Ultrastructural observations on stumpy (stm), a new chondrodystrophic mutant in the mouse

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SUMMARY

Stumpy (stm) is a new chondrodystrophic mutant in the mouse. Light microscopy of cartilage reveals a slightly increased mitotic rate, more chondrocytes than is usual per lacuna and a wide zone of hypertrophy. Electron microscopy shows that many chondrocytes are in close approximation with some tight junctions: in cartilage from 14-day-old mice there is much interdigitation and folding of the cell membranes of adjacent chondrocytes.

INTRODUCTION

In recent years there has been a renewal of interest in hereditary chondrodystrophy in both man and laboratory animals (see Rimoin, 1975, for a review). This has been largely due to the introduction of biochemical and ultrastructural studies on abnormal cartilage. It is becoming increasingly apparent, as descriptions of genetically distinct conditions accrue, that the chondrodystrophic phenotype can be produced by any one of a number of clearly distinct anomalies reflecting different ultrastructural or biochemical parameters.

Thus, the gene for nanomelia in the chick, and possibly chondrodystrophy (cho) in the mouse, affect acid mucopolysaccharide synthesis (Fraser & Goetinck, 1971; Seegmiller, Fraser & Sheldon, 1971; Seegmiller, Ferguson & Sheldon, 1972; Pennypacker & Goetinck, 1976), cartilage anomaly (can) in the mouse seems to affect collagen synthesis (Johnson & Hunt, 1974), brachymorphism affects sulphation of acid mucopolysaccharides (Orkin, Pratt & Gill, 1976), whilst the achondroplastic rabbit (ac) has a defect in mitochondrial oxidative phosphorylation (Bargman, Mackler & Shepard, 1972). Achondroplasia (cn) in the mouse is unique in having no known clear ultrastructural or biochemical defect (Silverberg & Lesker, 1975; Silverberg, Hasler & Lesker, 1976).

Stumpy is yet another genetically distinct chondrodystrophic dwarf with a distinctive cartilage phenotype.

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ORIGIN AND GENETICS

Stumpy arose in Cambridge in the control series of an experiment designed to test the mutagenicity of oral contraceptives. It is not allelic with achondroplasia (cn), brachymorphic (bm), stubby (stb) (Ferguson & Wallace, 1973) nor cartilage anomaly (can) (D. R. Johnson, unpublished).

The gene behaves as a simple recessive: female stm/stm are good mothers, but males are usually sterile. An account of the genetics and linkage of stumpy will appear elsewhere.

MATERIAL AND METHODS

The left knee and elbow regions of fourteen stm/stm mice aged 5–14 days and 12 normal litter-mates were fixed in Bouin's fluid, dehydrated, embedded in paraffin wax, sectioned longitudinally at 8 μm and stained with haematoxylin and eosin. The right knee and elbow were fixed in cacodylate-sucrose buffered glutaraldehyde at pH 7.2, post-fixed in osmic acid, dehydrated and embedded in Araldite or Spurr's resin. Ultrathin sections obtained with an LKB ultratome were stained with uranyl acetate and lead citrate and examined in an AEI EM6B electron microscope.

RESULTS

Light microscopy of immature stm/stm long bones shows reduced staining of the cartilage matrix. The number of cells per lacuna is increased, and a slight increase (14%) is seen in the number of mitotic figures. The zone of hypertrophy is extensive: hypertrophied cells seem to be a little larger than in normal litter-mates, and are surrounded by a dense rim of matrix (Figs. 1 and 2).

Electron microscopy shows normal-looking cells set in a normal-looking matrix (Figs. 3 and 4). However, a larger number of cells than normal lie in close approximation to their neighbour, and nests of four or eight adjoining cells were often seen. When two cells are adjacent, contact between them is maintained either by large areas of membrane which remain in close proximity but with a narrow intercellular gap, or, less commonly, by tight junctions (Figs. 5 and 6). In some cells, especially those from older (14-day) animals, the junctional region was complicated by folds in the membranes (Figs. 7

Figures 1-4

Figs. 1, 2. Sections through lower femoral epiphyseal plates of +/+ or +/stm and stm/stm mice respectively. In Fig. 2, note the wide zone of hypertrophy, dense deposition of matrix around hypertrophic cells and increased number of cells per lacuna.

Fig. 3. stm/stm chondrocytes: note normal appearance of cells and matrix.

Fig. 4. stm/stm chondrocytes showing areas of membrane contact.
Fig. 5. Tight junction between stm/stm chondrocytes.

Fig. 6. Area of contact between stm/stm chondrocytes showing close apposition of membrane and vesicles (V).

Figs. 7, 8. Tortuous membrane between stm/stm chondrocytes.
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and 8), which varied from simple peg and socket arrangements to complex interdigitations.

Although occasional chondrocytes (supposed to be the products of a recent division) were seen lying adjacent to one another in normal controls, tight junctions and membrane folding were not seen.

DISCUSSION

The main abnormality noted in stm cartilage appears to concern the failure of chondrocytes to move apart after mitosis. In normal sibs of stm mice, chondrocytes were seen side by side only rarely; and, in these cases, no involvement of membranes was seen.

The presence of large numbers of adjacent cells suggests a burst of mitosis and the mitotic rate in stm cartilage is slightly elevated. Also, I found (Johnson, 1977) that stumpy femora and tibiae are growing at normal rates or slightly faster over the period covered by the present observations: an abrupt cessation of growth follows at around 16 days.

Somewhat similar chondrocytes were described by Fahmy, Lee & Johnson (1971) in rat cartilage exposed to testosterone. These cells showed a high mitotic rate with many closely apposed cells, well-developed secretory activity and areas of membrane contact between adjacent cells. Fahmy found that testosterone served to accelerate the processes of division, maturation, hypertrophy, and degeneration. This is in accordance with the well-documented effects of steroid hormones; in general, small doses accelerate bone growth, large doses accelerate growth to such an extent that premature closure of the epiphyses occurs. However, Fahmy et al. did not describe tight junctions nor intercalations of membrane, which appear to be unique to the stm phenotype. The increase in mitosis, coupled with the wide zone of hypertrophy (Fig. 2), suggests the possibility of a temporary hormone imbalance in growing stm mice. In this case, the presence of male sterility may assume significance.

Stumpy fits well into the emerging picture of chondrodystrophy, a series of short-legged phenotypes produced by anomalies in cartilage metabolism. The nature of these anomalies appears variable: at present, there seem to be almost as many of them as there are mutants described. Perhaps we should be asking ourselves not what are the biochemical steps involved in the production of abnormal cartilage (although this knowledge will surely prove to be of value in the study of normal cartilage metabolism) but rather why cartilage is so vulnerable.

Consider as an example the achondroplastic (ac) rabbit. Bargman et al. (1972) showed that the primary defect in these animals is a failure to generate ATP at the cytochrome oxidase region (site III) of the terminal respiratory chain during oxidative phosphorylation. This mitochondrial defect must be present in all cells of the homozygous abnormal animal, and was, in fact,
demonstrated in liver mitochondria. Yet the most striking feature of the ac/ac rabbit is its achondroplasia. Bargman et al. suggest that, in tissues such as cartilage where oxygen tension is normally low, oxidative energy formation may be rate-limiting for growth.

It seems likely that this will not prove to be the only example of a rate-limiting process in cartilage: perhaps the present wide spectra of biochemical bases for achondroplasia have this in common, that they all represent breakdowns in rate-limiting steps on one of the many metabolic pathways present in the relatively isolated chondrocyte.

REFERENCES


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