A Model for Ulnar Dysmelia

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The treatment of pregnant rats with the carbonic anhydrase inhibitor, acetazolamide, produced gross limb malformations primarily affecting the forepaw, but also producing variable ulnar dysmelia. Analysis of the cytoarchitecture of the ulnar dysmelic limbs showed the presence of cartilaginous and fibrocartilaginous connections between the ulnar and radial chondroepiphyses, with variable deformation of the radial chondroepiphysis by the tethering effect (although the growth plate, per se, did not appear affected at the stage of development studied). The extremely variable experimental appearances duplicated most of the variations seen in the human disease analogue, and suggest this drug-induced embryopathy may be useful as a model for the study of postaxial forelimb deformities in the postnatal phase in order to adequately assess the structural changes of disparate growth between radius and ulna due to the presence of the cellular continuity between the two distal chondroepiphyses.

Intercalary and transverse reduction deformities of major bones of the appendicular skeleton may occur spontaneously in other animals besides man. Radial and fibular hemimelias seem to have spontaneous appearance rates comparable to man [1]. Certain genetic strains of inbred laboratory animals may have reduction deformities on a sufficiently regular basis to allow study of prenatal and postnatal growth dynamics of the deformed limb [2–5]. Teratogenic agents may also be used to induce selected skeletal abnormalities in the developing fetus [6–14].

Ulnar dysmelia, a postaxial deformity, is an infrequently encountered, highly variable, reduction defect which presents significant clinical problems to the treating physician [15]. Three types of ulnar deformity are recognized on the basis of roentgenographic evaluation: (A) hypoplasia; (B) partial aplasia, with ossification of the proximal part of the ulna present at birth; and (C) total aplasia, with ossification not present at birth, although it may appear later during postnatal development. The roentgenographically "absent" segment of the ulna in types B and C may be a large fibrocartilaginous anlage attached distally into the distal radial epiphysis, the ulnar side of the carpus, or both. Postnatal ossification of this band is not uncommon, even though there may be no radiographic evidence of formation of the primary ossification center during the first few months of life. The presence of this relatively large fibrocartilaginous anlage is very much unlike the comparable situation of radial hemimelia, in which remnants (osseous or cartilaginous) of the missing radius are rarely present. Thus, part of the syndrome of ulnar dysmelia is a relative delay in development of maturation of the cartilage at a very early stage, preventing normal transformation of some or all of the enchondral anlage into the primary ossification

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center. Further, this anlage may exert a considerable tethering effect on the more distal remaining structures, causing ulnar deviation of the wrist and hand, and subluxation or dislocation of the radial head, in utero, as well as progression of these deformities after birth. One of the authors (John A. Ogden) has advocated resection of the distal end of the fibrocartilaginous anlage during the first to second year of life to prevent or minimize these angular growth deformities and joint displacements [15]. It is also suggested that if the one bone forearm operation is to be undertaken, it should be deferred until a later age, since complications may be less likely to occur than at the time that the anlage is resected. Such delay also allows the surgeon an opportunity to adequately assess further development of hand/eye motor coordination in order to devise the best type of reconstructive procedure. However, the role of the fibrocartilaginous, distal ulnar remnant in producing these changing pathologic patterns, such as progressive subluxation-dislocation of the radial humeral joint, and surgical extirpation of the band remains controversial [15,16].

Since this particular deformity rarely occurs spontaneously in animals, experimental study is difficult. Layton and Hallesy [6] described predominantly right sided, postaxial reduction and duplication deformities of the forelimb following acetazolamide administration in rats. Vickers [12], in a similar study emphasizing digital and carpal defects, showed five basic patterns of malformation-dislocation, reduction, supernumerary defects, syndactyly and synarthrosis. None of these authors studied the ulnar defects in detail, concentrating instead on deformities of the paw. The postaxial involvement prompted further investigation of this drug-induced embryopathy as a possible model for the clinical syndrome of ulnar dysmelia. In particular, it was hoped that the evaluation of a satisfactory model would allow a better understanding of the cellular pathology, particularly as related to the formation of the fibrocartilaginous remnant, and the interrelation of the cells between the distal radius, ulna and carpus. Comprehension of the cellular connections, and the integrated growth patterns, in normal and abnormal anatomic relationships of the distal radius and ulna can only lead to increased appreciation of chronological changes that might influence clinical outcome in patients affected by ulnar hemimelia.

This study describes the cytologic changes affecting the ulna and radius, both distally and proximally, in a drug-induced paraxial, longitudinal hemimelia (ulnar), with emphasis on the variability of the abnormal fibrous or cartilaginous connection between the distal radial and ulnar chondroepiphyses. A detailed review of the relationship of the animal model to the human dysmelic state, as well as the possible biochemical mechanisms of deformity follows the presentation of the animal model.

MATERIALS AND METHODS

Female Sprague-Dawley rats, drawn from a closed colony maintained by random breeding were given acetazolamide, a carbonic anhydrase inhibitor, in dosages of 500–600 mgm/kg by intraperitoneal injection 240, 252, 264, and 272 hours following the morning (arbitrarily defined as 6 hours) on which vaginal smears were positive for overnight mating. Fetuses were subsequently removed twelve hours prior to anticipated birth and immediately fixed in ten percent formalin. Limbs were embedded in paraffin for serial sectioning at ten microns. These skeletal preparations were stained with methylene blue. The initial survey involved approximately 500 dysmelic limbs. Postaxial (ulnar) involvement was found in slightly over twenty percent of these limbs, and usually comprised only mild hypoplasia of the ulna, along with primary paw defects. There were no specific external characteristics which allowed easy

identification of those animals with significant ulnar dysplasia, since the forepaw digital reductions were often similar whether the ulna was hypoplastic or absent. While not used for this particular study, recently developed roentgenographic techniques, such as the water-cooled RSI tube, allow magnification of small specimens which may prove useful for screening such animal series in the future. For the final study grouping sixty-two serially sectioned forelimbs felt most likely to exhibit ulnar deformities were specifically reviewed. Of these, six limbs exhibited major structural deformities, while the other fifty-six showed minor involvement of the ulna.

RESULTS

The statistical analyses of the deformities have been presented previously [12]. Most of the ulnar reductions were minor and involved the distal end of the bone. The shortened distal ulnar chondroepiphysis was either rounded or tapered, and more slender than normal (Fig. 1). The most frequently absent portion was the styloid process. These mild deformities conform to the Type 1, or hypoplastic ulna, category described for the lesion in humans [15], and comprised the bulk of the evident ulnar deformities. Since the more severe ulnar deficiencies represented less than one percent of the fetuses, the emphasis of the remainder of this section will be descriptive histopathology, rather than the incidence of each particular structural change. While conforming to basic patterns, each limb with severe ulnar involvement exhibited specific minor variations not found in the others.

The forepaws showed reduction deformities of the ulnar-associated digits. Synarthroses were common in the remanent phalanges and metacarpals (Fig. 2). The paws were deviated toward the absent or shortened ulna, although the carpus usually still

FIG. 1.   A. Mild hypoplasia of ulna (U) compared to radius (R), with loss of ulnar styloid process. Note the blunt, rounded appearance of the ulnar chondroepiphysis (15X).   B. Slightly more severely involved ulna, with long tapering appearance. A definite cellular continuity is evident (*arrow*), although no significant deformation of the radius was evident in the serial sections (10X). The distal ulnar physis is more proximal than normal.
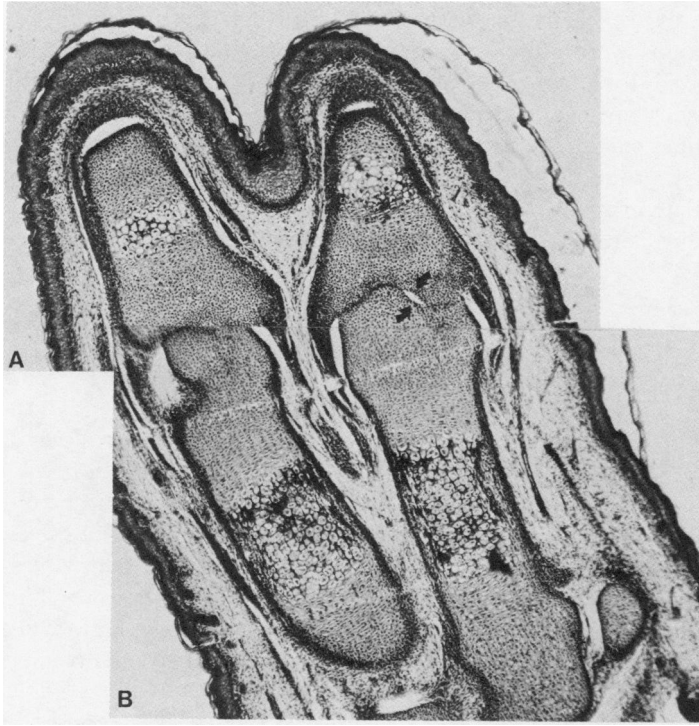
FIG. 2.   A. Distal phalanges showing early hypertrophic changes prior to vascular penetration and ossification. The interphalangeal joints have not formed, although abortive cavitation is present in one (*arrow*) (20X).   B. Proximal phalanges from same animal, showing ossification of the hypertrophic central region (20X). The proximal and distal phalanges are obviously at different stages of maturation, which undoubtedly makes certain regions more suceptible to teratogens than others.

articulated with the radius (Fig. 3). Sometimes a carpal bone was displaced proximally with the hypoplastic ulna (Fig. 4). The carpals were variably deficient and showed cartilaginous fusions (synchondroses) which undoubtedly would have mani-
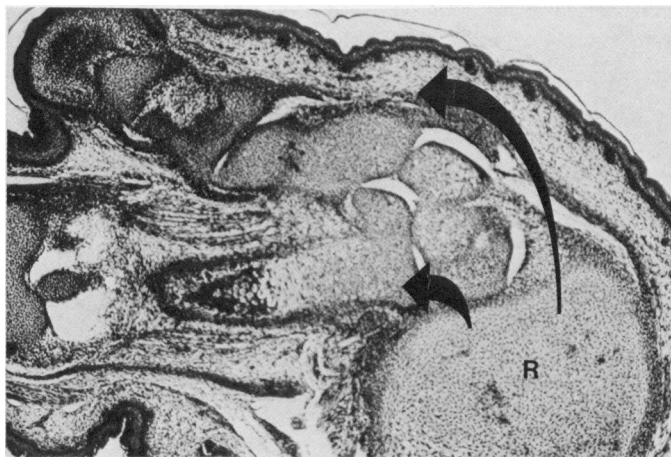

FIG. 3.   Ulnarward deviation of the remaining carpal to phalangeal elements (15X). The carpus is not dislocated from the distal radius.
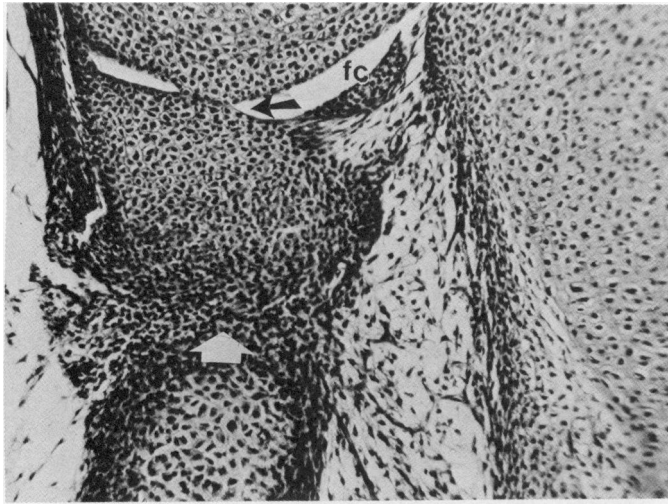
FIG. 4.   Severe hypoplasia of ulna with displacement of deformed carpal bone into region normally occupied by ulnar styloid. There is a large synchondrosis between the carpal bone and distal ulna (*white arrow*) and incomplete cavitation of the carpal/carpal joint (*black arrow*). An incomplete fibrocartilaginous bridge (FC) exists between the carpus and distal radial chondroepiphysis (20X).

fested as synostotic carpal fusions had the animals been allowed to reach skeletal maturation.

Most relevant to the use of this drug-induced embryopathy as a model of the human ulnar dysmelia was the finding of a deforming fibrocartilaginous band between the distal radius and ulna. Even when the ulna was only mildly hypoplastic, this cellular bridge appeared capable of causing some ulnarward deviation and deformity of the distal radial chondroepiphysis (Fig. 5). In other specimens, a larger synchondrosis was evident between the radius and ulna (Fig. 6). The larger cellular
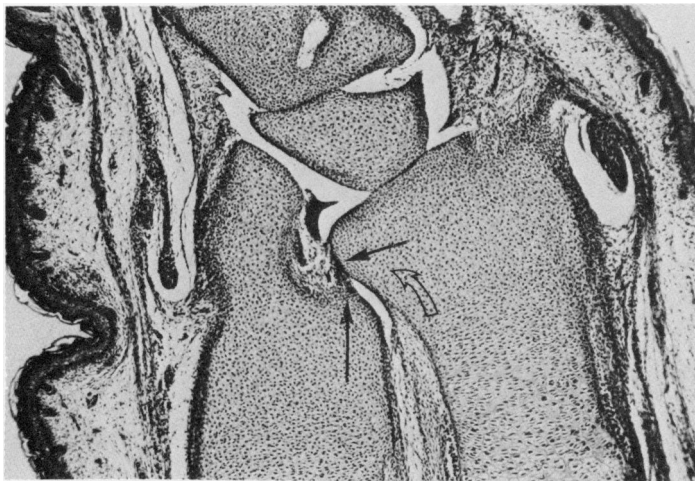

FIG. 5.   Mild ulnar hypoplasia (the ulnar styloid is still present) associated with a fibrocartilaginous band between the distal chondroepiphyses. The distal radius is beginning to show some mild deformation toward the ulna (15X).
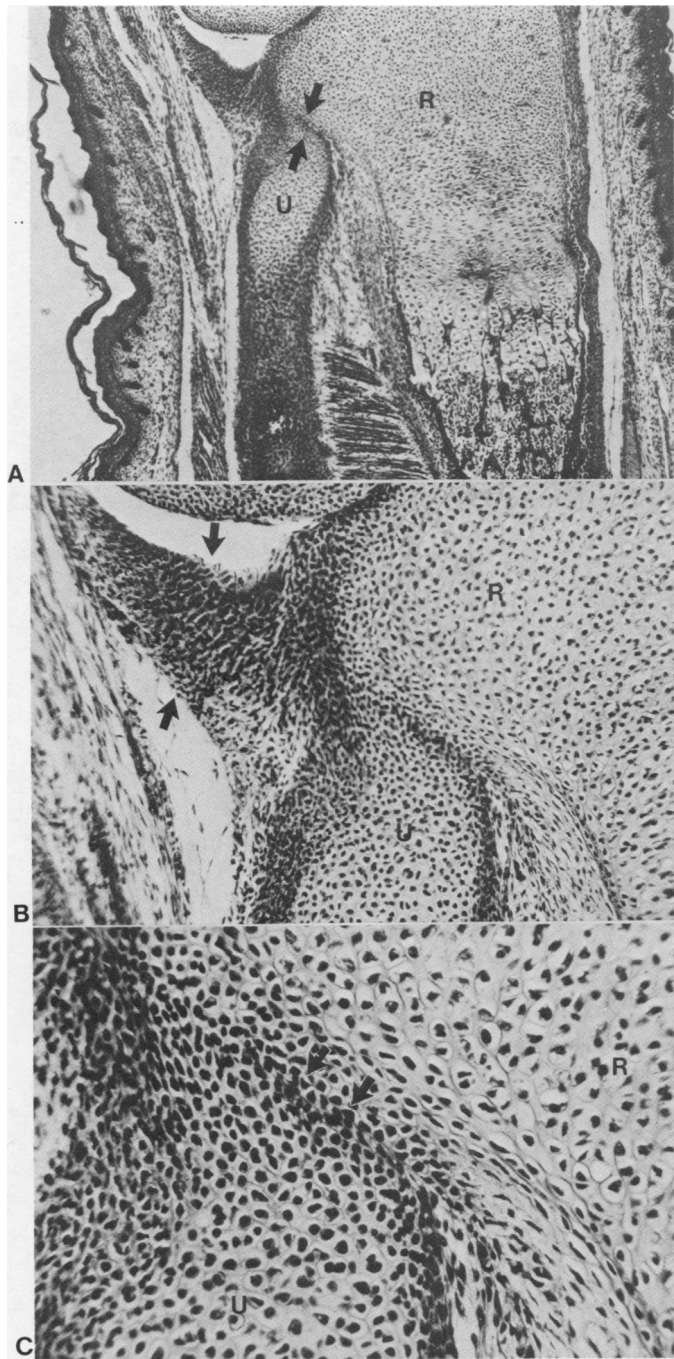
FIG. 6. Severely hypoplastic ulna with large cartilaginous communication between the distal epiphyses. The distal ulna did not have a recognizable growth plate distally. The distal radial chondroepiphysis is being deformed toward the tethering ulna. A malformed carpal bone is just above the ulna (15X). B. Higher magnification (30X) to show chondroepiphyseal fusion and rudimentary carpal bone (*arrow*) which is poorly defined hyaline cartilage. C. Higher magnification (60X) to show some compression of cells where interzone should have formed (*arrow*). The continuity of the hyaline cartilage cells is evident.
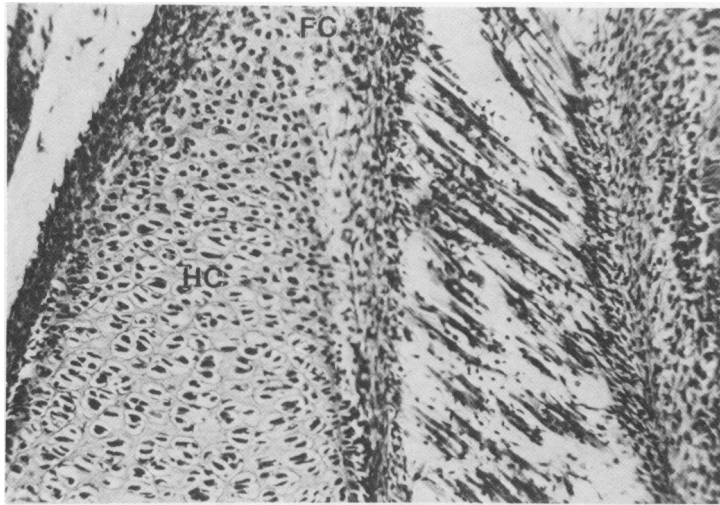
FIG. 7. Poorly formed distal ulnar chondroepiphysis showing some clone formation but complete loss of cell columnation. The hyaline cartilage (HC) is blending into fibrocartilage (FC) (30X).

continuity was also associated with deviation of the distal radial chondroepiphysis. Despite deformation of the hyaline cartilage component of the chondroepiphysis, the radial physis exhibited normal cytoarchitecture (cell column formation). The more severely affected the distal ulna, the more irregular the zone formation and cell column regularity. In some limbs, there was no demonstrable pattern to cell hypertrophy, and very little undifferentiated hyaline cartilage (Fig. 7). The diaphyseal bone sometimes blended into cartilage and fibrocartilage, with minimum evidence of a functioning growth plate.

Similar to the human dysmelia, the elbow was also affected. Comparable to Type 3 (complete ulnar absence), the radial head was dislocated (Fig. 8). When this happened, the proximal radius did not exhibit any of the characteristic contours, although a slight concavity of the radial head was evident. Irregularities of the proximal radial chondroepiphysis extended toward the distal humerus (Fig. 9). The distal humerus showed deformation of the trochlea when the ulna was completely absent, but milder changes when the ulna was hypoplastic.

The morphologic and histologic findings in this series demonstrated similar characteristics to the human lesion, particularly the capacity of cellular connections to cause structural deformation. However, the lesions tended to be either a hypoplastic or completely absent ulna, respectively analogous to Type 1 and Type 3 human lesions [15]. The partially present, late-ossifying Type 2 lesion was not seen. This may result from species differences in bone development. The rat forms very little osteon bone during skeletal maturation, and does not usually vascularize the chondroepiphyses with a cartilage canal system.

DISCUSSION

In the human ulnar dysmelia tends to be a unilateral, right-sided, male-predominant lesion, with the deficiency of the ulna ranging from a partial to complete postaxial longitudinal deficiency which may present with multiple, highly variable elbow, forearm, wrist and hand manifestations [15]. While the elbow may be functional, there is usually some limitation of motion, or the joint may be rigidly
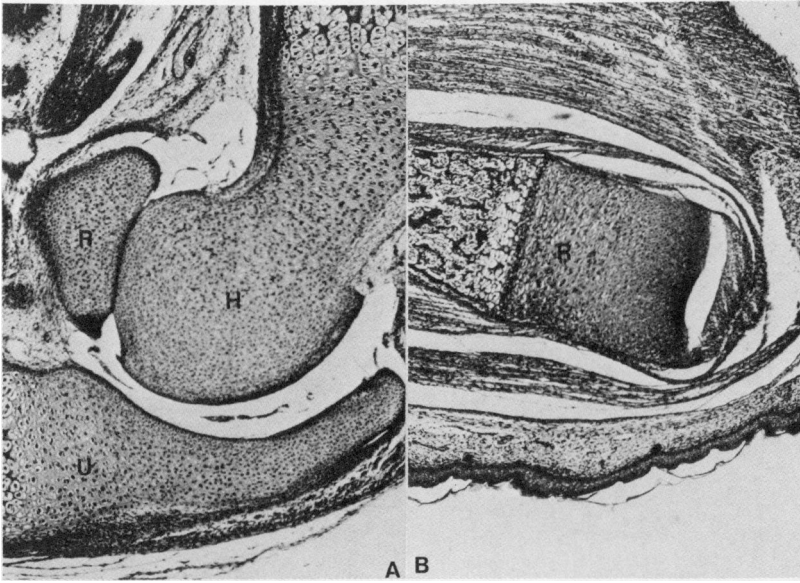
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FIG. 8. A. Minimally hypoplastic ulna showing normal radiohumeral joint with normal radial head contours (15X). B. Deformed, completely dislocated radial head in Type 3 (total ulnar absence). The radius has lost the curvilinear periphery (15X).

fixed, in either extension or flexion, by a synostosis and/or synchondrosis. The wrist and hand are usually deviated toward the dysmelic side, but the carpus rarely dislocates completely from the articular surface of the distal radius, in sharp contrast to radial hemimelia in which the carpus may completely dislocate off the remaining ulna.
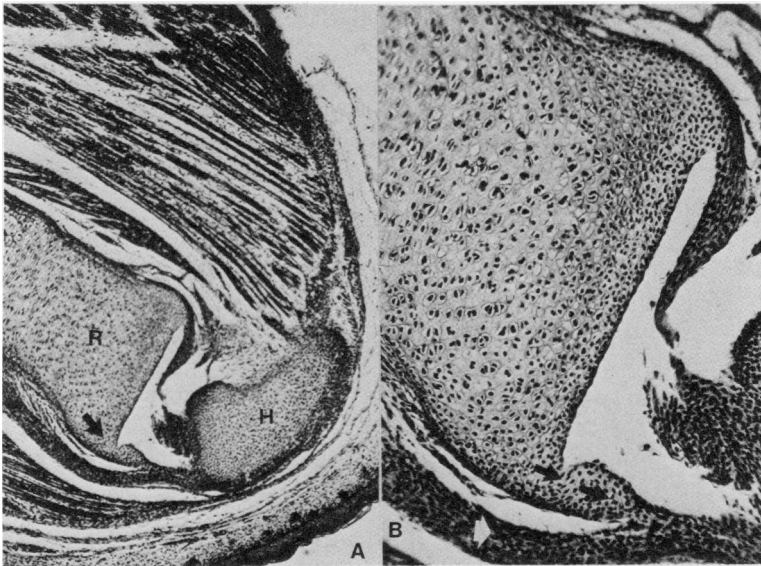
FIG. 9. A. Radial head associated with absent ulna. There is loss of the joint surface concavity and a cartilaginous extension toward the deformed distal ulna (*arrow*) (15X). B. Higher power (30X) showing cartilaginous extension (*black arrow*) which blends into a fibrous vestige (*white arrow*) of the absent ulna. This conjoined structure then attached to the distal humerus. The fibrous ulna could be traced to the mid-disphyseal level of the radius.

Absence of the carpal bones generally affects the ulnar side, with the pisiform being absent most frequently, followed by the hamate, triquetrum and capitate. If not absent, these bones may be synostotic, forming two or three large carpal masses. Metacarpal and phalangeal absence and deformity are highly variable. The fifth, or fourth and fifth metacarpals, and associated phalanges, tend to be absent most frequently. The majority of patients have a thumb. About twenty percent exhibit syndactyly. Monodactyly is rare.

Kummel [17], using elbow morphology, described a classification of ulnar deficiency: Type A—normal or near normal radiohumeral joint; Type B—radiohumeral synostosis; Type C—luxation of the radiohumeral joint. However, this classification scheme was based only on a single area of involvement, and failed to include the multiplicity of defects which are usually present, and which defy any systematic classification. This complex variability is depicted in Fig. 10.

In the majority of the human cases, the ulna was partially, rather than completely, absent. The least deformity was hypoplasia, with the entire bone, including proximal and distal epiphyses, being present and involved. The radius tended to be shorter than the unaffected side in both hypoplasia, partial and complete absence of the ulna. This variability was also observed in the experimental model. In many cases without initial roentgenographic evidence of an ulna, a primary ossification center formed sometime during the postnatal period, and often not until the child was several years old. This type of delayed primary (diaphyseal) ossification has been described by Rhodes and Elmer in brachypod (bp^H) mice [18]. They found that the fibula was severely retarded in its development, retaining a chondrogenic state until two weeks after birth, when the primary ossification center finally formed. The delay in transition from cartilage to bone was not the result of abnormal collagen or chondroitin sulfate molecules being synthesized, but rather an uncoordinated synthesis and degradation of these products. Associated with this metabolic failure (both quantitative and temporal) cellular regionalization into discrete zones following chondrification of the mesenchymal model did not occur. Such zone formation is

THE ULNAR DEFICIENT EXTREMITY
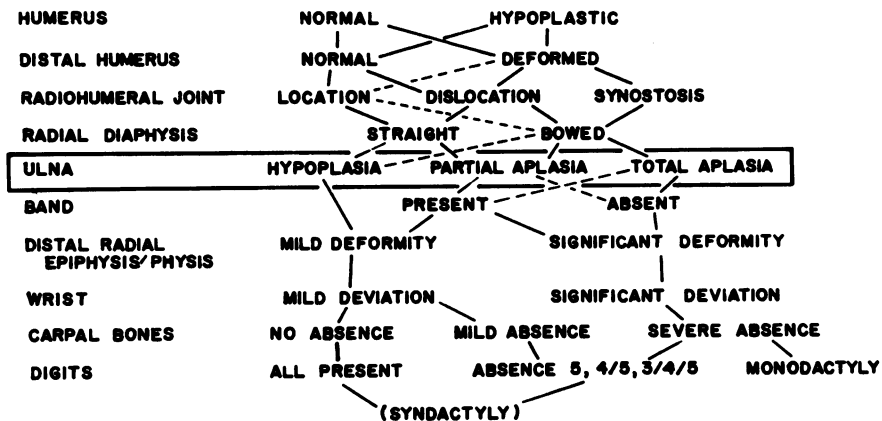
FIG. 10. Schematic depiction of anatomic variations possible in the human ulnar dysmelic extremity. Hypoplasia is defined as Type 1, partial aplasia as Type 2, and total aplasia as Type 3. The deformity is extremely variable and complex in the human, and is equally so in the animal model, although partial aplasia (Type 2) comparable to the human was not observed in the rats. The complete lines represent commonly encountered (clinically and experimentally) interrelationships, while the broken lines indicate infrequent concomitant incidence.

related to synthesis and secretion of the organic matrix of hyaline cartilage [19]. Irregular zone formation was noted in many of the affected rat ulnas, no matter what the severity of involvement.

In describing the pathomechanics of both partial and complete dysmelia of major long bones, particularly the fibula, some authors have emphasized the role of a distal, fibrocartilaginous remnant which may produce progressive structural deformity by a tethering effect [20–23]. Earlier pathologic descriptions of ulnar dysmelia have demonstrated the presence of a fibrous or fibrocartilaginous band extending from the ossified ulna to the distal radius or carpus [24,25]. More recent surgical observations have extended our understanding of this fibrocartilaginous anlage [15,26]. In our correlated clinical study [15] the progressive radiohumeral dislocation, radial bowing and "ulnar" deviation of the carpus and hand, as well as progressive ossification in partial absence (Type 2), or even de novo ossification in presumed complete absence (Type 3) of the ulna, strongly support the concept of a significant fibrocartilaginous anlage which becomes a prime factor in initial and subsequent structural deformation. Several of the experimental animals in this series demonstrated a definite correlation between fibrocartilaginous connections and structural abnormality. Even with relatively normal soft tissue interrelationships from the distal ulna to the distal radius and wrist, disproportionate ulna shortening (hypoplasia) may also cause significant structural changes in the radius and wrist.

Hovelacque [27] observed early developmental stages of the limb bud in tibial hemimelic mice and found a fibrous tract which was connected to the fibula by the interosseous membrane. In some of the involved fetuses cartilaginous nodules were observed in the proximal region and were in direct continuity with the more distal fibrous band. Similar genetic strains of mice with partial tibial hemimelia (and associated fibrocartilaginous continuations) have been associated with bowing of the fibula or fibular dislocation at the knee [2–5]. Complete replacement of the tibia by a fibrocartilaginous anlage connecting distal femoral epiphysis and talus, without knee and ankle joints, and cephalad dislocation of the fibula at the knee, has been observed in the human [23]. Delays in the normal developmental sequences of chondrification and ossification may significantly affect the adjacent bone when the tibia and fibula or radius and ulna are involved. The normal connections between the two bones may be replaced by cartilaginous, fibrocartilaginous or fibrous bridges, with the connection being the major factor causing deformation of the longer, more normal bone, due to disparate longitudinal growth rates.

While the evolutionary characteristics of the ulna, and particularly the humero-ulnar articulation, have been studied in detail in vertebrates [28], congenital absence of the ulna has been reported only sporadically in various animals such as the domestic pig [24,29]. It appears to be as infrequent a spontaneous lesion in other animals as it is in man. Warkany [30] produced absence of the ulna with conjoint radiohumeral synostosis by maternal irradiation in the rat. However, postaxial (ulnar) deficiencies occur most frequently in the fetuses of animals treated with a few specific teratogens, especially the carbonic anhydrase inhibitor, acetazolamide, and its chemical analogues. Layton and Hallesy [6] found the usual ulnar abnormality was a combination of absence of the fourth and fifth digits, shortening of the ulna, (Type 1) bowing of the radius, and a normal radiohumeral joint. At the extreme, one animal had complete absence of the ulna (Type 3), radiohumeral synostosis, and a monodigital paw. The defects primarily involved the right side, but when there was bilateral dysmelia, the defect was usually more severe on the right. However, subsequent work in another animal (guinea pig) showed approximately equal

involvement of right and left sides [9]. The female rat embryo appears to be more sensitive to acetazolamide toxicity than the male [7], which contrasts with the sex-incidence in the human. Other studies have corroborated postaxial upper, and less frequently lower, limb involvement in acetazolamide toxicity [11,13]. Singh and Sanyal [14] have found similar abnormalities using the cystostatic agent, cyclophosphamide. However, these studies centered primarily on the carpal/digital defects in Alizarin red stained gross specimens, with virtually no histological observations of the ulnar defects, which has been a major emphasis of the current study.

The basis for the asymmetric response is not certain, but it can be overcome in large measure by increasing drug dosage [12]. It appears unlikely that the differing responsiveness between the sides results from asynchronous forepaw development, since there is no perceptible difference in gross form during the early limb bud developmental stages suggesting that the evolution of one side precedes the other. However, at the cellular level there may be subtle, asynchronous cytoarchitectural (zone transformation) or metabolic changes which introduce susceptibility of the mesenchymal or cartilaginous anlage to a teratogen. Further, there may also be a subtle difference in the diffusion gradient within the vascular supply to the two sides, primarily due to aortic arch development.

The biochemical mechanism of action of acetazolamide is unclear, especially since carbonic anhydrase activity has not been found in the limb buds of rat fetuses at the time of maximum sensitivity to acetazolamide [31]. Modification of energy exchange reactions have been reported as mechanisms capable of causing limb deformity [8,32, 33]. The administration of 6-nicotinamide to developing chicken embryos caused variable micromelia. Histologic and electronmicroscopic examination suggested the limb malformations were probably induced by an inhibition of matrix synthesis by chondrogenic cells, as well as by actual cell death of core cells within the chondrogenic rudiment. Cell necrosis within some of the regions was often transitory, with damage to the rudiment being incompletely repaired by synthesis of matrix in cells near the perichondrium. It seems likely that this mechanism might be significant in partial defects (especially intercalary defects) of major longitudinal bones. However, these reactions do not appear specifically related to carbonic anhydrase chemistry, suggesting one or more intervening chemicals may be a factor, with carbonic anhydrase metabolism, probably acting elsewhere in the embryo, being related to the other chemical(s), which subsequently act on the end-organ (limb bud).

Maternal electrolyte imbalances due to the diuretic effect have been effectively ruled out as a primary causal factor [9,34–36]. Other carbonic anhydrase inhibitory compounds with diuretic effects, such as ethoxzolamide and dichlorophenamide, may also cause postaxial forelimb deformities [13,37]. The typical mammalian response to any of these compounds is respiratory and metabolic acidosis and depletion of total body potassium, with some sodium loss. However, Meng and Kroneberg [38] showed that carbonic anhydrase inhibitors also elevate blood glucose. Thus, the major effect of acetazolamide may be an alteration of carbohydrate metabolism, rather than electrolyte imbalance, sufficient to delay or prevent the chondrification and/or ossification processes within the endoskeletal anlagen.

Carbohydrate metabolism following potassium depletion is associated with increased plasma concentration of insulin [39]. The release of insulin itself, particularly in excess of normal physiologic levels, may have a significant role in the production of the teratogenic effect. Landauer et al. [40] and Runner [41] demonstrated that insulin also produces a variety of skeletal abnormalities in chickens and mice, contingent upon dose and time of administration. Zwilling and Debell [42], using

insulin-induced micromelia in chickens, suggested the major defect is in chondrification; limbs showed marked degeneration of chondrogenic tissue in the epiphyseal centra of long bones, as well as disturbances of matrix formation and cartilage hypertrophy in the diaphysis.

Another possible site of action of a teratogen is the early limb bud. The ectomesenchymal bud first differentiates by epidermal thickening to form the apical ectodermal ridge (AER) [43]. Zwilling and Ames [44] have shown that the AER subsequently stimulates outgrowth and differentiation of underlying mesoderm. Removal of the AER usually leads to formation of a limb bud lacking those distal structures for which mesodermal anlagen have not been induced at the time of surgical extirpation. Zwilling also hypothesized that there is an interplay between AER and differentiating mesoderm, with the latter supplying a "maintenance factor" for the former. The AER may be sensitive to some teratogens before any skeletal anlagen form, or it may be affected by lack of "maintenance factor" from a deficient or necrotic anlage. AER disruption may be a major factor eventuating in longitudinal limb deficiencies.

Radiohumeral fusion, carpal fusions, and digital fusions are relatively frequent in paraxial hemimelias. Such fusions have been noted in both embryonic and fetal periods [45]. The mesodermal skeleton induced by the AER is initially a continuous structure which eventually separates into distinct elements through a combination of chondrification and muscle activity. Paralyzed avian embryos do not form joints, and bridge the presumptive joint cavity by either fibrocartilage or hyaline cartilage [46–48].

Pringle [49], in discussing the "treasure house of nature," stressed the importance of a thorough search of the enormous variety of biological materials when looking for good experimental material that might answer a specific biological problem. While ascertaining such models may arise only by accident, as the initial discovery of this drug-induced dysmelia, a deliberate search for a suitable species is needed whenever studying any anatomical/physiological combinant variation. The August Krogh principle states that "for many problems there is an animal on which it can be most conveniently studied . . . but . . . most of them are unknown . . . and we must apply to the zoologists to find them and lay our hands upon them" [50]. Even relatively minor modifications of standard situations may present great advantages for studying selective phenomena without affecting basic principles.

However, animal material chosen for study, in which normal physiology and anatomy have not been altered, should be considered "examples" of disease, rather than "models." This would best describe animals exhibiting normal spontaneous anatomic mutations such as those associated with a particular longitudinal or intercalary hemimelia. In contrast a "model" is associated with a manipulated situation that approaches the real thing, but is not necessarily the real thing itself. Alloxan-induced diabetes is such a situation. Thus "models" are analogous to, but not identical with, the disease state concerning which information data is sought.

This drug-induced dysmelia reasonably duplicates the disease state found in human ulnar hemimelia, and affords a potential model to study, in detail, the pathophysiology of development of the disorder, as well as the changing anatomical patterns associated with time-dependent growth stages. Since it is an induced, or manipulated, situation it fulfills the aforementioned criteria for a "model."

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