*Archives of Disease in Childhood*, 1976, **51**, 887.

# Altered connective tissue in children with congenital dislocation of the hip

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**Fredensborg, N., and Udén, A. (1976).** *Archives of Disease in Childhood*, **51**, 887. **Altered connective tissue in children with congenital dislocation of the hip.** The umbilical cord was employed as a source of collagen in 10 children with congenital dislocation of hip. The amount of collagen and its solubility were measured in slices of the cords and in the umbilical veins and compared with the values in normal subjects. Both the amount of collagen and its solubility were decreased in children with congenital dislocation of the hip.

The laxity of connective tissue structures in children with congenital dislocation of the hip (CDH) is well known. Andrén (1960) showed pelvic instability and several investigators (Carter and Wilkinson, 1964; Wynne-Davies, 1970) have described general joint laxity in these children.

Hormonal influence of oestrogens as a factor in the aetiology of CDH was proposed by Andrén (1960) and Andrén and Borglin (1960, 1961a, b). Oestrogen-induced changes in the connective tissue of joint capsule and ligaments might be responsible for the increased laxity. Collagen is the major constituent of connective tissue and is responsible for the tensile strength of ligaments and joint capsule (Grant and Prockop, 1972). We have investigated the amount of collagen and the solubility of collagen in weak organic acid in order to assess the degree of cross-linking (Jackson and Bentley, 1960), since cross-linking is related to the physical properties of the connective tissue in structures such as joint ligaments. As biopsy material from ligament and joint capsule was unavailable the umbilical cord was used.

## Material and methods

Umbilical cords were collected from about 5000 consecutive newborns, and stored at −30°C until examined. Within 24 hours after delivery the infants were examined by a paediatrician for clinical signs of CDH. In 39 children (0·8%) CDH was diagnosed and confirmed by radiological examination. Of these the umbilical cords from the 10 (0·2%) with completely dislocatable hips, 7 girls and 3 boys, were selected and examined.

From the umbilical cord 5 or 6 slices 3–4 mm thick were taken for examination. Blood was rinsed off in distilled water at a temperature of 0°C. The slices were frozen in liquid nitrogen and crushed in a steel mortar. The crushed tissue was placed in a glass container with 4 times its wet weight of 0·5 mol/l acetic acid. Extraction was continued for 24 hours at 4°C with constant shaking. Separation was carried out in a centrifuge at 20 000 *g* for 1 hour and at a temperature of 4°C. The supernatant was decanted. An aliquot of each supernatant was dialysed against distilled water for 24 hours in order to separate the smaller polypeptides that are split off when the tissue is crushed in liquid nitrogen. The dialysis sack will allow peptides with a molecular weight $<12\,000$ to escape. Using a modification of the method described by Stegeman (Pikkarainen, 1968; Woessner, 1961) the amount of hydroxyproline was determined in the two fractions of the supernatant and in the sediment. The amount of collagen was calculated as hydroxyproline $\times 7{\cdot}1$.

In order to study a more homogeneous tissue we also examined the veins from the umbilical cords after careful dissection, as described above. Umbilical cords from 10 normal infants served as controls.

## Results

Large variations were found in both groups. The variation in collagen content was less in the veins than in the cord cross-sections (Table). The collagen content was less in CDH children when compared with controls, significantly so only in the vein samples. Extractable collagen was decreased in CDH children. The values of the nondialysed fraction differed significantly only in the cord

Received 1 March 1976.



Arch Dis Child: first published as 10.1136/adc.51.11.887 on 1 November 1976. Downloaded from http://adc.bmj.com/ on January 21, 2022 by guest. Protected by copyright.

TABLE

*Amount and solubility of collagen in umbilical cords of 10 CDH children and 10 controls (mean ± SD)*

| | Total collagen (mg/g wet weight) | Extractable collagen (mg/g wet weight) | |
|---|---|---|---|
| | | Nondialysed | Dialysed |
| *Cross-section of umbilical cord* | | | |
| CDH | 30·84±7·48 | 0·198±0·057 | 0·158±0·081 |
| Control | 38·88±11·34 | 0·275±0·041 | 0·197±0·022 |
| | 0·1>P>0·05 | 0·005>P>0·001 | P>0·1 |
| *Vein of umbilical cord* | | | |
| CDH | 25·30±3·50 | 0·241±0·075 | 0·124±0·056 |
| Control | 28·75±3·46 | 0·252±0·060 | 0·183±0·048 |
| | 0·05>P>0·01 | P>0·1 | 0·05>P>0·01 |

cross-sections. The dialysed collagen differed between the two sets of children both in the vein samples and in the cord sections. In the latter there was a significant difference in standard deviations ($P<0·001$).

## Discussion

The tensile properties of connective tissue depend on collagen. The collagen content of connective tissue is decreased after administration of oestrogens (Fischer, 1972; Henneman, 1968). Instability of the hip in animals can be provoked by oestrogen (Gustafsson, 1968, 1975; Gustafsson and Beling, 1969; Månsson and Norberg, 1961; Zaffaroni 1958). During pregnancy the fetus is exposed to high concentrations of oestrogens. An increased frequency of inguinal hernia in girls with CDH was reported by Fredensborg (1976), and Wagh *et al.* (1974) found a decreased amount of collagen in the anterior rectus sheath in patients with direct inguinal hernia.

The results of the present investigation have shown that the collagen content of connective tissue of children with CDH is decreased. This may explain the increased laxity of their joints and the instability of their hips. A possible explanation might be that the water content differed in samples from CDH children and controls, but when we measured the water content in another sample of veins of umbilical cords from CDH and control children, there was no difference. The difference in solubility indicates a difference in quality. There are, so far, 4 types of collagen. Type I is predominant in skin, tendons, and bones. Type III is the typical constituent of arterial walls and is the dominant fetal type of collagen. Fetal collagen is less soluble than adult collagen (Miller, 1973). The findings of the present study could be explained either by a different proportion of type I and type III collagen, a decreased synthesis of collagen, or a difference in cross-linking in children with CDH. Our findings support the theory that CDH is a consequence of changes in the connective tissue supposed to maintain the stability of the joint rather than of changes in the bony structures.

Financial support was received from the Swedish Medical Research Council (project no. B75.17X-2737-07C) and the Herman Järnhardt Foundation.

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