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Collagen Type III predominance in newborns with congenital dislocation of the hip

The collagen content and the relative distribution of collagen Types I and III were measured in umbilical cords from newborns with congenital dislocation of the hip (CDH) and healthy controls. The total content of collagen per mg dry tissue tended to be reduced. In newborns with CDH the collagen III/I ratio was higher than in the controls. If generalized, these alterations in collagen metabolism may explain the joint hypermobility seen in CDH. It is suggested that the redistribution of collagen Types III and I during normal fetal development and early infancy may be delayed in CDH.

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Several reports indicate that joint hypermobility is a general feature in children with congenital dislocation of the hip (CDH) (Andrén 1960, Carter & Wilkinson 1964, Wynne-Davies 1970, Felländer et al. 1970). Alterations in the connective tissue constituents, in particular collagen, of joint capsule and ligaments, have been claimed; but conclusive evidence is lacking. Fredensborg & Udén (1976) reported that the content of collagen and its acid solubility was decreased in the umbilical cord of newborns with CDH. Further, Skirving et al. (1984) observed that the relative distribution of collagen types was altered in the joint capsule of CDH children.

We report the collagen content and the relative amount of collagen Types I and III in the umbilical cords of newborns with CDH.

Material and methods

Umbilical cords were collected from newborns (Fredensborg & Udén 1976) and stored at −30°C until analyzed. Within 24 hours after delivery, all infants were examined for clinical signs of CDH. From 5 newborns with CDH, diagnosed clinically and confirmed radiographically, umbilical cords were selected for biochemical studies. Umbilical cords from 6 normal newborns were used as controls.

Total collagen. The hydroxyproline content was used as a measure of the total collagen content (Neuman & Logan 1950). Following rinsing in distilled water at 0°C, tissue samples of 8 mg dry-tissue weight were hydrolyzed in sealed tubes for 18 hours in 4 ml 6 M HCl at 118°C, and subsequently evaporated to dryness in vacuo at 60°C. After redissolution in water, the hydroxyproline content was determined using Ehrlich's aldehyde (Stegemann & Stalder 1967).

Collagen extraction and purification. Homogenized samples of umbilical cords weighing about 100 mg dry weight were extracted three times by horizontal shaking overnight at 4°C in 5 ml 0.5 M acetic acid, pH 3.4. Following each extraction the soluble extract, containing acid-soluble collagen (ASC), was isolated by centrifugation at 45,000, 4°C for 30 min in a Sorvall refrigerated centrifuge. The extracts were passed through Frisenette[R] filters (Frisenette Ltd., Denmark) and pooled. Bacterial growth was prevented by the addition of one drop of octanol to each extract. Aliquots were hydrolyzed in equal volumes of 12 M HCl, and the hydroxyproline content in ASC was determined as described above. Pepsin-soluble collagen (PSC) was extracted twice by limited pepsin digestion of the non-ASC sediment according to Sykes et al. (1976), followed by hydroxyproline determination as described.

Collagen Type III/I ratio. Pepsin-soluble collagen was subsequently purified from extracts by repeated salt precipitations (Heikkinen 1968) followed by dialysis against 0.2 M disodium phosphate and demineralized water. The relative proportion of Type III and I

Table 1. Collagen and protein content and collagen Type III/I ratio in umbilical cords of children with CDH and controls. Values are medians (ranges)

Group	Total protein[a] (mg/mg DW)	Total collagen[b] (μg/mg DW)	PSC[c] in per cent of total collagen	Collagen Type III/I ratio in PSC
CDH n=5	1.04 (1.00–1.14)	49.1 (32.5–64.0)	41.4 (32.2–62.8)	1.42[d] (0.81–1.58)
Controls n=6	1.06 (0.94–1.21)	53.5 (32.9–65.2)	41.7 (30.8–58.3)	1.06 (0.71–1.28)

[a]Total protein estimated as mg α-amino-nitrogen per mg dry tissue weight (DW).
[b]Total collagen estimated as μg hydroxyproline per mg.
[c]Pepsin soluble collagen.
[d]Higher than controls ($P = 0.04$).

collagen in pepsin-soluble collagen was measured using SDS polyacrylamide gel electrophoresis with delayed 2-mercaptoethanol reduction (Sykles et al. 1976). The relative distribution was determined densitometrically at 620 nm using a Chromoscan 200 (Joyce Loebl Co., UK) as the ratio between the alpha 1 (III) and the alpha 1 (I) + alpha 2 chains following staining with Coomassie Brilliant Blue in 20 per cent trichloroacetic acid.

Total tissue protein. The protein content (collagen and noncollagen) was measured as the alpha- amino-nitrogen content on the same tissue hydrolysates as employed for the hydroxyproline analysis, using monosodium glutamate as the standard (Moore & Stein 1948).

Statistics. Differences between groups were tested by means of the Mann-Whitney U test. Differences were considered significant provided $P < 0.05$.

Results

The content of total tissue protein did not differ between the groups (Table 1). The amount of collagen, as estimated by the hydroxyproline content in umbilical cords, was not diminished in children with CDH. Acid-soluble collagen was in both groups less than 1 per cent of the total collagen (values not listed), whereas pepsin solubilized about 41 per cent of the collagen in children with CDH and in control subjects. However, in the pepsin-soluble collagen the proportion of Type III to Type I collagen was augmented in umbilical cords from newborns with CDH compared with controls ($P = 0.04$). The SDS polyacrylamide gel electrophoresis pattern of PSC in a selected patient and control subject is shown in Figure 1.

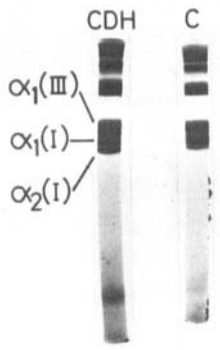

Figure 1. SDS-polyacrylamide gel electrophoresis patterns of PSC in a selected patient with CDH and a control subject (C). α_1 (I) and α_2 (I) chains of collagen Type I, and α_1 (III) of collagen Type III are indicated.

Discussion

Collagen is the matrix component mainly responsible for the extensibility and the tensile strength of various connective tissues (Oxlund & Andreassen 1980). As joint hypermobility is a general feature in newborns with CDH, systemic alterations in collagen metabolism might be of pathogenetic importance in this disease.

The reduction in collagen concentration in umbilical cords from newborns with CDH reported by Fredensborg & Udén (1976) was not conclusively confirmed in the present study. Collagen Type III, characteristic of fetal connective tissues, is not readily extracted by neutral salt or acid solvents, but can be effectively solubilized during limited proteolysis with an enzyme such as pepsin (Miller 1976). The low amount of acid-soluble collagen may thus suggest a high proportion of collagen Type III, with a high content of stable dehydro-hydroxylysinohydroxynorleucine cross-links, characteristic of fetal collagen (Pinnell 1978). In pepsin-soluble collagen, generally considered to contain the two collagen types in the same

proportion as in the native tissue (Junker 1985), the collagen Type III/I ratio was increased in newborns with CDH. This cannot be explained by a difference in pepsin solubility, as the proportion of this collagen fraction was equal in the two groups. Apparently, our result contrasts the finding by Skirving et al. (1984), who reported that the collagen Type III/I ratio of the hip joint capsule was diminished in CDH children aged 1–4 years. However, the distribution of collagen types during the development of scar and granulation tissue has a biphasic course, Type III collagen being predominant during the early stage of proliferation, whereas Type I prevails in the late stage of fibrosis (Miller 1976). Thus, the results of Skirving et al. (1984) most likely reflect a late stage of fibrosis and do not offer information as regards primary alterations in collagen metabolism in CDH (Ippolito et al. 1980).

As it is generally agreed that the acid-insoluble collagen pool determines the mechanical strength of the tissue (Vogel 1975), our results may indicate that the relative predominance of Type III collagen, if systemic, is of pathogenetic significance as to joint laxity in CDH. Imbalance of the synthesis or the secretion of collagen Types I and III have been reported in osteogenesis imperfecta and Ehlers Danlos syndrome Type IV (Uitto & Lichtenstein 1976).

The predominance of Type III collagen in fetal skin is dramatically reduced during normal fetal development and early infancy (Epstein 1974), after which the collagen Type III/I ratio remains constant at approximately 0.2 throughout life. As Type I collagen contains 25 per cent less hydroxyproline than Type III, our findings may be explained by a relative predominance of Type III collagen, and a simultaneous, absolute deficiency of Type I, characteristic of the immature, fetal collagen composition (Ebstein 1974). On the basis of our results, we suggest that this redistribution of collagens might be delayed in children with CDH. Such a concept is in agreement with the clinical experience that joint hypermobility subsides spontaneously during childhood, and dislocation of the hip is readily reduced by splinting without subsequent recurrence.

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