ACTA ORTHOPAEDICA SCANDINAVICA
SUPPLEMENTUM NO. 199, VOL. 54

From the Department of Orthopaedic Surgery (Head: Prof. Sven Olerud),
University Hospital, Uppsala, Sweden.

Experimentally induced hip dislocation in vitro and in vivo

A study in newborn rabbits

BY

STEFAN ASPLUND and ÅKE HJELMSTEDT

MUNKSGAARD. COPENHAGEN 1983

Translated by Maud Marsden

Key words: Hip joint, anatomy - Hip dislocation, congenital - Hip biomechanics

ISBN 87-16-09555-3
ISSN 0300-8827

Offsetcenter AB
Uppsala 1983

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INTRODUCTION

The literature concerning congenital dislocation of the hip (CDH) and its treatment is very extensive. Anatomical studies of this condition have been performed on autopsy material from foetuses and newborn infants (Holtzman 1895, Lepage & Grosse 1901, Le Damany 1912, Ortolani 1948, 1976, Stanisavljevic & Mitchell 1963, Laurenson 1964, Dunn 1969, 1976 b, McKibbin 1970, Campos da Paz & Karam Kalil 1976, Dega 1978, Ogden & Moss 1978, Ponseti 1978, Stanisavljevic 1981, and others). Observations during surgery have been reported by several authors, including Howorth and Smith (1932), Scaglietti and Calandriello (1962), Ferguson (1973) and Weinstein and Ponseti (1979), but for natural reasons the anatomical descriptions here have been incomplete. Roentgen-anatomical studies with the aid of arthrography and computed tomography have been of great help in the clinical management, but only exceptionally have these investigations been conducted on children below the age of six months (Severin 1941, Mitchell 1963, Astley 1967, Nakamura 1968, Grech 1972, Lönnerholm 1979, Browning, Rosenkrantz & Tarquinio 1982, Visser, Jonkers & Hillen 1982).

In animal experiments in vivo, hip dysplasia and/or hip dislocation have been induced by surgical procedures (Smith, Ireton & Coleman 1958, Smith, Coleman, Olix & Slager 1963, Riser 1975, Negri, Tricarico & Iorio 1977), external trauma (Langenskiöld, Sarpio & Michelsson 1962) or immobilization of the hind leg with or without concomitant administration of hormones (Wilkinson 1963, Sijbrandij 1965, Salter 1966, Michelsson & Langenskiöld 1972). The lesions have been studied radiologically and anatomically.

In a previous paper (Hjelmstedt, Asplund & Rauschning 1982) we presented a method of inducing hip dislocation in vitro in autopsy specimens and studying the result anatomically. We found it possible to produce deformation and dislocation similar to those described in autopsy investigations of CDH.

In the present study dislocation of the hip was induced in newborn rabbits both in vitro and in vivo. The aims were

to examine, in in vitro experiments, the relation between load and dislocation;

to develop an in vivo method for inducing dislocation and deformation of the hip;

to see whether dislocation of the same type and degree can be obtained with the in vitro method as with the in vivo method; and

to investigate the way in which different forms of post-mortem storage of specimens influence the results.

NOTATION AND ABBREVIATIONS

a = intercept of the regression line

b = slope of the regression line, i.e. the coefficient of regression

C = *centigrade*

CDH = congenital dislocation of the hip

CMC = carboxymethyl-cellulose gel

d = the diameter of the femoral head

D = the dislocation of the femoral head expressed as parts of the diameter d. Example: 1.0 d means a total dislocation and 0.5 d a partial dislocation where half of the head is out of the socket. See Fig. 5.

Dh = the mean of the dislocation D determined from three central sections *from one* and the same hip

Da = the mean of the dislocation Dh of the left and right hips of one and the same animal, where the load had been the same on both hips

g = gram

GAG = glycosaminoglycans

L_n = the load in newtons

L_r = the relative load expressed as parts or multiples of W

mg = milligram

N = *newtons*

p = probability

q = the distance in mm between the central point of the femoral head and the anterior margin of the acetabulum (see Fig. 5)

r = coefficient of correlation

rh = the radius of the femoral head in mm (see Fig. 5)

SD = standard deviation

S_{yx} = standard deviation from regression

W = the weight of the animal, i.e. the product of the mass and the acceleration due to gravity. W is expressed in newtons.

μm = microns

DEFINITIONS

DEFORMATION - The alteration in the shape of the skeletal parts subjected to stress.

DISLOCATION - Partial or complete displacement of the femoral head. In our own experiments this was measured as shown in Figure 5 and is called dislocation when the upper normal limit of Dh = 0.109 d was exceeded.
The word dislocation thus includes both subluxation and luxation, terms which are avoided in this paper.

DYSPLASIA - This can have several different meanings. In the present paper the word is used in the sense of an abnormal shape irrespective of whether this has been caused by external or internal factors, or both.

RELATIVE LOAD - As the animals differ in size, it is appropriate to relate the load to the weight W of the animal. This is done by expressing the load as parts or multiples of W. This expression is called the relative load L_r as distinct from the load L_n, which is expressed in newtons.

APPARATUS, MATERIAL AND METHODS

1. In vitro and storage series

Apparatus. The experimental apparatus (Fig. 1) is the same as was used previously in corresponding experiments on human specimens (Hjelmstedt, Asplund & Rauschning 1982). It consists of two load devices which are fixed above to a frame, which is connected to a base by four metal rods. The base is supplied whith holes and screws for attachment of the specimen (sp). The load device consists of a force gauge (f) with a measurement range of 0 - 10 N (Ametek T) and a screw device (s) for setting the desired load. The force is transmitted from the load device to the femur through a metal rod (m) and the orientation of the force is set by means of a ball-and-socket joint (j).

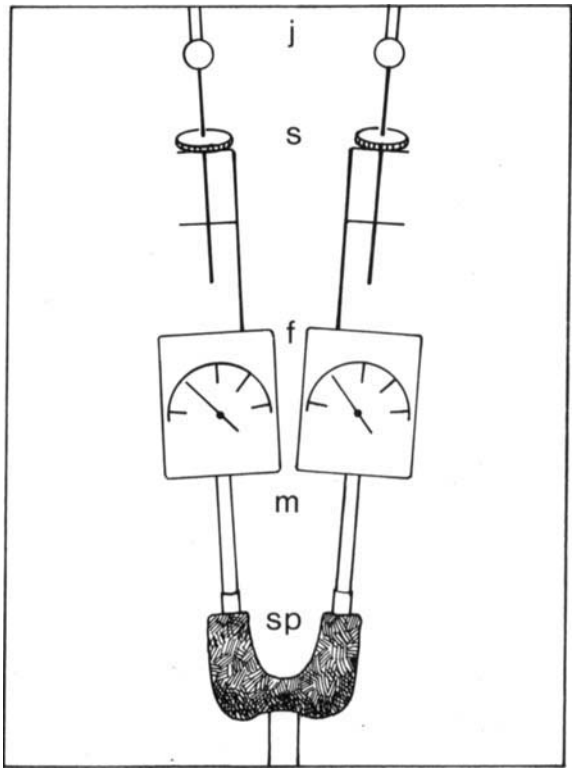


Figure 1. Schematic drawing of the experimental apparatus. j = ball-and-socket joint, s = screw device, f = force gauge, m = metal rod, sp = specimen. See text for details.

Material, preparation and procedure. New Zealand rabbits 7 - 12 days old (mean age 9.6 days) and with a body weight of 120 - 223 g (mean 173 g) were used.

The rabbit was killed with an overdose of ketamine. A specimen consisting of the femora, pelvis and lumbar vertebral column, with all surrounding muscles intact, was removed by dissection. A pin was inserted in each femoral shaft parallel to the condylar axis to permit subsequent measurement of the hip rotation. The specimen was fixed in position lying on a thin wooden block, which gave support to the vertebral column and the medial parts of the pelvic girdle but did not prevent dislocation of the hip. The wooden block was screwed onto the base of the apparatus. The femoral condyles were removed and a metal tube of appropriate size was threaded over the end of the femur. Into this tube was inserted a thin metal rod, the end of which came to rest against the femur. This rod was connected to the load device. The load device was set in the desired position, whereafter loading was begun, under continuous monitoring of the force gauge. During the first minutes the deformation occurred quickly and the load was kept constant by continuous adjustment of the screw device. The rate of deformation decreased progressively and after about 30 minutes the adjustment was only necessary every fifth minute. At the end of the experiment the deformation had practically ceased. The experiment was performed at room temperature, $+20^{\circ}$ C. During the experiment the specimen was covered with gauze moistened with Ringer solution to prevent it from drying. Only bilateral experiments were done. The hip flexion was 90° in all instances. The abduction was 0 to 3° and the rotation varied between neutral and 5° outward rotation. The relative load (L_r) varied between 0.25 x W and 2.5 x W (L_n = 0.3 - 5.0 N). For each specimen the load was the same on the left and right hips. The experiment lasted for 3 hours. All experiments in the in vitro series were started within one hour after the death of the animal. In the storage study, the experimental conditions were varied as described later.

At the end of the experiment the apparatus, with the still loaded specimen, was placed in a freeze box at -20° C. After about 30 minutes the specimen was frozen through. It was then demounted and examined roentgenologically, whereafter it was embedded for cryosectioning. An attempt was made to get more rapid freezing in some cases by pouring liquid nitrogen over the loaded specimen. This method was abandoned at an early stage, however, as it was difficult to get even and complete

freezing of the specimen, and two of these experiments had to be excluded from the series because of technical failure.

2. In vivo series

Material, procedure and preparation of specimens. New Zealand rabbits 7 - 12 days old (mean age 9.2 days) and weighing 120 - 240 g (mean 165 g) were used.

The experiments were performed in two series. In series I, which was a pilot study, the different experimental conditions were varied. With the guidance of the results in series I, the experimental conditions in series II were standardized.

The knee joint was immobilized in extension with a dorsally applied plastic splint (Crystona), which was fixed with adhesive tape. Most of the extremities were also fixed with the hip flexed, by means of a strip of adhesive tape around the trunk. In series II, in addition the two hind legs were fixed to one another in inward rotation (Table I, Fig. 2). Note that fixation of the legs in inward rotation was not equivalent to inward rotation of the hip joints. This was because part of the rotation took place at the knee joint, and also because some of the rotation was lost through slipping of the skin in relation to the underlying muscle and bone. At the end of the experiment 5 mg of ketamine were injected intramuscularly. With this form of anaesthesia the muscle tone was maintained. The rabbit was then lowered in the anaesthetized state into liquid nitrogen (-196^{o} C) and became deep-frozen almost instantaneously. After the freezing, a specimen comprising the pelvis and both hind legs was excised. During the course of the dissection the specimen was dipped from time to time in liquid nitrogen to prevent thawing. The specimen was then examined roentgenologically with an anteroposterior (a.p.) projection of the femur, and the abduction or adduction was measured on the roentgenograms. After exarticulation at the knee joints, the rotation of the hips was determined from the position of the femoral condyles. The specimen was then divided sagittally at the midline and embedded for cryosectioning.

Table I. Summary of the experimental variables in the in vivo series I and II

	Series I	Series II
Bilateral experiments	6	10
Unilateral experiments	4	-
No. of extremities fixed to the trunk in flexion	12	20
No. of extremities not fixed to the trunk	4	-
No. of extremities fixed in inward rotation	2*	20
Hip joint:**		
flexion	65-140° mean 102°	90-110° mean 96°
outward rotation	0- 55° mean 22°	-30-+15° mean -1°
abduction	-15-+20° mean 7°	-15-+12° mean -4°
experimental period	1 min - 24 h	3 h
No. of dislocated hips	9	18

* Fixed both in flexion and in inward rotation

** Refers to all immobilized extremities

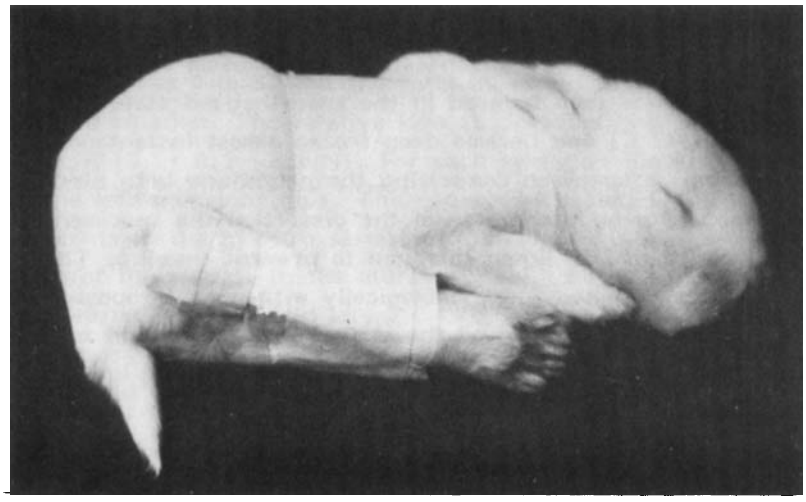


Figure 2. Immobilized rabbit from the in vivo series II with both hind legs fixed in inward rotation and pronounced flexion. In relation to the pelvis, however, the flexion was only about 90°.

3. Roentgenological examination

All specimens were examined roentgenologically in the frozen state after completion of the experiment (Fig. 3). Kodak X-omatic roentgen film was used (exposure data: 50 kilovolts, 1.2 sec). The roentgenograms were taken with the femur in the a.p. projection, i.e. in the projection in which the dislocation could be expected to be most distinctly evident. Most of the specimens had a hip flexion of about 90^{o}, which meant that the osseous parts of the pelvis were superimposed upon one another on the roentgen film and in many cases the acetabulum was difficult to demarcate exactly. Moreover, the acetabulum was not completely mineralized, which made the evaluation even more difficult. All femoral heads had a distinct ossification centre, although the degree of mineralization varied. Differences in dislocation between different specimens were usually clearly evident, but because of the difficulty in finding reproducible measurement points, the roentgenograms were not used to determine the degree of dislocation. On the other hand, the abduction or adduction of the hip was easily measured.

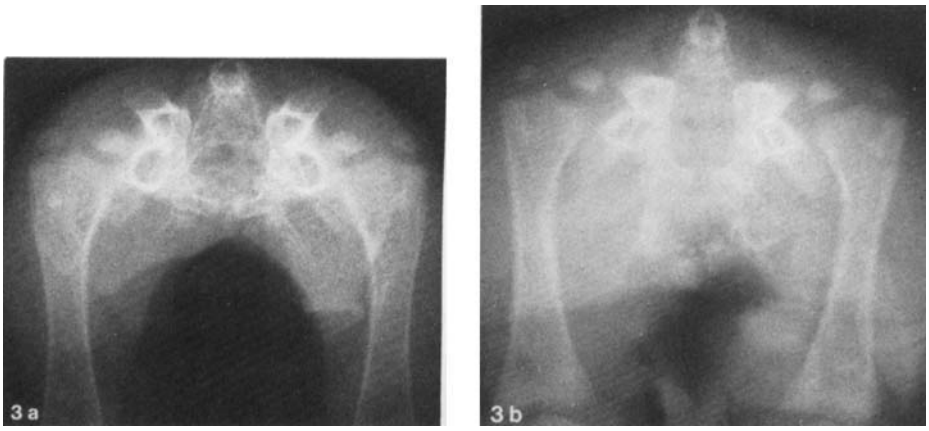


Figure 3. Roentgenograms of hip specimens. Frontal view of the femur. Hip flexion about 90^{o}. a) Unloaded specimen without dislocation. b) Specimen from the in vivo experiments, with dislocation.

Note the incomplete mineralization of the femoral head and acetabulum, which makes it impossible to demarcate these structures exactly.

4. Cryosectioning

Microtome. For cryosectioning , a heavy-duty cryomicrotome (LKB 2250, Bromma, Sweden) with a 35° microtome knife was used. To facilitate repeated adjustment of the specimen in all planes during the course of cutting, a microtome stage with a ball-and-socket joint was employed, allowing tilting of the stage by 15° in any direction from the horizontal plane. Most functions of the cryomicrotome can be guided from a remote control panel. Among other things, the number and thickness of sections can be predetermined within the range of 1 - 999 µm and 1 - 999 sections respectively.

Embedding. The frozen specimen was divided sagittally through the vertebral column, giving two hip specimens. These were frozen-fixed separately onto a slab of frozen carboxymethyl-cellulose gel (CMC). The positioning of the hip specimens was of greatest importance for obtaining a correct sectioning plane, i.e. the plane parallel to the femoral neck and the shaft, in which the dislocation could be expected to be most pronounced. The position of the femoral shaft could be observed on the exterior of the specimen. The position of the femoral neck could be estimated from the measured hip rotation, with correction for anteversion. According to Wilkinson (1962) the anteversion in five-day-old rabbits is 10°. The slab with the specimen was placed in a microtome stage box at -70° C. To prevent thawing of the small specimen, cooled CMC was added in stages until the specimen was completely embedded in frozen gel.

Sectioning. The ice block containing the specimen was mounted in the cryomicrotome and was allowed to equilibrate to its temperature of -20° C. The specimen was sectioned down to the hip joint level and fine-adjustment of the sectioning plane was made. The subsequent sections were 50 µm thick. After every fifth section (0.25 mm), the cut surface of the block was slightly thawed in order to remove ice crystals, and was then photographed.

5. Photographic documentation

The section surface was photographed with a 35 mm SLR camera (Olympus OM 2 N) to which an 80 mm macrolens was attached via an extension tube. Kodachrome 25 reversal film was used. Two automatic electronic flash units (Olympus T 32), placed one on either side of the specimen at an angle of 45^{o} to the surface of the section, served for illumination. A measuring scale was placed at the edge of the section surface to permit subsequent measurements. The photography was done manually.

6. Measurement of dislocation

The dislocation was measured on the photograph of the section surface, which was magnified about 30 times on a screen. Of several conceivable ways of measuring the dislocation, we chose the following. In a normal rabbit hip the femoral head is essentially spherical, i.e. it has a circular shape on the section surface (Fig. 4). On loading, however, some deformation of the femoral head takes place, with flattening and sometimes the formation of a crease. It is then not possible to measure the

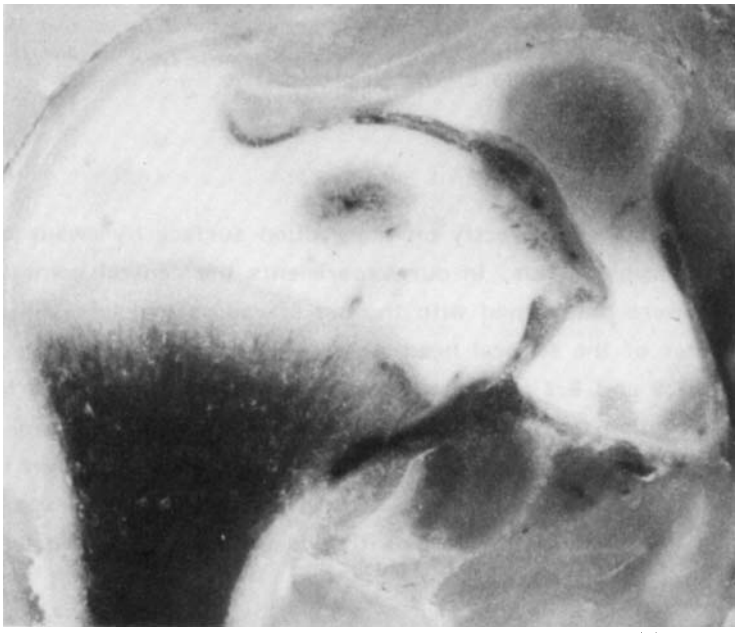


Figure 4. Cryosection of unloaded hip joint. Note that the articular surfaces are not in direct contact but are separated by a layer of fluid. The femoral head is essentially circular, and its diameter can be measured with the aid of a radius templet.

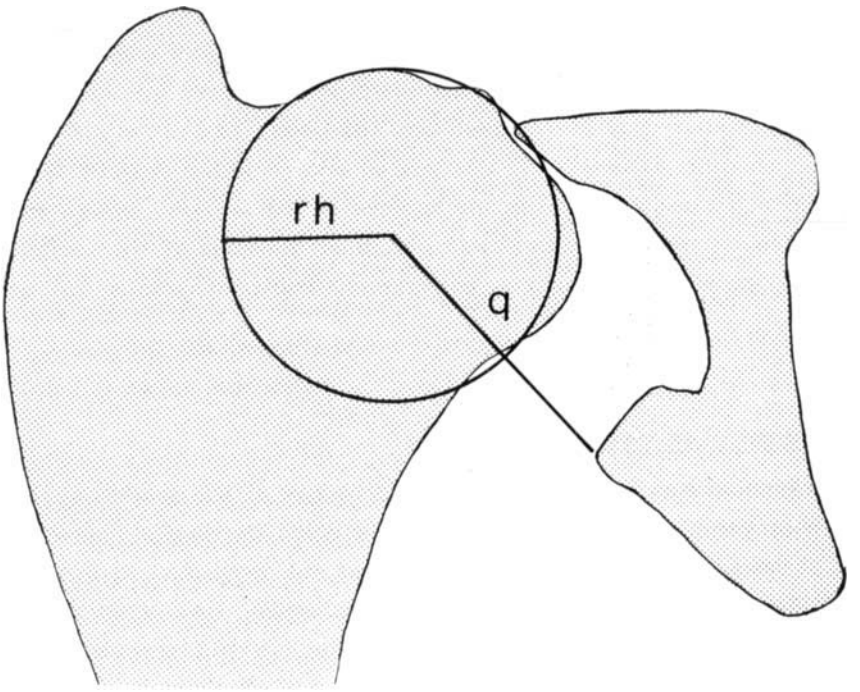


Figure 5. Schematic illustration of the way in which the dislocation is measured. The radius (rh) and the diameter (d) of the femoral head and the central point are determined with the aid of radius templets. q = the distance between the central point of the femoral head and the ventral margin of the acetabulum. The dislocation expressed in mm is q-rh and as expressed as parts of the diameter it is $\frac{q-rh}{d}$.

radius of the femoral head directly on the section surface by means of compasses, for example. Thus, in our experiments the central point and the radius (rh) were determined with the aid of radius templets (Fig. 5). Since the diameter of the femoral head as measured on the sections varied between 3.9 and 5.2 mm, it was considered most appropriate to express the dislocation D in relation to the diameter d of the femoral head instead of in millimetres. The method of measurement is shown in Fig. 5. D was determined on the three central sections and it is the mean value (Dh) of these determinations that is used in the presentation of the results.

7. Normal limit of the joint space

If Dh = 0 d this means that the femoral head should have complete contact with the ventral margin of the acetabulum. However, even in an unloaded hip there is always a small distance between the two articular surfaces, representing a normal joint space (Fig. 4). Moreover, when the joint is frozen this distance may increase to some extent as a result of expansion of the joint fluid between the articular surfaces, which will displace the femoral head in relation to the acetabulum. Consequently, it may be difficult to distinguish between a slight dislocation in a loaded hip and a normal joint space in a hip that is not loaded. With the aim of determining an upper normal limit for Dh, the following study was performed.

Ten New Zealand rabbits from four litters were used. They were 7 - 11 days old (mean 8 days) and weighed 125 - 240 g (mean 161 g). The animals were killed with an overdose of ketamine and the hips were placed in approximately 90^{o} flexion and neutral abduction and rotation, after which they were frozen. One hip joint from each animal was cryosectioned and the joint space, expressed as Dh, was determined. The mean Dh was 0.057 d (range 0.029 - 0.105 d) and the standard deviation (SD) 0.026 d. The upper normal limit for Dh was therefore set at 0.109 d (0.057 d + 2 SD).

IN VITRO EXPERIMENTS

The aims of this investigation were

to ascertain whether the previously used method of inducing hip dislocation in a short time in human specimens was also applicable to small specimens from young rabbits; and if so,

to study the relationship between load and dislocation.

Experimental set-up

Twenty-eight hip joints from 14 rabbits were loaded and cryosectioned and the dislocation was measured, as previously described. The magnitude of the load was varied with respect to the weight of the animal (W) from corresponding to approximately 0.25 x W to 2.5 x W, so that seven different load categories were obtained, with two animals in each category. Expressed in newtons, the load varied between 0.3 N and 4.0 N (Table II). All hips were loaded in the position of 90^{o} flexion, 0 - 3^{o} abduction and 0 - 5^{o} outward rotation. The experimental period was three hours.

Table II. Division into categories according to relative load in the in vitro series

Relative load	Animal 1		Animal 2	
	Load (N)	Weight (N)	Load (N)	Weight (N)
0.25 x W	0.3	1.2	0.4	1.7
0.50 x W	0.65	1.3	0.75	1.5
0.75 x W	1.25	1.65	1.25	1.7
1.0 x W	1.5	1.5	1.5	1.5
1.5 x W	2.7	1.8	3.15	2.1
2.0 x W	2.8	1.45	4.0	2.05
2.5 x W	3.9	1.6	3.9	1.55

Results

Dislocation occurred in all experiments and Dh varied between 0.121 d and 0.730 d (upper normal limit 0.109 d). The dislocation in the different load categories is given in Table III. It increased with the magnitude of the load and is presented graphically in Figure 6 as a function of the logarithm of the relative load L_r and in Figure 7 as a function of the logarithm of the load L_n expressed in newtons. As the dislocation in the right hip and that in the left hip may be regarded as dependent variables, the dislocation in each animal is reported as the mean value for the two hips (Da) and is given as a point in Figures 6 and 7. It was also these mean values that were used in calculating the regression lines.

Deformation of the femoral head and/or of the acetabular cartilage was observed in all experiments. The part of the femoral head that lay against the dorsal margin of the acetabulum became flattened, and in cases of pronounced flattening a crease was seen lateral to the flattened area. The dorsal acetabular margin was folded backwards to a greater or lesser extent as a result of the pressure from the femoral head. The deformation increased with increasing load and is described in more detail in Figures 8 - 11. In no case was any macroscopic damage to the joint capsule or the ligament of the head of the femur observed.

Table III. The dislocation in the in vitro series

Relative load	No. of hips	Dislocation Dh			
		min. (d)	max. (d)	mean (d)	SD (d)
0.25 x W	4	0.121	0.236	0.168	0.051
0.50 x W	4	0.231	0.353	0.275	0.054
0.75 x W	4	0.354	0.461	0.415	0.046
1.0 x W	4	0.400	0.473	0.432	0.031
1.5 x W	4	0.495	0.705	0.619	0.091
2.0 x W	4	0.575	0.602	0.588	0.012
2.5 x W	4	0.711	0.730	0.722	0.009

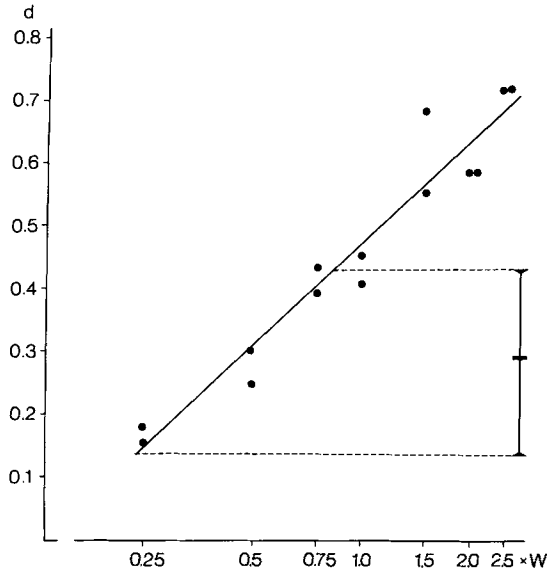


Figure 6. The relationship between relative load (L_n) and dislocation in the in vitro experiments. Logarithmic scale (natural logarithms). The mean dislocation in each animal (Da) was used, for calculating the regression line, and is indicated by a point. The numerical values for Da are given in Table VIII in the Appendix. a = 0.473, b = 0.24, r = 0.97. The mean and range for the dislocation (Dh) in series II of the in vivo experiments are presented on the right-hand side of the diagram.

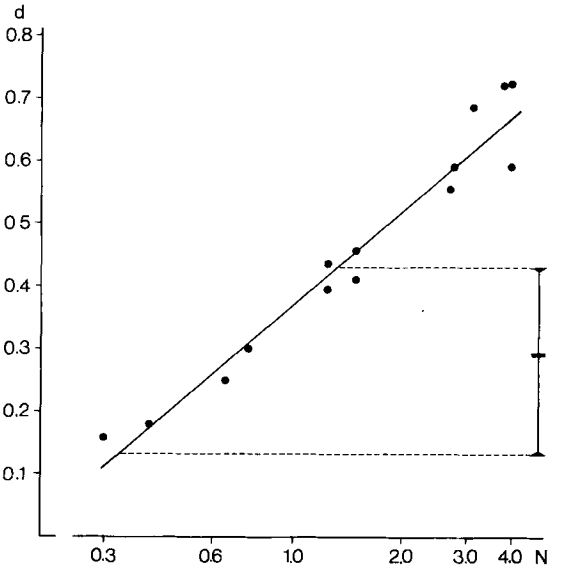


Figure 7. The relation between load (L_n) and dislocation in the in vitro experiments. Logarithmic scale (natural logarithms). The mean dislocation in each animal (Da) was used for calculating the regression line, and is indicated by a point. The numerical values for Da are given in Table VIII in the Appendix. a = 0.372, b = 0.22, r = 0.97. The mean and range for the dislocation (Dh) in series II of the in vivo experiments are presented on the right-hand side of the diagram.

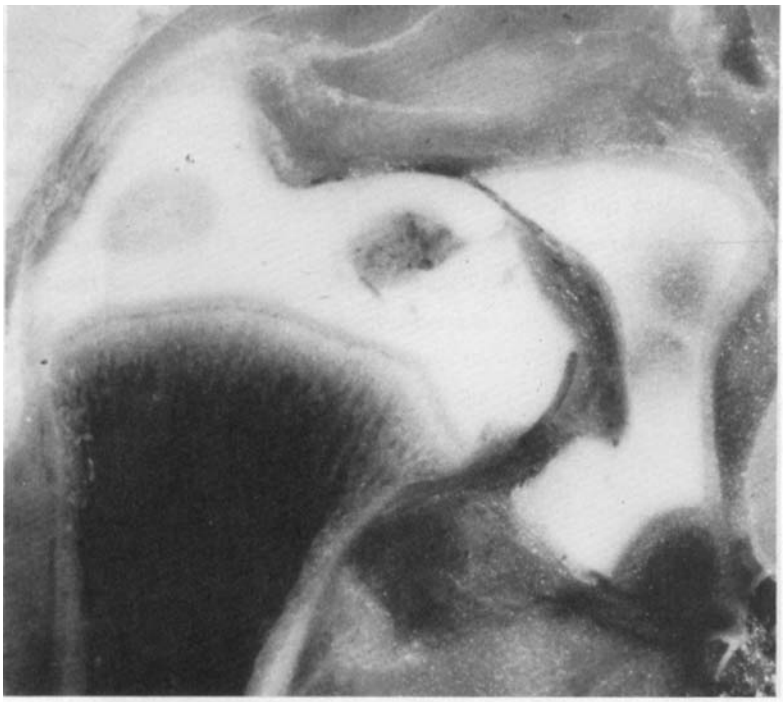


Figure 8. A hip joint after loading with 0.25 x W (0.3 N). There is slight dislocation. Dh = 0.174 d. The posterior acetabular margin is slightly deformed (cf. Fig. 4).

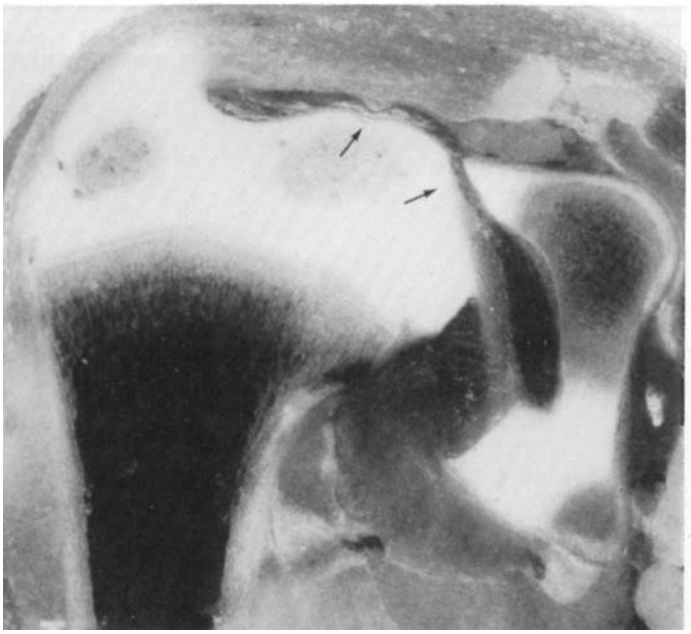


Figure 9. A hip joint after loading with 1.0 x W (1.5 N). There is considerable dislocation. Dh = 0.437 d. The ligament of the head of the femur is tense but intact. The femoral head is slightly deformed (→), while the posterior acetabular margin is greatly deformed.

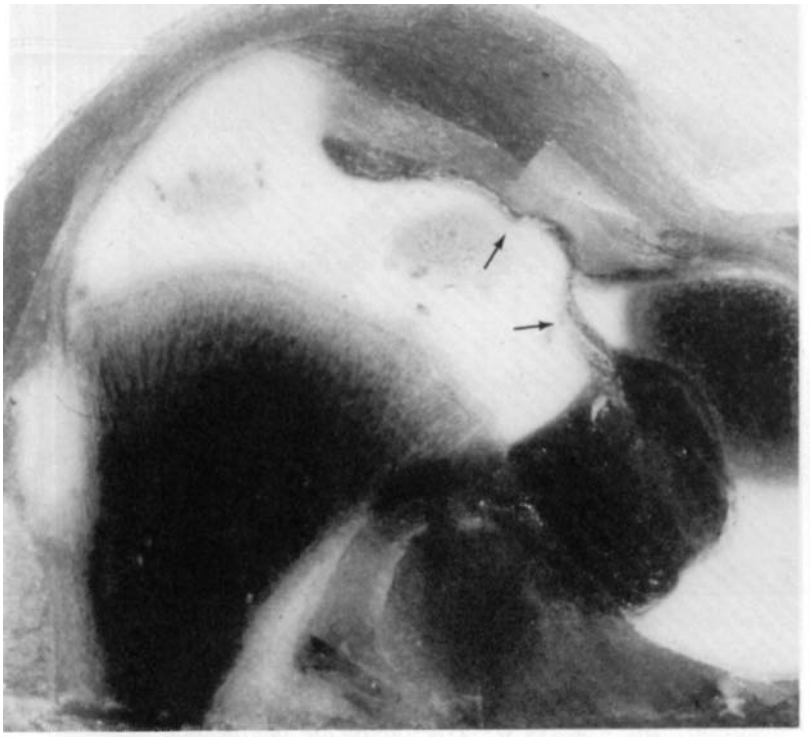


Figure 10. A hip joint after loading with 2.5 x W (3.9 N). There is pronounced dislocation. Dh = 0.730 d. The femoral head and posterior acetabular margin are greatly deformed. Note the crease formation (→) and the deformation towards the acetabular margin.

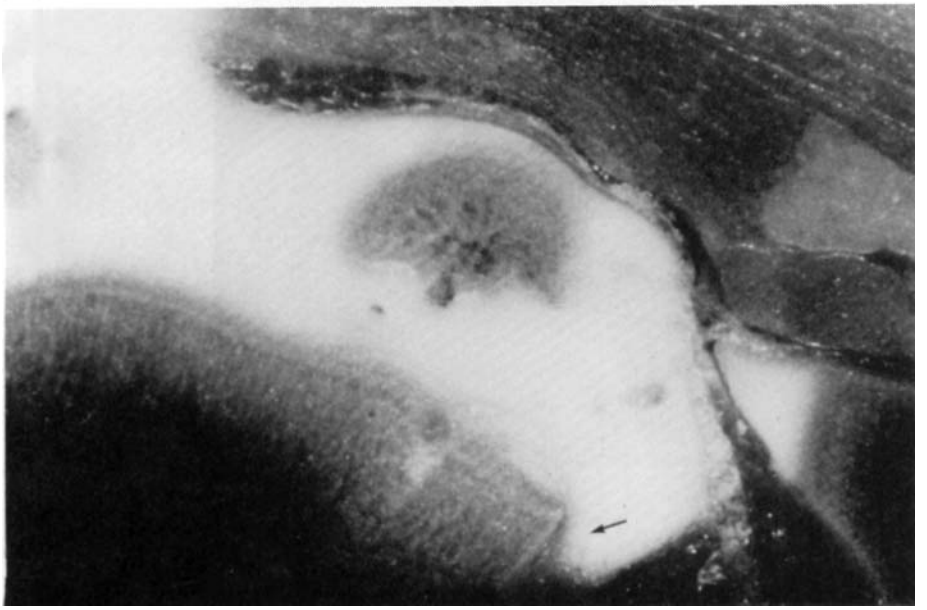


Figure 11. Detail of a hip joint loaded with 2.5 x W (3.9 N). The femoral head shows considerable deformation and distally the cartilage has been forced outside the supporting metaphysis (→).

Discussion

The apparatus and method have been described in detail by Hjelmstedt, Asplund and Rauschning (1982) and with minor modifications they have been found to be well applicable in experiments on our small rabbit specimens. With this method the entire intact hip joint is subjected to a mechanical load causing deformation of the cartilage and extension of the joint capsule and the ligaments. These investigations seem to be the first of this type on intact neonatal joints. Methods with a similar principle have been used, however, by Takei (1979) and Takei and Terayama (1981) in studies of cartilage deformation in knee joint specimens from adult humans and pigs.

In order to study the relation between force and dislocation, the load was varied. With our technique both hips were loaded equivalently so as to prevent asymmetrical deformation of the pelvis and thereby large differences in abduction between the hips. The force acts in the direction of the femoral shaft and therefore dislocation occurs more readily in an adducted than in an abducted hip. If the abduction varies greatly, the results in different hips will not be comparable. Neither can the same specimen be subjected to repeated experiments. For these reasons comparisons of dislocation at different loads must be made between different individuals. We tried to use as similar young rabbits as possible, but the body weight nevertheless varied between 120 g and 215 g. The hips from a rabbit of low body weight are smaller and when they are subjected to a defined load the dislocation and deformation can be expected to be greater than in a heavier rabbit. Smith (1954) and Moss and Ferguson (1980) found a correlation between body weight and ligamental strength in animal experiments. In the planning of the present experiments the load was therefore related to the weight W of the animals. This relative load L_r is expressed as parts or multiples of W. The dislocation was found to be approximately proportional to the logarithm of both the relative load L_r and the load L_n in newtons. The correlation coefficients were also equal, 0.97. The explanation may be that the scatter of the weight (W) was too low to have an effect on the results. Thus the weight of 11 of the 14 animals was 1.3 to 1.8 N.

As early as in 1847 Wertheim studied the relation between load and elongation in tension tests on different body tissues, including tendons. Our experiments demonstrated an approximately logarithmic relationship between load and dislocation. Similar results have been obtained by se-

veral authors in experiments on different types of collagenous tissue (e.g. Morgan 1960 & Hirsch 1974). In these investigations the same specimen was subjected to a progressively increasing load and/or repeated tests. In our experiments this was not possible. Instead, comparisons were made between specimens that were subjected to constant loads. It should be pointed out that our investigations also differ from earlier ones in that our specimens were from newborns and not from adults.

The relation between time and dislocation at constant loading could not be measured directly in our experiments, but the dislocation seemed to occur very quickly at first, and then gradually decline (see in vitro methods). Armstrong, Bahrani and Gardner (1979) studied cartilage deformation at constant loading of intact hip joint specimens from adults. They found that the deformation of the cartilage, after the initial onset, increased slowly and approached a steady state value exponentially. There was still a slow creep after 30 minutes . Corresponding findings were made by Kempson, Freeman and Swanson (1971) in indentation tests on femoral head specimens from adults. The deformation of the articular cartilage did not cease until after 2 - 3 hours. In previous tension tests with constant force on the ligament of the femoral head from newborns, we found pronounced initial elongation followed by a slow creep phase which lasted several hours (Hjelmstedt, Olofsson & Asplund 1981).

It must be emphasized that our experiments were performed on neonatal joints. In newborns the relative amount of cartilage is substantially greater than in older individuals, which naturally has an impact on the occurrence of deformation. There are other important differences, however. Both cartilage and connective tissue in the newborn have a different chemical composition and other biomechanical properties than those in older individuals. Thus, in studies of cross-linking in collagen from cattle of different ages, Verzár (1962) found that the degree of cross-linking between the collagen molecules from calves 1 - 3.5 months old was very low and increased rapidly with increasing age. Elliott and Gardner (1979) determined the content of glycosaminoglycans (GAG) in human articular cartilage from newborn, growing and fully grown individuals. They found that at birth GAG constituted about 50 per cent of the dry weight of the cartilage and that they decreased during the period of growth, constituting approximately 15 per cent of the dry

weight of the cartilage in adulthood. In newborns GAG consisted almost entirely of chondroitin sulphate, but during growth this was partly replaced by keratan sulphate. Lust, Pronsky and Sherman (1972) carried out biochemical studies of cartilage and connective tissue from hip joints of growing dogs, and observed that the contents of hexosamines and DNA in the femoral head and acetabulum decreased with increasing age. The content of hydroxyproline, a measure of the collagen content, almost doubled during the first year of life. The water content of the cartilage decreased by about 10 per cent during the same period. About 30 per cent of the collagen fraction from the joint capsule and ligaments at birth was water-soluble collagen, while the corresponding proportions in the acetabular cartilage and the cartilage of the femoral head were 20 and 11 per cent respectively. At the age of one year the water-soluble collagen had been almost completely replaced by insoluble collagen.

There are very few reports in the literature on comparisons of the biomechanical properties of cartilage and other collagenous tissue between growing and adult individuals. Göcke (1928), however, observed in indentation tests on costal cartilage that the deformation was considerably greater in specimens from a six-month-old child than in those from a 40-year-old. Rollhäuser (1950) and Blanton and Biggs (1970) found a lower tensile strength in tendons from neonates than in adult tendons. In tension tests on bovine articular cartilage, Roth and Mow (1980) found differences in biomechanical properties between specimens from growing animals and those from fully grown ones. These findings demonstrate that the results of biomechanical investigations in growing individuals are not representative of those in adults, and vice versa.

Is is tempting to compare the findings in our animal experiments with previous observations on human specimens in vitro (Hjelmstedt, Asplund & Rauschning 1982). In this connection the difference in the mineralization of the femoral head and acetabulum must be kept in mind (Fig. 12). The rabbits in our study had a well developed ossification centre in the femoral head, while this is lacking in human specimens from neonates. The mineralization of the acetabulum also seems to be more extensive than in man. These circumstances explain why, for instance, the deformation of the posterior acetabular margin is less pronounced in the rabbit hips than in the human ones. Another discrepancy between the two species is the anatomy of the pelvis. Also of importance is the differ-

ence in post-mortem storage conditions between the two experimental series. The human specimens were first stored at $+4^{o}$ C for some days and then at -20^{o} C for a few weeks. The animal specimens, on the other hand, were examined within one hour post-mortem. There was a distinct similarity, however, in the type of dislocation and deformation between the rabbit and human hips, although the deformation seemed to be of a lesser degree in the rabbit specimens.

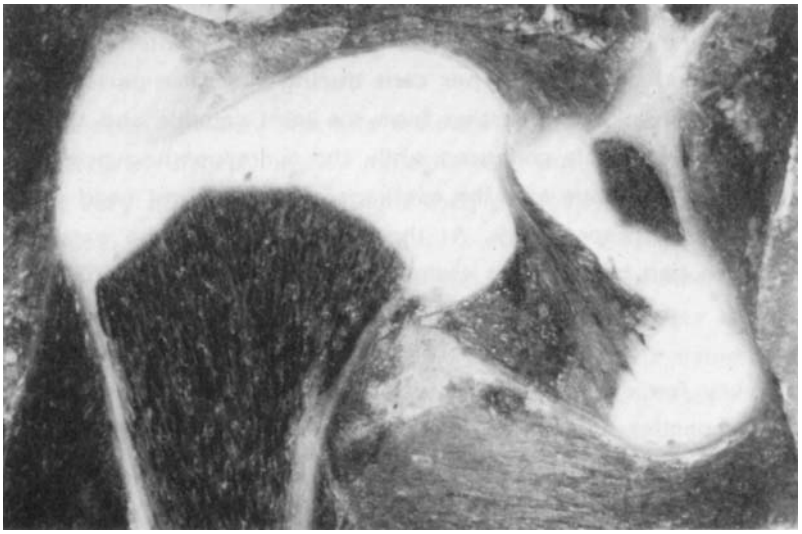


Figure 12. A hip joint from a human specimen, with dislocation after three hours under load. There is no ossification centre in the femoral head. There is also a difference in acetabular mineralization as compared with rabbit specimens.

Conclusions

1 The in vitro method of inducing hip deformation and dislocation in man is also applicable in corresponding investigations in young rabbits.

2 Deformation and dislocation can be induced in a short time with varying loads and without macroscopic damage to the joint capsule or ligaments.

3 The deformation of the femoral head and acetabulum increases with increasing load.

4 The magnitude of the dislocation is approximately proportional to the logarithm of the applied load.

IN VIVO EXPERIMENTS

The aim of this investigation was to develop an in vivo method of inducing hip dislocation and deformation in a short period of time.

As stated in the description of the method, the in vivo experiments were carried out in two series. The first series (series I) was a pilot study, in which the different experimental conditions were varied. In the second series (series II) a standardized method was used.

Series I - Experimental set-up

In the pilot study it was investigated whether dislocation could be induced by immobilization of the knee joint in extension, and if so, in what position the hip should be fixed in order to obtain the highest frequency of dislocation. The experiments were performed on 10 rabbits. There were six bilateral and four unilateral experiments - i.e. 16 extremities were immobilized. Twelve immobilized extremities were fixed in flexion and two of them were fixed both in flexion and in inward rotation. The experimental period varied from one minute to 24 hours (Table I).

Results

Dislocation occurred in nine of 16 hips and in these the Dh value varied between 0.136 d and 0.390 d (mean 0.217 d). The mean flexion in the dislocated hips was 103^{o} and in the non-dislocated ones 99^{o}. The corresponding values for abduction were 4^{o} and 10^{o} and for outward rotation 14^{o} and 31^{o} respectively (see also Table I). The degrees of abduction and rotation seemed to be of importance for the occurrence of dislocation, as seen in Tables IV and V. Dislocation was most easily induced if the outward rotation was 20^{o} or less and the abduction less than 10^{o}. Variations in the degree of flexion within the range 65 - 140^{o} had less impact on the occurrence of dislocation. The experimental period, three hours, i.e. the same as in the in vitro experiments, was found to be sufficiently long to induce dislocation.

Table IV. Relation between abduction and dislocation in in vivo series I

Abduction	No. of hips		
	Dislocation	No dislocation	Total
< 10	7	2	9
≥ 10	2	5	7
Total	9	7	16

Table V. Relation between outward rotation and dislocation in in vivo series I

Outward rotation	No. of hips *		
	Dislocation	No dislocation	Total
≤ 20	7	3	10
> 20	1	4	5
Total	8	7	15 *

* Information on rotation is missing for one hip with dislocation.

Series II - Experimental set-up

On the basis of the results in series I, a series of experiments was performed under standardized conditions. Twenty extremities from 10 rabbits were immobilized with the knees extended. The legs were fixed against one another in a position of inward rotation, adduction and flexion (Fig. 2). The experimental period was three hours (Table I).

Results

Dislocation occurred in 18 of 20 hips and the Dh for these 18 varied between 0.137 d and 0.431 d (mean 0.292 d). The mean flexion in the dislocated hips was 96^{o}, abduction -5^{o} and outward rotation -1^{o}. In one

of the two non-dislocated hips the flexion was 90^{o}, abduction 12^{o} and outward rotation -5^{o}, and in the other one the corresponding values were 100, 0 and 5^{o} respectively (see also Table I).

In the dislocated hips deformation of the cartilage of the femoral head and acetabulum was observed. The part of the femoral head that lay against the acetabular margin had become flattened, and when this flattening was pronounced, a crease was seen lateral to the flattened area. The dorsal acetabular margin was also deformed (Figs 13 a and 14 a). The space that appeared between the femoral head and the ventral part of the acetabulum was filled with synovial fluid. Whether this meant an increased amount of fluid was difficult to judge, but in at least two joints this was considered to be the case. In no instance was macroscopic damage to the ligament of the femoral head or to the joint capsule observed. In one hip joint, however, the synovial fluid was sanguinolent.

Discussion

Experimental induction of dysplasia and dislocation of the hip has previously been reported by several authors.

Dislocation has been produced by surgical or external trauma. Smith, Ireton and Coleman (1958) and Smith, Coleman, Olix and Slager (1963) excised the joint capsule, the ligament of the femoral head and/or the posterio-superior portion of the acetabular cartilage in puppies and observed dysplasia or dislocation in a high percentage after one to six months. Intraoperative stretching of the joint capusle and ligaments to breaking point resulted in the same changes. Excision of the posterior acetabular margin in rabbits led to posterior dislocation and secondary morphological alterations as in CDH (Negri, Tricarico & Iorio 1977). Riser (1975) performed tenotomy on the short outward rotators of the hip in puppies, and this caused dysplasia and subluxation. Traumatic dislocation of the hip with rupture of the capsule and ligaments in newborn rabbits has been found to result after two to three weeks in a morphological picture as seen in CDH (Langenskiöld, Sarpio & Michelsson 1962).

Another experimental model that has been used is immobilization of the hind leg in different positions. Sijbrandij (1965) immobilized one of the

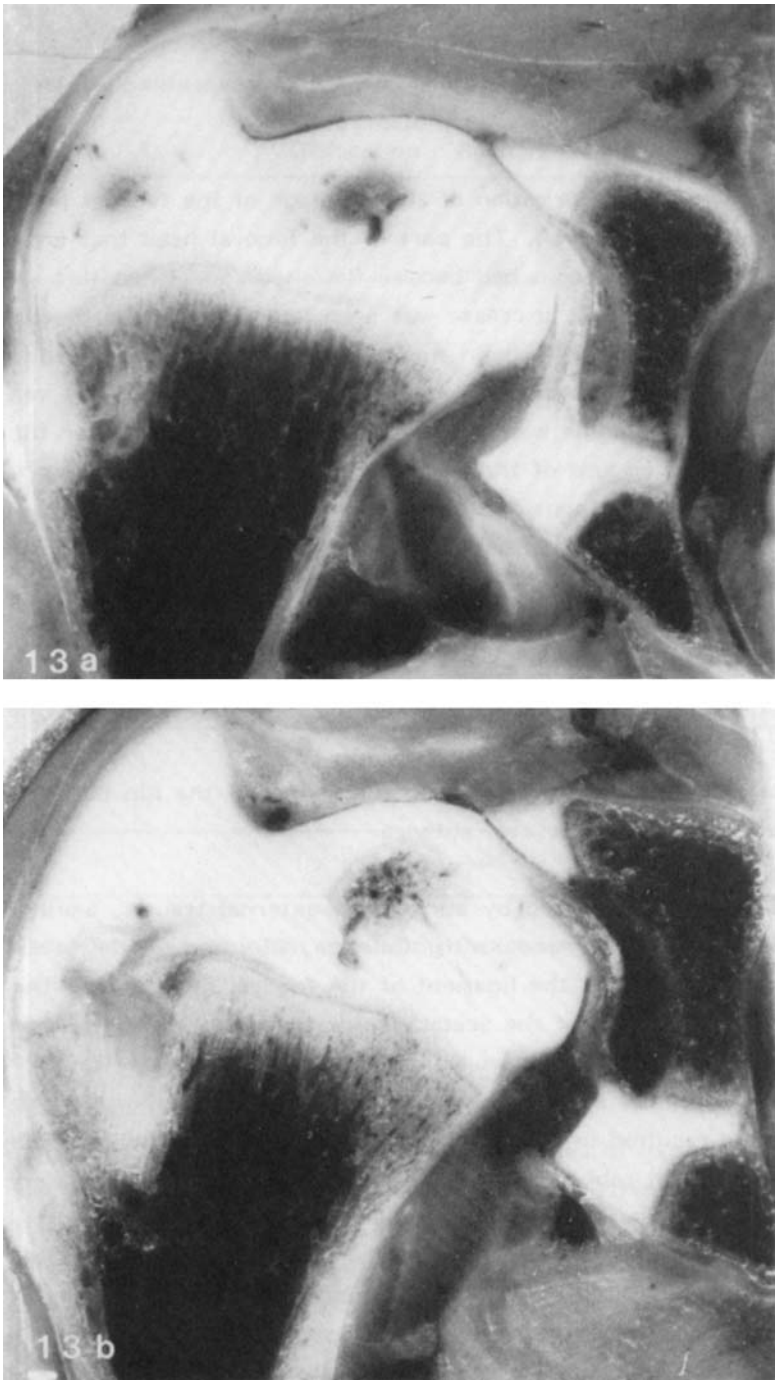


Figure 13. a) In vivo specimen with moderate dislocation of the femoral head. Dh = 0.182 d. There is a slight flattening of the femoral head and backward curving of the acetabular margin. Compare with the normal hip in Figure 4. b) In vitro specimen subjected to a load of 0.5 x W (0.65 N). The dislocation is somewhat more pronounced (Dh = 0.231 d), but otherwise the deformation is similar to that in Figure 13 a.

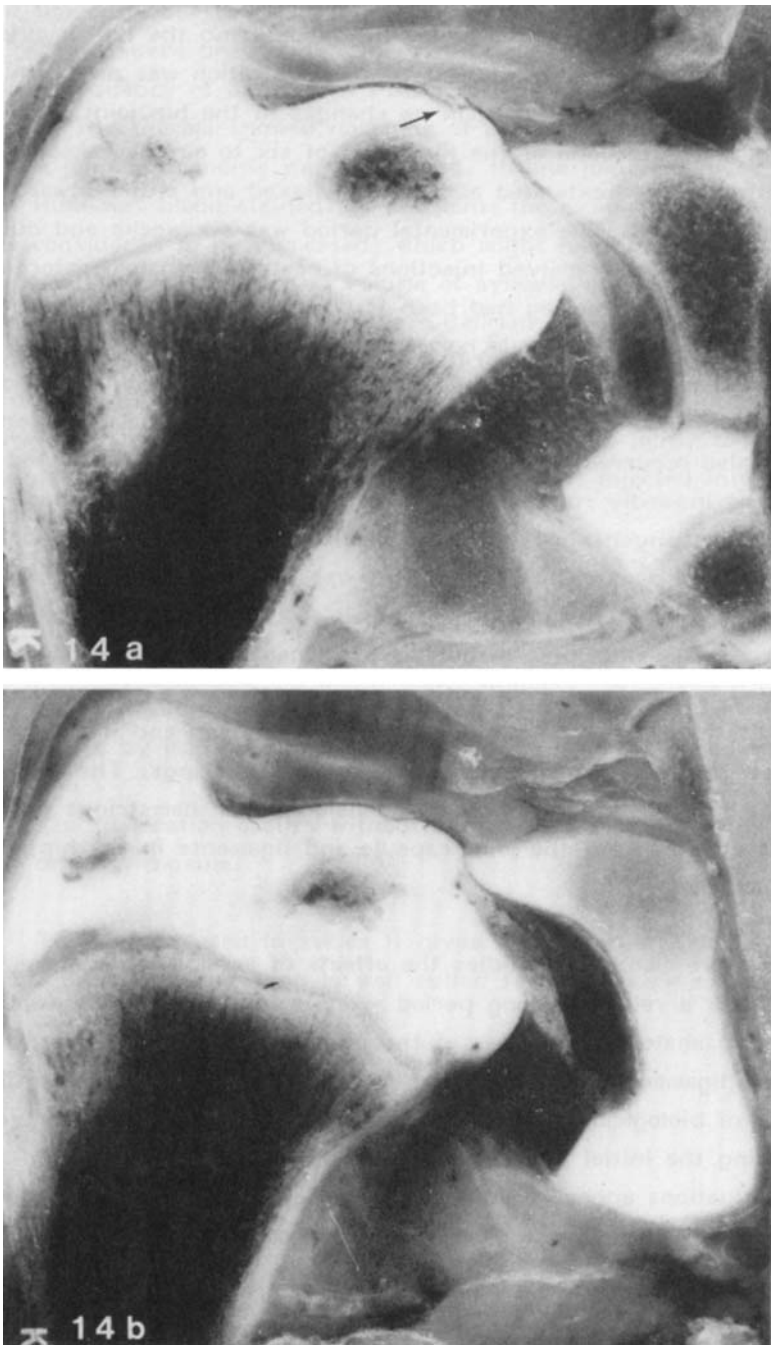


Figure 14. a) In vivo specimen with pronounced dislocation of the femoral head. Dh = 0.420 d. The deformation of the femoral head is more marked than in Figure 13 a and a distinct crease is seen (→). b) In vitro specimen with corresponding deformation and dislocation. Dh = 0.418 d. A load of 1.0 x W (1.5 N) had been applied to the hip.

hind legs of three-week-old rats with the ankle, knee and hip in extension, and observed dislocation after 10 weeks. The author considered the dislocation to have been caused by the hip extension. Salter (1966) induced hip dysplasia in newborn pigs by immobilizing the hip in extension. The changes were reversible if the immobilization was discontinued. Immobilization in flexion resulted in no changes in the hip joint. Wilkinson (1963) immobilized one of the hind legs of six to eight week old rabbits with the knee extended and the hip flexed and either inwardly or outwardly rotated. The experimental period was six weeks and during this time some animals received injections of oestrone and progesterone. Among the animals whose leg had been fixed in outward rotation, dislocation only occurred in female hormone-treated ones. Wilkinson attributed the dislocation mainly to the position of the immobilized leg and the laxity of the joint resulting from the hormone treatment. However, dislocation also occurred in a few animals which had been immobilized with the legs inwardly rotated and which had received no hormones. Michelsson and Langenskiöld (1972) immobilized the knee in extension in rabbits 7 - 60 days old. The hip joint was freely movable, but was held spontaneously in flexion. In almost all of the youngest rabbits dislocation occurred within one to four weeks. When the hamstring muscles were divided before the immobilization, no dislocation was observed. Normally rabbits hold the knee joint in pronounced flexion and if the knee is extended, increased tension develops in the hamstrings. The authors therefore concluded that the increased tension of the hamstrings produced a slow stretching of the joint capsule and ligaments in the hip, leading to dislocation.

In all the above-mentioned studies the effects of immobilization were examined after a relatively long period - one week to several months. The observed anatomical changes of the femoral head, acetabulum, joint capsule and ligaments have to a large extent been secondary, with distinct signs of biological remodelling. Short-term studies have their value in elucidating the initial phase of the development of hip dislocation. No such investigations appear to have been carried out previously and we have therefore no other results with which to compare our findings.

By using ketamine anaesthesia and killing the animal by instantaneous deep freezing, the muscle tone was maintained throughout the experiment. Rabbits as young as was feasible were used, since the hip joints are quickly mineralized and the possibility of cartilage deformation therefore decreases with time. Michelsson and Langenskiöld (1972) also noted

the highest frequency of dislocation in one to three week old rabbits. By fixing the immobilized extremities to one another in inward rotation and flexion under the abdomen, abduction and extreme outward rotation of the hips were prevented - in series I these latter positions gave a lower frequency of dislocation (Tables IV and V). The method seemed gentle, the animals showed no signs of pain and no macroscopic injuries to the joint components were observed. In one joint, however, the synovial fluid was blood-stained. In two joints the amount of synovial fluid was considered to be increased, which might have been a sign of synovial irritation. An increased volume of synovial fluid can in itself contribute to greater instability. Lust, Beilman, Dueland and Farrel (1980) and Lust, Beilman and Rendano (1980) found that this volume was larger in dogs with subluxated hips than in those with normal hips. When the synovial fluid was aspirated from a subluxated hip joint, the instability diminished, When, on the other hand, fluid was injected into intact, normal hip joints, subluxation ensued.

Conclusions

1 Hip dislocation and deformation can be induced in vivo in three hours by immobilization alone.

2 The dislocation occurs without macroscopic damage to the ligaments or joint capsule.

3 The method used in series II gives dislocation and deformation in a high frequency and is well suited for short-term experiments.

COMPARISON BETWEEN THE FINDINGS IN THE IN VIVO AND IN VITRO INVESTIGATIONS

The aim of this comparison was

to see whether the same type and degree of dislocation could be induced with the in vitro method as with the in vivo one, and if so,

to determine approximately the magnitude of the force that causes the dislocation in vivo.

By comparing the results from the in vivo series II and the in vitro series, these factors could be analysed. The material and methods have been presented separately.

Result

Twelve of the 28 hips in vitro showed a dislocation corresponding to that in the in vivo cases, whereas in the other 16 it was of a greater degree. In hips with approximately the same dislocation, the type of deformation was also found to be similar in the two series, as seen in Figures 13 and 14. As demonstrated previously, in the in vitro series there was an approximately logarithmic relationship between load and dislocation. In the in vivo series the dislocation was known, but not the force. However, the force acting in vivo could be determined approximately by the use of data from the in vitro series (see Figs 6 and 7). According to these calculations the dislocating forces in the in vivo series varied between 0.25 x W and 0.85 x W, corresponding to 0.35 to 1.3 N.

Discussion

This comparison showed that with the in vitro method changes could be induced corresponding to those found in the in vivo series II. The in vivo method is more physiological, since the hip dislocation is produced in living animals subjected solely to immobilization of the hind legs. A drawback of the method is that the dislocating force cannot be regulated - that is, raised above a certain level - or measured by

direct methods. Furthermore, the method can only be used in animal experiments. The in vitro method used on fresh specimens was found to give similar results and here the force can be regulated as desired, both with respect to magnitude and to orientation. The examiner can thus control the experimental conditions. An important advantage is that the method can be used on human specimens. However, such investigations cannot be performed on fresh specimens, but only on those that have been stored in one way or another for one to several days. This raises the question whether the storage might alter the mechanical properties of the specimen, a problem which led to a special study of these conditions.

The cartilage deformation and the extension of the ligaments and joint capsule that are observed in short-term experiments may conceivably reflect the initial stage in the development of CDH. The changes, at least in the in vivo experiments, are probably reversible if the dislocating force is removed.

In in vivo experiments, when the immoblization had been discontinued after one to three weeks, Michelsson and Langenskiöld (1972) noted normalization of the hip joints in about 10 per cent of the animals. It should be pointed out, however, that this was a question of regression of pronounced skeletal changes and that the normalization took place during the course of several weeks. Salter (1966) immobilized the hind legs of pigs and observed dysplasia of the acetabulum after six weeks. The dysplasia was reversible if the immobilization was discontinued. Concerning hip instability in newborn infants, Barlow (1962) found that over 60 per cent recovered in the first week of life and 88 per cent in the first two months. MacKenzie and Wilson (1981) reported spontaneous normalization of 47 per cent of infants with hip instability within four weeks. Clinically we know that there is laxity of the joint at birth, but anatomically we do not know if there is any dysplasia.

In in vitro indentation tests on articular cartilage from adult individuals, under optimal experimental conditions, the cartilage completely recovered after removal of the load (Elmore, Sokoloff, Norris & Carmeci 1963, Kempson, Freeman & Swanson 1971). Takei (1979) loaded human knee specimens from adults. The specimens were frozen under load, and deformation of the articular cartilage was observed. When the specimens were thawed, the cartilage regained its original shape. In tension tests on the medial collateral ligament from adult dogs, Woo, Gomez and Ake-

son (1981) found complete regression of the deformation after removal of the load.

There seems to be a difference in the restoration of the tissues, however, between immature and adult individuals. In indentation tests on costal cartilage, Göcke (1928) observed considerably greater residual deformation in specimens from a six-month-old child than in those from a 40-year-old. Rollhäuser (1950) found in tension tests on tendons that the residual deformation after unloading was greater in tendons from newborns than in those from adults. We also found in our tension tests on the ligament of the femoral head that the recovery was not complete (Hjelmstedt, Olofsson & Asplund 1981). As mentioned previously (Verzár 1962), the degree of cross-linking in collagen from newborn calves is very low and increases rapidly with age. According to Ferry (1961), the creep recovery is not complete for uncross-linked polymers. This should indicate that full restoration does not take place after extension of immature collagen tissue. The purely mechanical properties of the tissues are not the only factors of importance for normalization, however. Biological adaptation will almost certainly take place when the forces around a joint are altered.

Conclusions

1 With the in vitro method hip deformation and dislocation of the same type and degree as with the in vivo method can be induced in fresh specimens.

2 The dislocating forces in vivo can be estimated approximately at 0.25 x W to 0.85 x W, corresponding to 0.35 to 1.3 N.

EXPERIMENTAL STUDIES OF THE EFFECT OF POST-MORTEM STORAGE

For obvious reasons biomechanical studies on human specimens cannot be performed immediately post-mortem. Usually the specimens are not available until after a few days and as a rule they have then been stored at $+4^{o}$ C. If the experiment cannot be done straight away, it is usual to store the specimen at -20^{o} C until required.

The aim of this in vitro study in rabbits was to examine the influence of post-mortem storage on the degree and type of deformation and the magnitude of dislocation induced.

Experimental set-up

Thirty rabbits were divided into 10 test groups. Each group comprised three animals from the same litter and of approximately the same body weight. The three animals were killed simultaneously and the hips from one of them were subjected to a load within one hour, as described in the section on in vitro methods. The other two animals were stored at $+4^{o}$ C for five to six days. Load test was then performed on one of these animals and the other one was placed in a freeze box at -20^{o} C. After a further 21 - 30 days the frozen animal was slowly thawed at $+4^{o}$ C. When the animal had assumed room temperature, a load was applied to the hip joints. As in the in vitro series, the load was related to the animal's weight W. This relative load L_r was of the same magnitude in each test group. The material was divided into five load categories, namely 0.75 x W, 1.0 x W, 1.5 x W, 2.0 x W and 2.5 x W, with two test groups in each category (Table VI). Expressed in newtons, the load (L_n) was 1.2 - 5.0 N. The experimental period was three hours. In all experiments the hip joint was in 90^{o} flexion, $0 - 3^{o}$ abduction and $0 - 5^{o}$ outward rotation at loading.

In this way all 60 hip joints were loaded, with 20 hips in each of the three storage classes: 1) fresh specimens, 2) refrigerated specimens and 3) frozen specimens. All specimens were cryosectioned and the dislocation was measured as previously described.

Table VI. Distribution of the specimens into load categories and storage classes in the storage series

Storage class	Relative load				
	0.75xW	1.0xW	1.5xW	2.0xW	2.5xW
Fresh specimens	2	2	2	2	2
Refrigerated specimens	2	2	2	2	2
Frozen specimens	2	2	2	2	2

Results

By the division into test groups comprising three comparable animals that were subjected to the same relative load and by allocating one animal to each storage class, a direct comparison between the dislocation in the three storage classes could be made. Table VII presents the mean animal weight (W), magnitude of the load in newtons (L_n), and dislocation (Da) in the three storage classes. The dislocation increased with the magnitude of the relative load in all classes. In Figure 15 this is illustrated graphically as a function of the logarithm of the relative load (L_r). The mean Da for each animal is given in the figure and the regression lines were calculated from these values. The scatter of the results from the fresh specimens was relatively low, while the refrigerated and frozen specimens showed greater scatter. At analysis of variance a statistically significant difference ($p < 0.01$) in dislocation was found between the storage classes.

Table VII. Mean weight, load and dislocation in the three storage classes

Storage class		Weight (N) mean	Load (N) mean	Dislocation Da (d) mean
Fresh specimens	(n=10)	1.74	2.8	0.551
Refrigerated specimens	(n=10)	1.74	2.8	0.623
Frozen specimens	(n=10)	1.75	2.8	0.734

n = number of animals

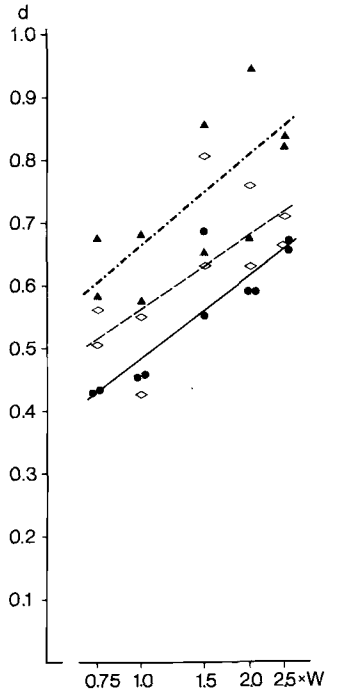


Figure 15. The relation between relative load and dislocation in the storage study. Logarithmic scale (natural logarithms). The dislocation is given as Da, for which the numerical values are presented in Table XI in the Appendix.

	Da	Regression line				
Fresh sp.	●	———	*a=0.485*	*b=0.19*	*r=0.89*	S_{yx} *=0.049*
Refrigerated sp.	◇	— — —	*a=0.563*	*b=0.17*	*r=0.69*	S_{yx} *=0.088*
Frozen sp.	▲	—·—·—	*a=0.664*	*b=0.21*	*r=0.73*	S_{yx} *=0.095*

Note the difference in scatter between the storage classes.

The deformation of the femoral head and acetabulum increased with increasing load in all storage classes. The difference between the fresh specimens and the refrigerated specimens was small, while the frozen specimens displayed the greatest deformation. Often the frozen specimens had imbibed blood and the joint cavity was filled with sanguinolent fluid. In the specimens subjected to the highest load, striation of the femoral head cartilage was also seen (Fig. 16 c). Otherwise, in no instance were macroscopic injuries to the ligament or joint capsule observed. Three hips from the same test group are illustrated in Figure 16, where the differences in dislocation and deformation between the storage classes are clearly evident.

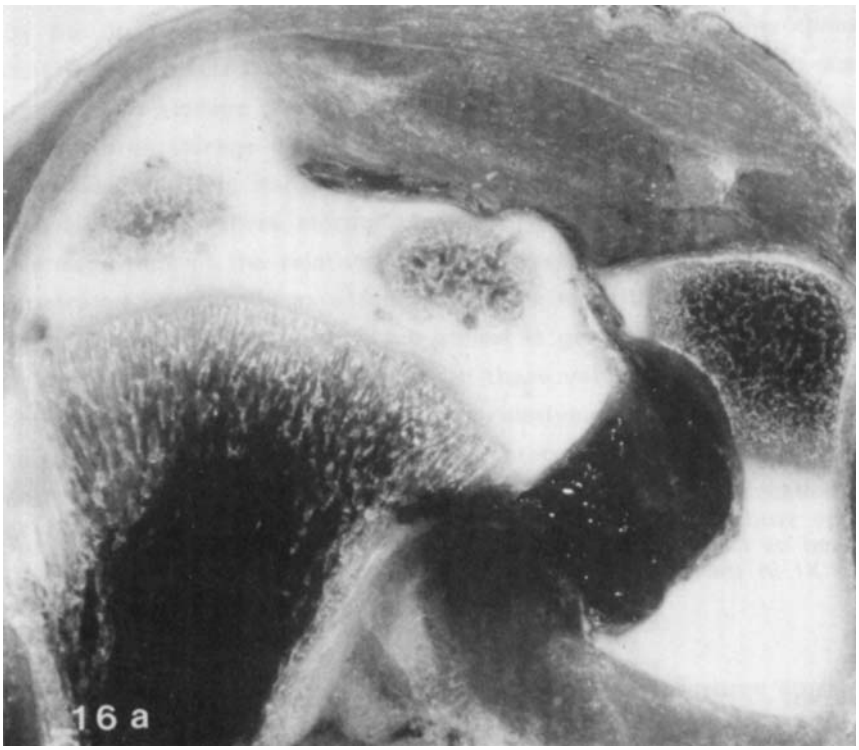
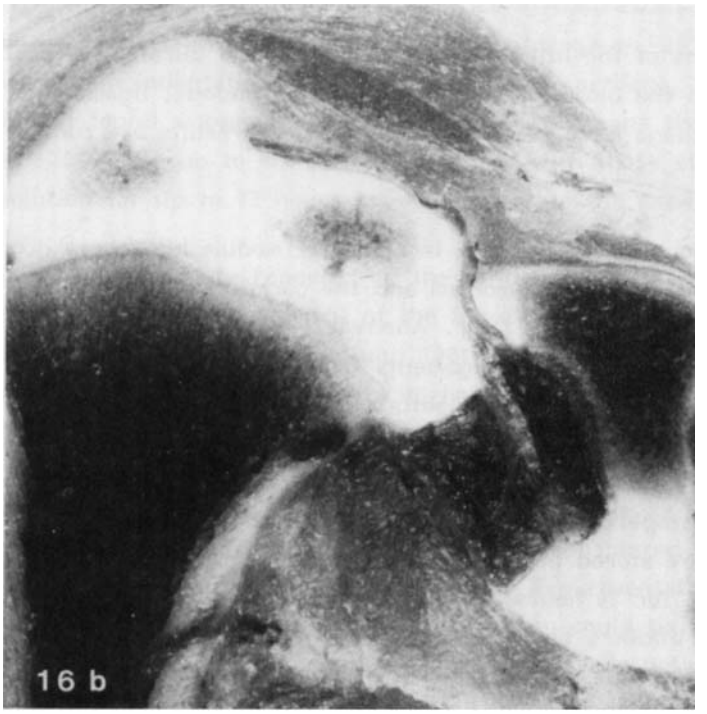


Figure 16. Results of storage experiments with a relative load of 2.5 x W in a test group of three rabbits. a) Cryosection of specimen loaded within one hour post-mortem. b) Cryosection of specimen stored at +4° C for six days prior to loading. c) Cryosection of specimen stored at +4° C for six days and at -20° C for a further 25 days prior to loading.

The dislocation (Dh) was 0.649 d in a) 0.731 d in b) and 0.883 d in c). The cartilage deformation was least pronounced in the fresh specimen and most pronounced in the specimen that had been frozen. Note the striation in specimen c) (→).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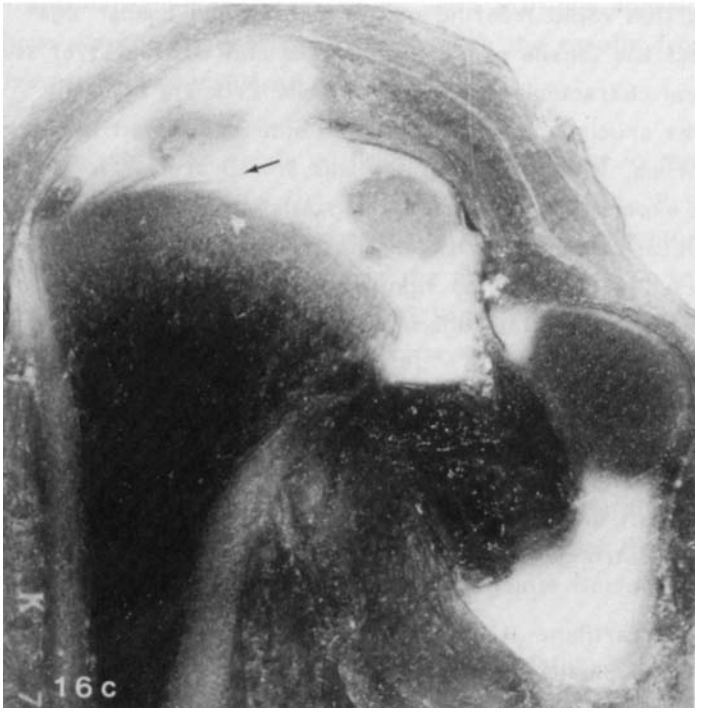
16 c

Discussion

Many investigations of the influence of the method and duration of post-mortem storage on the biomechanical properties of tendons, ligaments and articular cartilage have been reported, and the results are contradictory.

Wertheim (1847) found no difference in elasticity module between a dog tendon tested immediately post-mortem and the corresponding contralateral tendon tested five days later. Annovazzi (1928) found that the physical properties of knee joint ligaments from dogs altered rapidly post-mortem. Storage of intact knee joints from rabbits at room temperature for four days did not change the tensile strength of the cruciate ligaments (Viidik, Sandqvist & Mägi 1965). On the other hand, changes in biomechanical properties were found in all free-dissected cruciate ligaments that were stored in phsyiological saline at $+20^{o}$ C for five hours or at $+4^{o}$ C for 24 hours, or stored at -20^{o} C, compared with fresh specimens (Viidik & Lewin 1966). Galante (1967) studied specimens of annulus fibrosus and found that after five hours' storage in physiological saline at $+20^{o}$ C they became more extensible and the residual deformation was significantly greater. Rapid freezing to -60^{o} C did not, on the other hand, alter the tensile properties. Tkaczuk (1968) also demonstrated that rapid freezing of the longitudinal lumbar ligament did not affect the tensile properties. Noyes and Grood (1976) studied the mechanical characteristics of the anterior cruciate ligament from monkeys. The cruciate ligament from one side was tested within one hour post-mortem, while the other leg was stored at -15^{o} for four weeks before the experiment. There was no statistically significant difference in mechanical properties between the fresh and frozen specimens. Moss and Ferguson (1980) found no significant difference in tensile strength between bovine cruciate ligaments examined two days post-mortem and those examined after 5 - 18 days. In biomechanical studies on cruciate ligaments from monkeys, Barad, Cabaud and Rodrigo (1982) compared the effect of deep freezing to -80^{o} C with that of storage at $+4^{o}$ C for 24 hours. The right knee was compared with the left. No statistically significant difference was found, but the refrigerated specimens tended to be stronger and more rigid than the frozen ones.

Storage of articular cartilage at -20^{o} C for several weeks did not alter its fluid transport properties (Maroudas 1968). Kempson, Spivey, Swanson and Freeman (1971) performed indentation tests on femoral heads

from humans. They noted no difference in the results between testing 48 hours post-mortem and testing after storage at -20^{o} C for three weeks. In indentation tests on human tibial cartilage, Hori and Mockros (1976) found a lower elasticity module in specimens that had been stored at -20^{o} C for up to six weeks, compared with those stored in Ringer solution for up to 72 hours at $+4^{o}$ C prior to the experiment.

We have previously reported on the influence of post-mortem storage in tension test on the ligament of the femoral head from newborn infants (Hjelmstedt, Olofsson & Asplund 1981), but otherwise we have not found any reports on storage studies on immature tissue, nor on any biomechanical studies performed on whole joint specimens from newborns, apart from our human experiments described elsewhere (Hjelmstedt, Asplund & Rauschning 1982). Moreover, the results of previous storage studies have been contradictory, and we therefore considered it necessary to investigate the storage question with our experimental model, so that, above all, the results of experiments in man could be better evaluated. As mentioned earlier, comparisons had to be made between results from different animals, as it is not possible with our technique to load the right and left hips from the same animal on different occasions. By the division into test groups comprising three animals from the same litter and of approximately the same weight, and with one animal in each of the three storage classes, we tried to make the results from these storage classes as comparable as possible.

This investigation sheds no light on the changes in mechanical properties that may occur during the first hour post-mortem, or on alterations of these properties during experiments at room temperature. Matthews and Ellis (1968) found no change in the elasticity module in tendons from cats at repeated tests in the first three hours post-mortem. Neither did Woo, Gomez and Akeson (1981) observe any change in the mechanical properties of the collateral ligament from dog knees during the first six hours post-mortem. As mentioned previously, Viidik, Sandqvist and Mägi (1965) found no alteration of the tensile strength of the anterior cruciate ligament on storage of intact knee joints at room temperature for four days. These investigations indicate that there was no change of the mechanical properties of the hip joints during our experiments.

There was a significant difference in dislocation between the specimens stored in the different ways. The refrigerated specimens showed 13 per cent greater dislocation, on the average, than the fresh ones, and the

the frozen specimens were dislocated 18 per cent more than the refrigerated ones. The greatest difference, 33 per cent, was found between the frozen and fresh specimens. The scatter of the measurement data was also greater for the refrigerated and freezer-stored specimens than for the fresh ones. The correlation coefficients for the latter two storage classes were lower than for the fresh specimens. Thus, in in vitro experiments of this type fresh specimens should be used. With specimens that have been stored in a refrigerator a somewhat greater degree of deformation and dislocation is obtained. This has to be accepted, however, in in vitro experiments on human specimens. On the other hand, specimens that have been frozen should preferably not be used.

Conclusions

1 The mechanical properties of the specimens are altered after long periods of storage at $+4^{o}$ C and at -20^{o} C.

2 The experiments should, if possible, be performed on fresh specimens.

3 If this is not possible, specimens that have been stored intact at $+4^{o}$ C for up to six days can be accepted. The measurement data will then show a greater scatter and the deformation and dislocation will be somewhat increased. Following storage in the frozen state these errors will increase further and therefore experiments on previously frozen specimens should be avoided.

GENERAL DISCUSSION

Introduction

The cause of CDH is still unclear. A number of aetiological and pathogenetic factors have been suggested in the literature. It is generally considered nowadays that CDH does not have one single cause but is the result of several interacting factors or a sequence of events.

Genetic, racial and ethnic factors all play a certain role. Anatomical deviations such as an increased anteversion angle of the femoral neck, primary acetabular dysplasia, or laxity of the joint capsule and ligaments, either primary or hormone-induced, have also been attributed some significance. Mechanical factors that exert their action in utero, during delivery or post-partum have been pointed out as possible causes, especially in recent times. Dunn (1972, 1974, 1976 a) regarded CDH as a congenital deformity and came to the conclusions "that quite gentle forces, if persistently applied, are capable of producing deformities; that the foetus is particularly vulnerable because of its extremely rapid rate of growth and because of its relative plasticity; that while prenatal deforming forces might on occasion be of intrinsic origin due to muscle imbalance, most foetuses are exposed to extrinsic forces in the later weeks of pregnancy because of their increasing size and the diminishing volume of amniotic fluid".

Relevance of the in vitro experiments for the condition in living individuals

In vitro experiments constitute a substantial part of our experimental biomechanical study. Important questions are the relevance of these experiments for hip deformity and dislocation in living individuals, and the advantage of the in vitro method over in vivo studies. The latter might seem to correspond better to the clinical conditions than the in vitro experiments. Good agreement was found, however, between the in vivo and in vitro results in our short-term experiments with respect to deformation and dislocation. Thus the in vitro results from fresh specimens can be considered relevant for the in vivo conditions. The advantage of the in vitro method is that the magnitude and orientation of the dislocating force is known. The method also permits considerably greater

loading than is possible with the in vivo procedure. Furthermore, it provides an opportunity for biomechanical investigations also in man, where such in vivo experiments obviously cannot be performed.

It is seldom possible to carry out studies on completely fresh human specimens, and there is generally a preceding period of storage at $+4^{o}$ C. It may thus be asked whether the biomechanical properties alter during this storage period. In an attempt to answer this question a special study was conducted on the influence of storage on the degree of dislocation and deformation. The results showed that in specimens that had been stored at $+4^{o}$ C for five to six days the dislocation was 13 per cent greater than in fresh specimens. This difference is undesirably great, but nevertheless may be considered acceptable. On the other hand, storage in the frozen state can hardly be accepted, as in our experiments this resulted in 33 per cent greater dislocation than in completely fresh specimens.

Relation between force and dislocation

In the in vitro experiments an approximately linear relationship was found between the logarithm of the load and the degree of dislocation. This implies that a large increase in load will give a relatively smaller increase in dislocation. The acting force in vivo could not be measured, but had to be estimated on the basis of the observed dislocation. In doing this, it was hypothesized that a defined force gives an equivalent degree of dislocation, whether it acts on a hip in vivo or in vitro (see Figs 6 and 7). However, the hip specimens in the in vitro series had a temperature of 20^{o} C during the experiments, while the hips in vivo were of body temperature. This temperature difference might imply a difference in the biomechanical properties of the tissues. In indentation tests on articular cartilage, Elmore, Sokoloff, Normis and Carmeci (1963) found that temperature variations between 13 and 50^{o} C had no effect on the results. No corresponding investigations seem to have been performed on ligaments.

The dislocating forces acting in vivo were estimated at between 0.25 x W and 0.85 x W. These are moderate forces to which a child's hips very well might be exposed in utero, during delivery or on immobilization of the legs and hips that has been or still is the custom in certain ethnic groups. Thus hip instability and deformation in newborns may conceivably be caused by mechanical factors alone.

The importance of the time factor for the deformation and dislocation

In previous animal experimentation (Wilkinson 1963, Sijbrandij 1965, Salter 1966, Michelsson & Langenskiöld 1972) it has been found that hip dysplasia and/or dislocation can be induced by different types of immobilization of the knee and/or hip joint in young animals within a period of six days to six weeks. Both our in vivo and in vitro experiments showed that a considerable degree of deformation and dislocation can occur in three hours after immobilization alone or after application of a moderate load in seven to 12 day old rabbits. Some stretching of the capsule and ligaments also seems to take place, although this could not be measured here. In previous in vitro studies on human specimens (Hjelmstedt, Asplund & Rauschning 1982), however, laxity of the joint was observed after three hours of provocation, and experiments on the ligament of the femoral head (Hjelmstedt, Olofsson & Asplund 1981) have demonstrated that considerable elongation of the ligament occurs after moderate loading. As regards the time factor, this implies that laxity, deformation and dislocation could occur during a relatively short period in the breech position in utero or during a breech delivery. Whether it could also occur during delivery with a vertex presentation cannot be answered by our rabbit experiments, which were performed with the hips in pronounced flexion. Sijbrandij, however, induced dislocation and dysplasia in immobilization experiments with the hip in extension for 10 weeks. It is not known whether similar changes can occur in a very short time, but the conditions should be analogous with those at immobilization in flexion. Salter also induced dysplasia in pigs by immobilizing the hip and knee in extension, but found no changes when the hip was immobilized in flexion.

Our short-term experiments would therefore seem to give a reasonable explanation for the hip instability immediately post-partum. Deformation and dislocation that occur during a short period may be expected to regress if the biomechanical conditions are normalized. Spontaneous stabilization takes place in a short time in hip instability in humans. According to Barlow (1962), over 60 per cent become stabilized during the first week of life and 88 per cent within two months. In a large material of about 53 000 newborn infants MacKenzie and Wilson (1981) found spontaneous normalization of 1 341 out of 2 850 primarily unstable hips within four weeks. Thus it would seem to be the action of forces post-partum that decides whether normalization occurs or not.

If the deformation and dislocation persist for a long period, according to findings in animal experiments (Wilkinson 1963, Sijbrandij 1965, Michelsson & Langenskiöld 1972) and clinical experience (Campos da Paz & Karam Kalil 1976, Ogden & Moss 1978, Ponseti 1978) a remodelling of the hip joint, including the joint capsule, takes place. In animal experiments, Michelsson and Langenskiöld (1972) observed a relatively slow regression of the dysplasia in 10 per cent of the cases if the experiments were terminated within one to three weeks. Salter (1966) found that dysplasia was still reversible after six weeks of immobilization.

Time is an important factor in biomechanical studies of viscoelastic material. The methods employed here unfortunately do not permit a detailed study of the relation between duration of loading and deformation. On the other hand, tension tests on ligaments and indentation tests on cartilage give an idea of this relationship. Few such studies have been undertaken on neonatal tissue, which differs biomechanically from adult specimens. A more penetrating study of these factors is planned.

CONCLUSIONS

1 The method of inducing hip deformation and dislocation in vitro in human specimens is also applicable in corresponding investigations in young rabbits.

2 Deformation and dislocation can be induced in vitro in three hours with varying loads and without macroscopic damage to the joint capsule or ligaments.

3 The deformation of the femoral head and acetabulum increases with increasing load.

4 The magnitude of the dislocation is approximately proportional to the logarithm of the applied load.

5 Hip deformation and dislocation can also be induced in vivo in three hours solely by immobilization of the hind legs, and without macroscopic damage to the joint capsule or ligaments.

6 The method used in in vivo series II gives dislocation and deformation in a high frequency and is well suited for short-term experiments.

7 Hip deformation and dislocation induced in fresh specimens with the in vitro method are similar in type and degree to those produced by the in vivo method.

8 The dislocating forces in vivo are estimated approximately at 0.25 x W to 0.85 x W, corresponding to 0.35 to 1.3 N.

9 The mechanical properties of the specimens are altered by a long period of storage at $+4^{o}$ C or by storage at -20^{o} C.

10 In vitro experiments should, if possible, be performed on fresh specimens. If this is not feasible, specimens stored at $+4^{o}$ C for up to six days can be accepted. The measurement data will then show a greater scatter and somewhat increased deformation and dislocation.

After storage of the specimen in the frozen state these errors will be even greater, and therefore experiments on previously frozen specimens should be avoided.

SUMMARY

I In an in vitro study hip deformation and dislocation were induced in specimens from 8 - 11 day old rabbits by a method that had been used previously on human specimens (Hjelmstedt, Asplund & Rauschning 1982). The hip joints were loaded within one hour post-mortem in an experimental apparatus with a constant force, which was maintained for three hours. The load was varied, with respect to the weight W of the animals, between 0.25 x W and 2.5 x W, so that seven different load categories were obtained, with two animals in each category. At the end of the experiment the specimen was deep frozen while still under load. Dislocation and deformation occurred in all hips. The dislocation of the femoral head was measured on cryosections and was expressed in parts of the diameter of the femoral head. The degree of dislocation was approximately proportional to the logarithm of the applied load. The deformation increased with increasing load. No macroscopic damage to the joint components was observed.

II In an in vivo study hip dislocation and deformation were induced in a short period in 7 - 12 day old rabbits. An experimental model similar to that used by Wilkinson (1963) and Michelsson and Langenskiöld (1972) in long-term experiments was employed. The study was performed in two series. In series I the experimental conditions were varied and dislocation occurred in nine out of 16 hips. The importance of the degrees of rotation and abduction of the hip for the occurrence of dislocation was noted. On the basis of the results from this series, the experiments in series II were standardized. The knee joint was immobilized bilaterally in extension with a plastic splint and the two hind legs were fixed to each other in inward rotation and flexion with a strip of adhesive tape around the trunk. After three hours the rabbits were killed by shock-freezing in liquid nitrogen. The hips were cryosectioned and the dislocation was determined in the same way as in the in vitro experiments. Dislocation occurred in 18 out of 20 hips.

III At a comparison between the findings in in vivo series II and the in vitro series it was found that 12 of the 28 hips in vitro showed a dislocation corresponding to that in the in vivo cases, while in the other 16 the dislocation was of greater magnitude. In cases with approximately the same degree of dislocation the type of deformation was also similar in the two series. From data obtained in the in vitro series, the dislocating force acting in vivo was calculated to be between 0.25 x W and 0.85 x W, corresponding to 0.35 to 1.3 N.

IV In vitro experiments on human hip specimens cannot be performed immediately post-mortem. As a rule the specimens have been stored at $+4^{o}$ C for several days and sometimes they have even been stored in the deep-frozen state before the experiment. In order to assess the effect of post-mortem storage on the degree of dislocation and deformation, the following study was undertaken in rabbits. Thirty rabbits 7 - 12 days old were divided into 10 test groups, each group containing three animals from the same litter and of approximately the same body weight. The three animals were killed at the same time and the hips from one of them were loaded within one hour post-mortem. The other two animals were stored at $+4^{o}$ C for five to six days. Load test was then performed on one of these two animals and the other was stored at -20^{o} C. After a further 21 - 30 days the frozen animal was thawed and its hips were loaded. The relative load was of equal magnitude in each test group. A division into load categories was made as in the in vitro series, and the load was varied between 0.75 x W and 2.5 x W. The deformation was studied on cryosections and the degree of dislocation was determined. There was a significant difference in dislocation between the three storage classes - fresh specimens, refrigerated specimens and frozen specimens. The refrigerated specimens dislocated 13 per cent more and the frozen specimens 33 per cent more than the fresh specimens. The measurement data also showed that the scatter of the values was greater in the two former classes than in the fresh specimens. The deformation was greatest in the frozen specimens, while the refrigerated and fresh specimens were less deformed. Thus, in in vitro experiments fresh specimens should be used. If this is not possible, specimens that have been kept at $+4^{o}$ C can be accepted, but those that have been frozen should be avoided.

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Appendix. Table VIII. Detailed table of data in the in vitro series

Rabbit No.	Load (N)	Weight (N)	Relative load	Dislocation Dh, right (d)	Dislocation Dh, left (d)	Da (d)
48	0.3	1.2	0.25xW	0.174	0.139	0.157
64	0.4	1.7		0.121	0.236	0.179
37	0.65	1.3	0.5xW	0.231	0.264	0.248
42	0.75	1.5		0.251	0.353	0.302
58	1.25	1.65	0.75xW	0.406	0.461	0.434
67	1.25	1.7		0.437	0.354	0.396
41	1.5	1.5	1.0xW	0.418	0.400	0.409
52	1.5	1.5		0.437	0.473	0.455
31	2.7	1.8	1.5xW	0.495	0.610	0.553
55	3.15	2.1		0.705	0.664	0.685
25	4.0	2.05	2.0xW	0.575	0.602	0.589
61	2.8	1.45		0.580	0.596	0.588
65	3.9	1.6	2.5xW	0.711	0.730	0.721
66	3.9	1.55		0.728	0.718	0.723

mean difference $Dh_{right} - Dh_{left} = -0.023$ d SD = 0.044 d

Appendix. Table IX. Detailed table of data in in vivo series I

Rabbit No.	Side	Knee immobil.	Fixation in flex.	Fixation in inw. rot.	Hip joint's (degrees) Flex.	Abd.	Outw.rot.	Experimental period (hrs)	Dislocation Dh (d)
14	right	yes	no	no	90	2	?	0	0.205
	left	no							0.100
19	right	yes	yes	no	115	15	10	6	0.224
	left	yes	yes	no	115	10	10	6	0.020
20	right	yes	yes	no	120	10	15	3	0.390
	left	yes	yes	no	120	12	15	3	0.049
21	right	yes	yes	no	140	5	10	3	0.136
	left	no							0.052
22	right	yes	no	no	65	-5	20	3	0.096
	left	yes	no	no	75	7	20	3	0.232
24	right	yes	yes	no	90	5	30	3	0.162
	left	yes	yes	no	100	18	30	3	0.046
28	right	yes	yes	no	90	10	55	24	0.038
	left	yes	yes	no	90	-15	10	1 min	0.204
29	right	yes	no	no	100	4	35	24	0.024
	left	no							0.029
30	right	yes	yes	no	105	20	55	24	0.004
	left	no							0.035
34	right	yes	yes	yes	100	5	20	0.3	0.209
	left	yes	yes	yes	110	5	0	0.3	0.194

Appendix. Table X. Detailed table of data in in vivo series II

Rabbit No.	Side	Hip joint's Flex. (degrees)	Abd. (degrees)	Outw. rot. (degrees)	Dislocation Dh (d)
35	right	90	-7	0	0.295
	left	90	10	10	0.285
36	right	90	5	0	0.305
	left	80	8	10	0.287
38	right	90	-3	0	0.291
	left	90	-2	15	0.282
39	right	90	-7	-10	0.382
	left	95	-7	10	0.336
40	right	105	-4	10	0.420
	left	110	-4	10	0.431
43	right	90	-15	0	0.182
	left	90	-5	-5	0.207
44	right	105	-8	-5	0.291
	left	105	-7	-5	0.356
45	right	105	-12	0	0.281
	left	105	-4	-5	0.300
46	right	90	-12	-25	0.137
	left	90	12	-5	0.039
47	right	105	-11	-30	0.189
	left	100	0	5	0.100

Appendix. Table XI. Detailed table of data in the storage series

Test group	Rabbit No.	Storage +4° C (24 h)	Storage −20° C (24 h)	Load (N)	Weight (N)	Relative load	Dislocation Dh, right (d)	Dislocation Dh, left (d)	Dislocation Da (d)
	58	–	–	1.25	1.65		0.406	0.461	0.434
1	59	5	–	1.25	1.65	0.75 x W	0.538	0.586	0.562
	60	5	24	1.4	1.8		0.633	0.717	0.675
	85	–	–	1.2	1.55		0.415	0.442	0.428
2	86	5	–	1.2	1.55	0.75 x W	0.507	0.507	0.507
	87	5	22	1.2	1.5		0.589	0.574	0.582
	52	–	–	1.5	1.5		0.437	0.473	0.455
3	53	6	–	1.5	1.5	1.0 x W	0.538	0.559	0.549
	54	6	29	1.5	1.5		0.742	0.616	0.679
	81	–	–	1.7	1.6		0.425	0.492	0.459
4	82	5	–	1.7	1.65	1.0 x W	0.391	0.455	0.423
	83	5	22	1.7	1.65		0.544	0.603	0.574
	31	–	–	2.7	1.8		0.495	0.610	0.553
5	32	6	–	2.7	1.8	1.5 x W	0.645	0.616	0.631
	33	6	21	2.95	1.95		0.687	0.616	0.652
	55	–	–	3.15	2.1		0.705	0.664	0.685
6	56	6	–	3.2	2.2	1.5 x W	0.803	0.804	0.804
	57	6	30	2.7	1.8		0.875	0.833	0.854
	25	–	–	4.0	2.05		0.575	0.602	0.589
7	26	6	–	3.8	1.9	2.0 x W	0.624	0.636	0.630
	27	6	28	4.2	2.1		0.662	0.688	0.675
	61	–	–	2.8	1.45		0.580	0.596	0.588
8	62	5	–	3.0	1.5	2.0 x W	0.833	0.680	0.756
	63	5	25	3.0	1.55		0.907	0.985	0.946
	75	–	–	4.8	1.85		0.649	0.656	0.653
9	76	6	–	5.0	1.95	2.5 x W	0.731	0.685	0.708
	77	6	25	4.3	1.7		0.883	0.886	0.885
	78	–	–	4.7	1.8		0.664	0.672	0.668
10	79	6	–	4.3	1.7	2.5 x W	0.662	0.661	0.662
	80	6	25	4.9	1.9		0.818	0.825	0.822

Mean difference $Dh_{right} - Dh_{left} = -0.008$ d. SD = 0.040 d