J. Inher. Metab. Dis. 7 (1984) 27–31

Congenital Dislocation of the Hip: A Possible Inborn Error of Collagen Metabolism

A. P. SKIRVING*, T. J. SIMS and A. J. BAILEY
Orthopaedic Hospital, Winford, Avon and Agricultural Research Council, Langford, Bristol, BS18 7DY, UK

Significant changes in the type of collagen, the fibril diameter and the nature of the crosslinks have been demonstrated in the joint capsules of subjects with congenital displacement of the hip (CDH). These changes are probably tissue specific since no detectable change could be observed in the skin of these subjects compared to controls. These preliminary biochemical studies clearly demonstrate that CDH involves an error in collagen metabolism.

Hip joint laxity is a characteristic feature of the congenital dislocation of the hip (CDH). The hip joint capsule has generally been found to be thicker in CDH patients but only a few histological studies have been carried out. Ippolito *et al.* (1980) reported that a higher proportion of the collagen fibrils in the CDH joint capsule were smaller than in age-matched controls, although the fibre bundles were found to be larger. Elastic fibres were also examined and found to be of uneven size and irregularly distributed.

The mechanical properties of most human tissues are primarily dependent on the strength of the collagen fibre. Defects in the collagen at the molecular level have been shown to dramatically affect these properties of the tissue. These defects can be due to a deficiency in an enzyme involved in one of the many post-translational modifications of collagen and in the gene expression of the genetically different types of collagen.

As far as we are aware, no biochemical studies have been previously carried out on collagen in CDH patients. In this preliminary study we have examined the joint capsule for differences in the genetic types of collagen present, the nature of the stabilizing crosslinks and changes in the diameter of collagen fibrils. Differences from normal hip joint capsule collagen have been found with no such change in skin from CDH patients.

MATERIALS AND METHODS

Biopsy samples of hip joint capsule were obtained at surgery from patients with CDH within the age range 1 to 4 years. Age- and site-matched controls were obtained from cadavers with no previous history of joint problems. Skin samples were also obtained both from CDH patients and age- and site-matched controls. The samples were sealed in plastic containers and kept at −20° C until required for analysis.

Correspondence to: Dr A. J. Bailey, ARC Meat Research Institute, Langford, Bristol BS18 7DY
* Present address: Dept of Surgery, Royal Perth Hospital, Perth, Western Australia 6001

Portions of hip joint capsule and skin samples were chopped finely, homogenized and washed in several changes of physiological saline. They were then defatted in 2 : 1 (v/v) chloroform/methanol mixture, dried and stored at −20° C while awaiting analysis.

Cyanogen bromide digestion of tissue samples

Portions of the defatted tissue were weighed, suspended in 70% formic acid and digested with an equal weight of cyanogen bromide at 30° C for 4 h. Excess cyanogen bromide was removed by rotary evaporation and the samples re-dissolved in distilled water and freeze-dried. The digests were then analysed by SDS polyacrylamide gel electrophoresis.

SDS Polyacrylamide gel electrophoresis and determination of the type III/I collagen ratio

Electrophoresis was carried out using the procedure described by Laemmli (1970). CNBr peptides were dissolved in 2% (w/v) SDS and resolved on a 10% polyacrylamide slab gel in Tris/HCl buffer (pH 8.8) with a 3% stacking gel (pH 6.8). Gels were stained with Coomassie Brilliant Blue R250 and destained in a methanol–acetic acid–water mixture.

The gels were scanned using a Chromascan densitometer (Joyce Loebl U.K.) and the relative proportion of Types I and III collagen were determined by measurement of the areas under the peaks for the two peptides αI CB8 and αIII CB6 (Figure 1) according to the method of Light (1982).

Reduction and identification of covalent intermolecular crosslinks

Portions (100 mg) of the washed, defatted samples were weighed and homogenized in cold (4° C) physiological saline. A stock solution of tritiated sodium borohydride (NaB^3H_4) was prepared in cold physiological saline and an aliquot of this added immediately to each of the suspended samples (1 : 30 borohydride/sample, w/w). After 2 h the reaction was terminated by addition of glacial acetic acid to pH 4.0. The samples were then dialysed, freeze-dried, weighed and hydrolysed in 6*N* HCl for 24 h.

Journal of Inherited Metabolic Disease. ISSN 0141-8955.

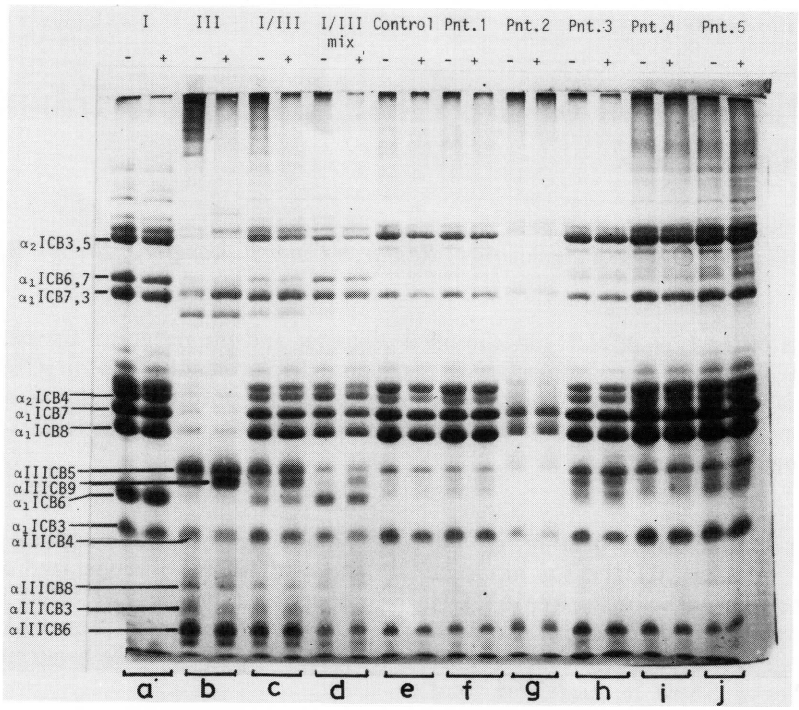

Figure 1 SDS polyacrylamide gel electrophoresis patterns of cyanogen bromide peptides from normal and CDH hip joint capsules. All samples were electrophoresed prior to (−) and after (+) reduction with 2-mercaptoethanol. (a) Human type I collagen standard; (b) human type III collagen standard; (c) and (d) mixtures of types I and III collagens; (e) control hip joint capsule; (f)–(j) hip joint capsules from CDH patients

Identification of the crosslinks was achieved by ion-exchange chromatography as described by Robins *et al.* (1973). Essentially, the hydrolysate was analysed using a two-column ion-exchange system. The hydrolysate was applied to a Zeolit 225 ion exchange column and developed with volatile pyridine-formate buffers (Figure 2).

The radioactive fractions were collected and re-chromatographed on a Jeol 6AH amino acid analyser using citrate buffers. The elution positions on both types of column were compared with standard samples of the authentic crosslinks.

Determination of collagen content

The total collagen content was determined from the hydroxyproline content of the acid hydrolysate using the method of Grant (1964).

Electron microscopy of collagen fibrils and measurement of fibre diameter

Small portions of skin and hip joint capsule (2 mm^3) were fixed in buffered glutaraldehyde, washed and

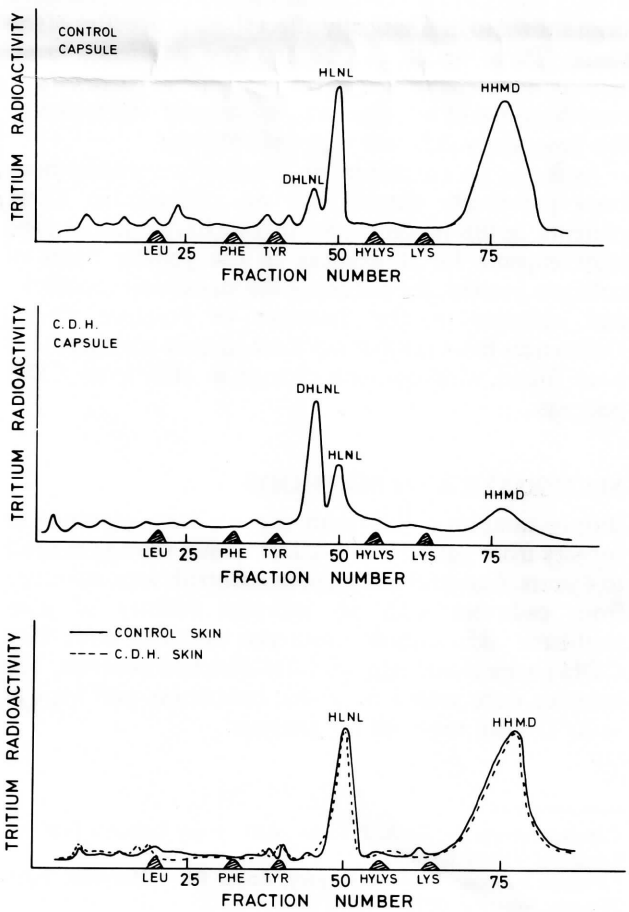

Figure 2 Elution patterns of radioactive (^3H) reducible components separated by ion exchange chromatography using pyridine/formate buffers from the acid hydrolysates of hip joint capsule and skin. DHLNL, hydroxylysino-5-keto-norleucine; HLNL, hydroxylysino-norleucine; HHMD, histidino-hydroxymerodesmosine (hatched areas denote positions of normal amino acids)

stained in bulk with phosphotungstic acid followed by uranyl acetate. After dehydration in alcohol the tissue was embedded in resin, and sections examined on a Philips EM 400T electron microscope.

Collagen fibre diameter measurements were taken from electron micrographs using a digitizer drawing board (VersaWriter) coupled to an Apple microcomputer.

RESULTS

Collagen types

The ratio of the collagen types was determined by densitometry of the acrylamide gels shown in Figure 1.

Joint capsule Analysis of the ratio of Type III to Type I collagen in the joint capsules revealed a dramatic decrease in the ratio, from 0.3–0.44 in the controls to 0.14–0.29 in the CDH subjects (Table 1); within-batch comparisons suggested CDH subjects had a ratio about half that of controls.

Skin In contrast, the ratio of the collagen types was unaltered in the skin of CDH patients compared with controls.

Intermolecular crosslinks

Joint capsule An average of 50% increase in the proportion of the hydroxylysino-hydroxynorleucine crosslink occurred in the CDH joint capsules compared with controls. This increase was not reflected in the other two crosslinks, hydroxylysinonorleucine and histidino-hydroxymerodesmosine, where a decrease of about 20% and 40% was noted (Table 2).

Skin The crosslinks present in the skin collagen of CDH patients were identical to those of the controls.

Collagen fibre diameter

Electron micrographs of skin and capsule from control and CDH subjects are shown in Figure 3.

Figure 4 shows the distribution of collagen fibre diameters for hip capsule. The fibre diameters from CDH capsule fell into three distinct groups with peaks at 33.0, 41.0 and 50.0 nm, and with an average diameter of 38.8 nm. Collagen fibres from the age-matched control capsule exhibited a much broader distribution with a slightly lower average of 34.8 nm. No differences could be detected in the fibre diameters in skin from CDH and control subjects. The average diameter for both was 36.0 nm.

Table 1 Ratio of the Type III to Type I collagen in the joint capsule and skin of CDH patients and age/site-matched controls

	Age (months)	*Sex*	*Ratio Type III/I*	
			Joint capsule	*Skin*
Batch 1				
Control 1	13	F	0.3	0.16
CDH patients				
1	12	F	0.14	0.13
2	13	F	0.17	0.10
3	42	F	0.18	0.14
4	20	F	—	0.14
			Average 0.16	Average 0.13
Batch 2				
Control				
1	13	M	0.37	—
2	22	F	0.44	—
3	13	M	—	0.31
4	22	F	—	0.31
			Average 0.41	Average 0.31
CDH patients				
5	13	F	0.29	0.34
6	14	F	0.28	0.24
7	15	F	0.18	0.31
8	17	F	0.26	0.32
9	24	F	—	0.33
			Average 0.25	Average 0.31

Analyses were carried out in two separate batches so comparison should only be made within batches

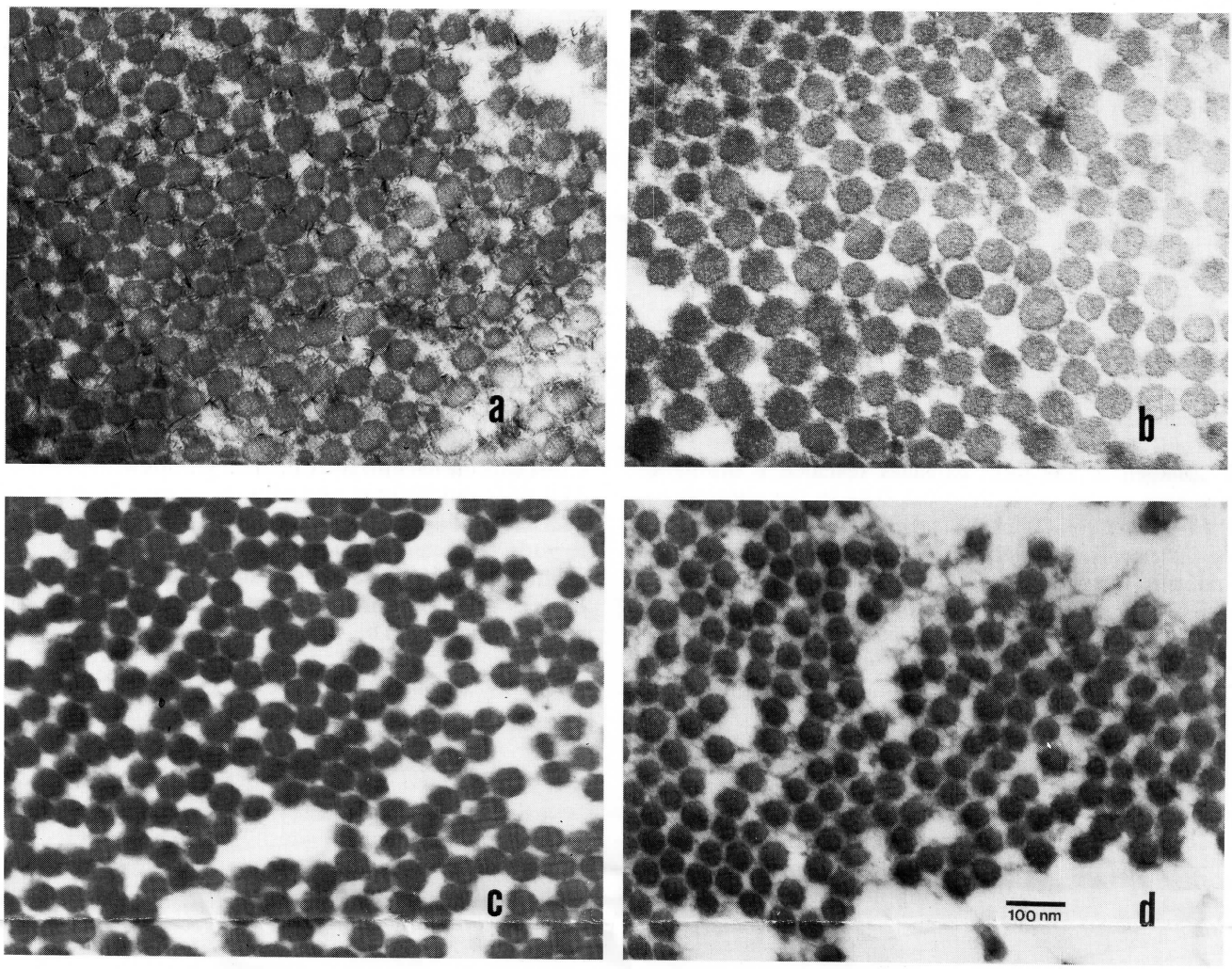

Figure 3 Electron micrographs of transversely sectioned collagen fibrils from hip capsule and skin (magnification × 64 000). (a) Control capsule; (b) CDH capsule; (c) control skin; (d) CDH skin

Table 2 Ratio of the individual reduced intermolecular crosslinks hydroxylysino-hydroxynorleucine (DHLNL); hydroxylysinonorleucine (HLNL) and histidinohydroxymero-desmosine (HHMD) in joint capsule of CDH patients and age/site matched controls

	DHLNL	*HLNL*	*HHMD*
Control	1.0	1.0	1.0
CDH			
Joint capsule	1.5	0.8	0.6
Skin	1.0	1.0	1.0

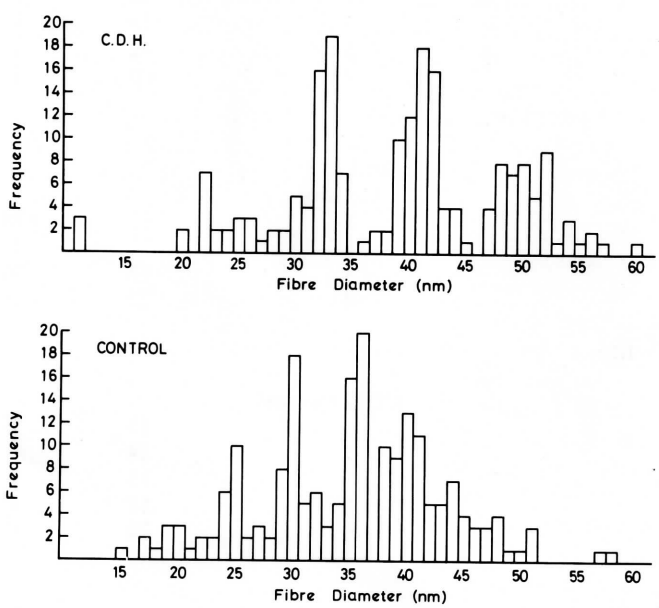

Figure 4 Size/frequency histograms of collagen fibrils in (a) CDH hip joint capsule, (b) control hip joint capsule

DISCUSSION

In this preliminary study we have demonstrated a change in the ratio of the major collagen types, in the type of crosslinks and in the fibril size, in the capsule of CDH patients. These defects may not be strictly tissue-specific but no changes were found in the dermal collagen of the CDH subjects. Although insufficient data are available on collagen types and their relationship to mechanical stability, it is clear from these results that in CDH patients there is an error in the metabolism of the collagen.

Several workers have reported age-related changes in the ratio of Type I to Type III in the skin. Initially there is a high proportion of Type III (about 50%) which decreases in childhood to about 15–20%, and thereafter remains constant. On the other hand, there is a steady increase in the proportion of Type III collagen in the vascular system with increasing age during growth. It has therefore been suggested that a high proportion of Type III collagen is essential for flexibility, as would be required in the embryonic dermis and aorta. In contrast, in the CDH patients there is a decrease in both the proportion of Type III collagen and in the mechanical stability of the joint capsule. Similarly, the Ehlers-Danlos syndrome Type IV or Ecchymotic form is characterized by a dramatic weakening of the vascular system, and biochemically there is a decrease in Type III. Clearly the relationship of collagen types and mechanical stability is not understood.

The mechanical stability is also determined by the nature and extent of the intermolecular crosslinks. However, although the type of crosslink changed in the CDH capsule compared with the control, there was no overall decrease in the amount of the crosslinks to account for a reduced stability in the tissue. The high level of the hydroxylated crosslinks probably reflects the greater immaturity of the tissue compared with the controls.

The diameter of the collagen fibres undoubtedly also plays a role in the mechanical properties of tissues. The present study demonstrated a difference in the distribution of fibre diameters in the CDH capsule compared with those in the control. On average, the fibres from CDH capsule were 11.5% wider than the controls. In a study by Cotta (1961) much larger differences in fibre diameter were found. He reported an increase in fibre diameter of up to three-fold. In contrast, Ippolito *et al.* (1980) reported a slightly smaller diameter for fibres from CDH patients, but concluded that this difference would hardly explain the changes observed in the capsule and acetabulum.

Although the relationship of fibre size to mechanical stability has not been established it may be that the size is critical in certain specific tissues but the differences observed here are sufficient to account only in part for a decrease in mechanical stability.

One may conjecture that the inability of the tissue to synthesize the correct ratio of Type I/III collagen may have a profound effect on the accretion of fibrils to form fibres of the optimum diameter necessary for mechanical stability.

It has also been suggested that a relationship exists between collagen fibre size and crosslinking. In studies by van den Hooff *et al.* (1959) and Shore *et al.* (in press) collagen fibrils from lathyritic tissues, where normal crosslinking has been inhibited by administration of the lathyrogen β-aminopropionitrile, showed an increase in diameter as well as a bimodal distribution compared with a unimodal distribution in normal tissue. Although we did not observe in our CDH capsules the sort of inhibition of crosslinking found in lathyrism, we did nevertheless observe significant changes in the type of crosslinking. It could then be conjectured that a perturbation of the normal crosslinking might be reflected in altered fibre size. In our view crosslinking is unlikely to affect fibre size directly, and a more plausible explanation might be that the fibres are weaker and therefore attempt to compensate for the stress on them by increasing the fibre size.

Before any conclusions can be drawn, other joints should be examined in a similar manner, and tissues other than skin to demonstrate whether the effect is tissue-specific or a generalized connective tissue defect. A similar biochemical analysis of the acetabulum should also be undertaken. It should also be possible to demonstrate that fibroblasts from the capsule exhibit similar biochemical changes when cultured *in vitro*.

It cannot be certain whether the biochemical changes in the joint capsule in CDH, or indeed the mechanical changes, are primary or secondary to the dislocation of the hip. However, these changes in the collagen are significant and further studies are clearly warranted.

MS received 29.7.83
Accepted for publication 21.11.83

References

Cotta, H. Elektroenoptische Untersuchungen über das Bindegewebe der Gelenkkapsel—I Mitteilung: Das Fibrillensystem. *Z. Orthop.* 94 (1961) 303–317

Grant, R. A. Estimation of hydroxyproline by the Auto Analyser. *J. Clin. Pathol.* 17 (1964) 685–686

van den Hooff, A., Levene, C. I. and Gross, J. Morphologic evidence for collagen changes in chick embryos treated with β-aminopropionitrile. *J. Exp. Med.* 110 (1959) 1017–1022

Ippolito, E., Ishii, Y. and Ponseti, I. V. Histologic, histochemical and ultrastructural studies of the hip joint capsule and ligamentum teres in congenital dislocation of the hip. *Clin. Orthop.* 146 (1980) 246–258

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227 (1970) 680–682

Light, N. D. Estimation of types I and III collagens in whole tissue by quantitation of CNBr peptides on SDS-polyacrylamide gels. *Biochim. Biophys. Acta* 702 (1982) 30–36

Robins, S. P., Shimokomaki, M. and Bailey, A. J. The chemistry of the collagen crosslinks: age-related changes in the reducible components of intact bovine collagen fibres. *Biochem. J.* 131 (1973) 771–780

Shore, R. C., Moxham, B. J. and Berkovitz, B. K. (1974). Changes in collagen fibril diameters in a lathyritic connective tissue. *Connective Tissue Res.* (In press.)