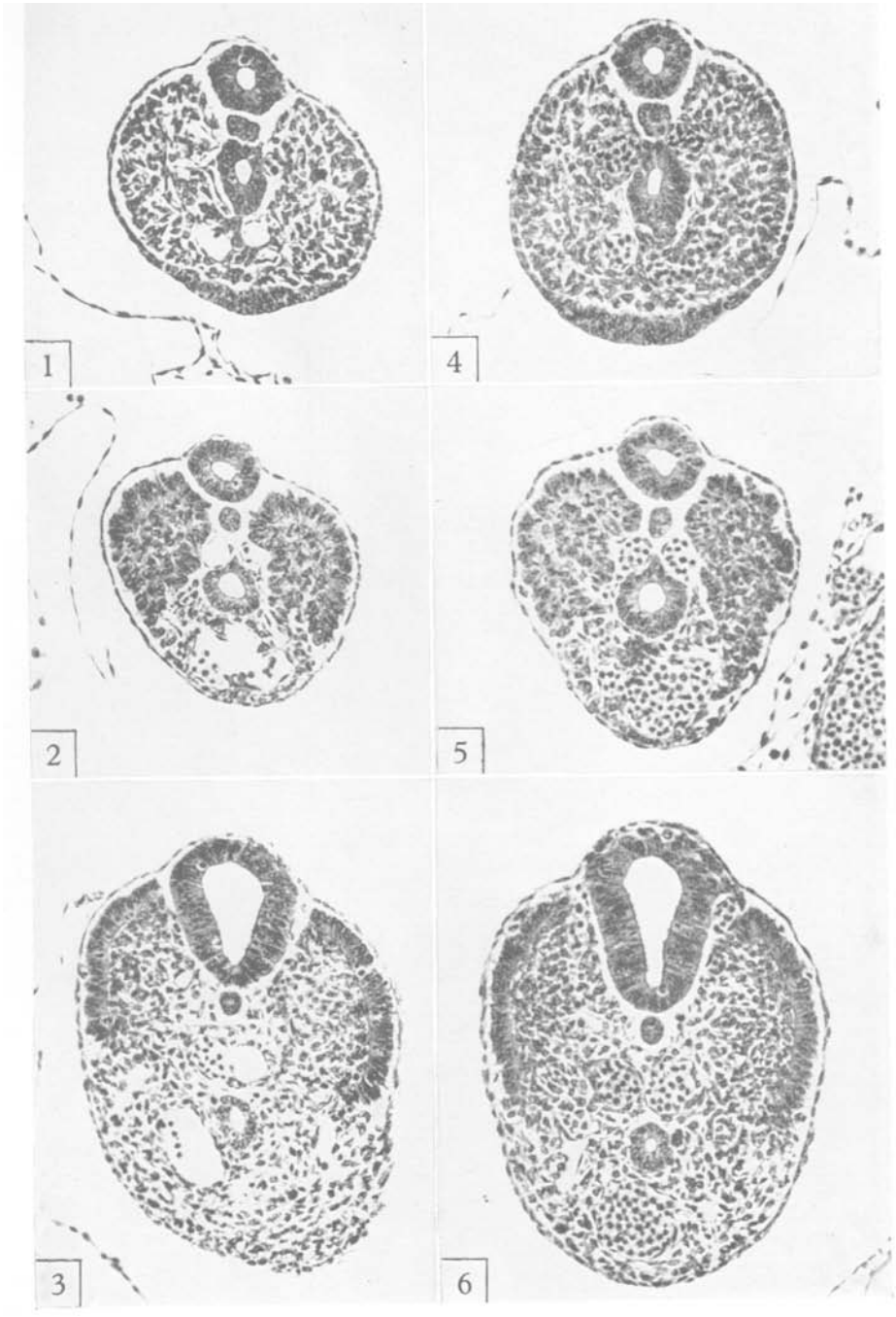

NORMAL PUDGY

Transverse sections through the tails of two 11-day-old embryos. Nos. 1–3 normal No. 1436 (C.R.L. 5·0 mm.), Nos. 4–6 pudgy No. 1437 (C.R.L. 5·0 mm.). In the normal the sections pass through the unsegmented paraxial mesoderm and the ventral ectodermal ridge, through the penultimate somite, and through a dermomyotome respectively; those in the pudgy embryo pass through approximately corresponding levels as indicated by arrows in Text-fig. 2. Bouin fixation; imbedded by Peterfi's method; sections 7·5 micra thick; haematoxylin and eosin. Photographs × 120, enlarged to × 160.

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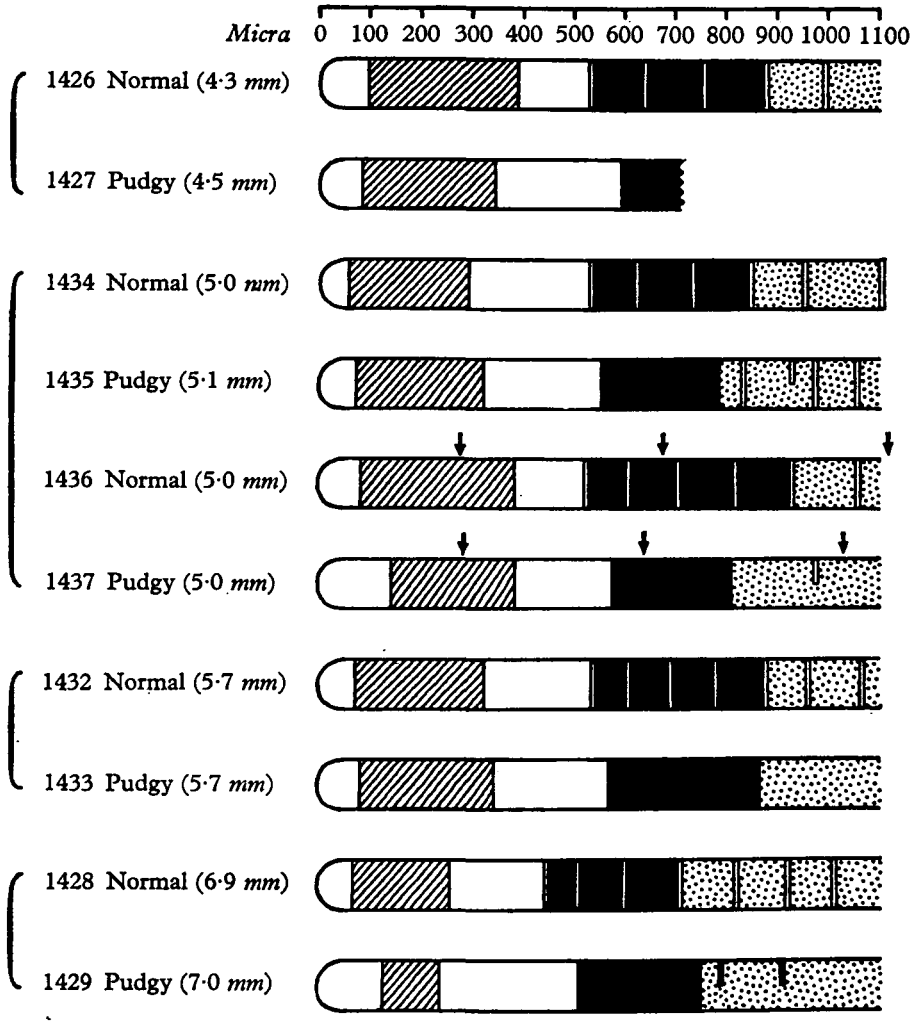
To sum up. In the tail of pudgy embryos, the paraxial mesoderm is formed into solid somite tissue with some delay and in a less orderly fashion. Segmentation does not start until the somite tissue is ventro-medially breaking up to form



Text-fig. 2. Tail diagrams of normal and pudgy embryos aged 10–12 days. Littermates bracketed together. The unsegmented tail tip is shown in white, with the VER hatched. Somites or somite tissue black, sclerotomes stippled. Intersegmental fissures shown in the upper half of a diagram only are rudimentary. Scale in micra starts from the tail tip on the left. In the case of embryo 1427 the proximal part of the tail could not be analysed on account of its curvature. Position of sections in Plate I indicated by arrows.

sclerotomes, and, even then, the fissures separate only consecutive dermomyotomes from each other (and not always completely); the sclerotome tissue, so far as one can see, remains continuous.

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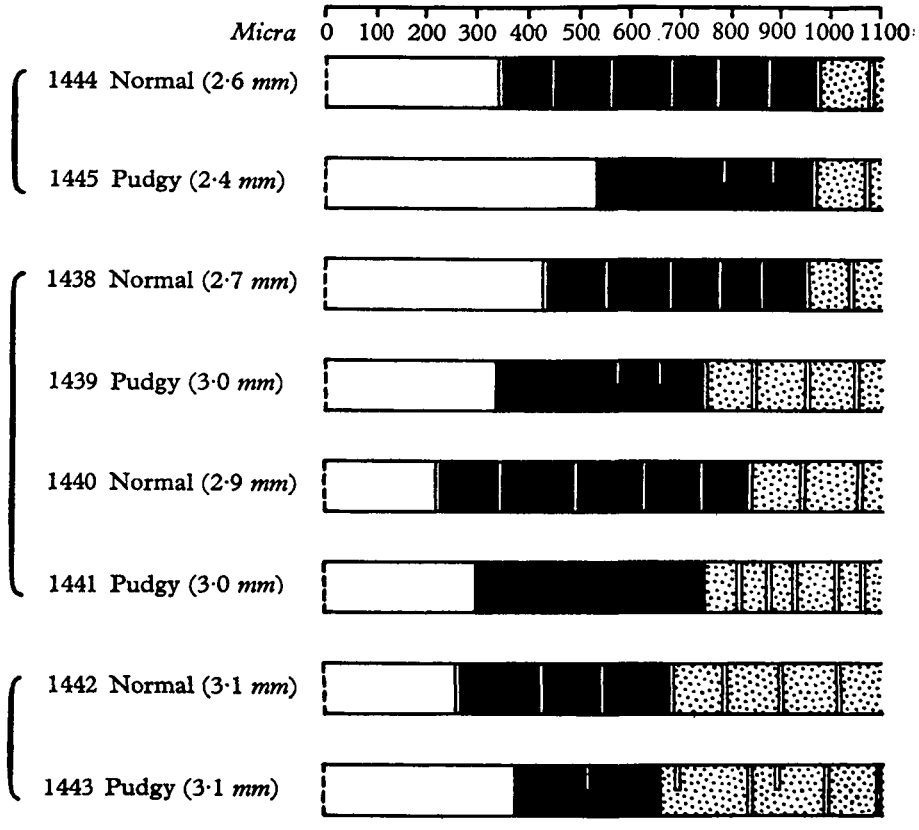
At the 9½-day stage (Text-fig. 3) there is hardly any tail bud and the segments in process of formation are the lumbo-sacral ones. As the posterior end of the body is somewhat curved relative to the trunk region, in which segmentation is in progress, the first section which opens the coelom has been used as the starting-point of the scale in micra. The difference between normal and pudgy embryos is similar to that in the tail region. The main difference is that rudimentary inter-



Text-fig. 3. Diagram of segmentation in the process of formation in the lumbo-sacral region of 9½-day-old normal and pudgy embryos. Conventions as in Text-fig. 2, except that the scale in micra starts from the section which first opens the coelom. The position of the third intersegmental fissure from the left in embryo 1444 is by interpolation only as the series was damaged in this neighbourhood.

segmental fissures in the somite block occur in three out of the four pudgies. The anomalies are thus rather less extreme than in the tail, in conformity with the anatomical situation in the adult animal.

In addition to those already described, seven more pudgy embryos, together with seven normal litter-mates, were sectioned in various planes; they included the ages of just under 11 to 12½ days (C.R.L. from 4·7 to 8·3 mm.). They showed, as expected, that the chaotic behaviour of the cartilaginous axial skeleton is pre-

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ceded by a similar disorder of the sclerotomes. An additional fact is that the spinal ganglia which, in parasagittal sections of normal embryos, are quite separate from each other, form an almost continuous mass in the pudgies in which their segmental origin is more or less concealed.

DISCUSSION

From the descriptions given above, there cannot be any doubt that the state of the axial skeleton of pudgy mice is basically due to an anomaly of segmentation. The question which immediately arises is whether this is due to anomaly of the unsegmented mesoderm which makes it difficult to subdivide into individual somites, or whether there is some defect in the dynamics of the segmentation process as such, the material to be segmented being normal. This question must remain open at present. I have not been able to discover a consistent difference in the structure of the unsegmented paraxial mesoderm distal to the block of somite material of normal and pudgy mice. Needless to say, other methods might bring such differences to light. On the other hand, both the outer shape and the internal structure of the somite block of pudgy mice is less regular than that of the corresponding somites of normal embryos. However, these irregularities might be either cause or effect of the disturbance of segmentation.

The morphogenesis of the vertebrae of pudgy mice does not seem to be disturbed as such. Wherever there is an opportunity, the regional specificity of the vertebrae asserts itself, and occasionally, such as in the caudal vertebra in Text-fig. 1, a completely normal element may be formed. Evidently, the structural abnormalities are entirely due to the non-separation of the sclerotomes and the consequent scrambling of the chondrogenic material; where circumstances permit, normal or near-normal morphogenesis takes place.

A condition virtually indistinguishable from pudgy is stub (*sb*/*sb*) in the mouse (Dunn & Gluecksohn-Schoenheimer, 1942). There is complete agreement so far as one can judge from the published account. Unfortunately, stub is now extinct so that no direct tests for allelism are possible. For that reason, a distinct name has been used for the present mutant. It is quite possible, if not indeed probable, that pudgy is an allel or a recurrence of stub. Stub was lost before its development had been studied.

Apart from stub in the mouse, by far the most similar inherited condition is short-spine in cattle (Mohr & Wriedt, 1930). The gene is recessive and lethal at or soon after birth. The greatly shortened vertebral column, together with normal head and normal appendicular and girdle skeleton, give the affected calves an elk-like appearance. The anomalies of the telescoped vertebral column, of the ribs and of the sternum are so similar to those of pudgy mice that similar developmental processes are probably responsible for both of them.

It has already been mentioned that the sex-linked mutant Bent-tail in the mouse is probably due to a rather mild disturbance of segmentation (Grüneberg, 1955*b*). Another rather slight involvement of somite formation occurs in the mutant Crooked-tail (*Cd*/+) of the mouse (Matter, 1957) where some of the

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somites are reduced in size and deformed, with cell pycnosis in places; these abnormal somites give rise to reduced and deformed blastemata and ultimately cartilaginous and osseous vertebrae. An anomaly of segmentation even more extreme than that of pudgy is found in Rib fusions (*Rf*/*Rf*) recently described by Theiler & Stevens (1960); in these lethal homozygotes, the paraxial mesoderm never forms into solid somite tissue at all; nonetheless, sclerotomes of a kind are formed and a highly telescoped vertebral column with extensive rib fusions is the ultimate result; as in pudgy, the spinal ganglia also tend to coalesce with each other; some involvements of the CNS like exencephaly have not been encountered in pudgy.

In the chick embryo, segregation of somites can be inhibited by various amino-acid antagonists, and particularly by leucine antagonists (Herrmann, 1953; Rothfels, 1954; Herrmann *et al.*, 1955). Recent work by Deuchar (1960) suggests that these substances act by interfering with adenosine triphosphatase activity in the somite mesoderm, and that the activity of this enzyme is one of the main factors in somite formation. The pudgy gene might act in a similar fashion.

SUMMARY

The recessive mutant pudgy in the mouse has a greatly shortened vertebral column with highly irregular fusions between vertebrae and fragments of vertebrae. Ribs and sternum are also involved, but the rest of the skeleton is quite normal. The deformities arise from a defective segmentation. Although the paraxial mesoderm forms somite tissue with an epithelially arranged outer layer, this material either shows only an abortive segmentation into somites or, in the tail, none at all. A belated segmentation into dermomyotomes ultimately takes place, but the sclerotomic material remains continuous and gives rise to erratically abnormal blastemata which then chondrify and ultimately ossify.

I am deeply indebted to Drs W. L. and L. B. Russell, who turned the pudgy mutant over to me for study. The sectioned material and the photomicrographs were made by Miss June Denny, and the drawing by Mr A. J. Lee, to both of whom I wish to express my appreciation. The work was partly supported by a grant from the Rockefeller Foundation which is gratefully acknowledged.

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