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Changes in oxidative activities of chondrocytes during the early development of natural murine osteoarthritis

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Summary. A high incidence of natural osteoarthritis of the knee joint is found in male mice of the STR/ORT strain. The condition affects mainly the medial tibial cartilage and by the age of 27 weeks most male mice of this strain show some osteoarthritic change. Analysis of the oxidative metabolism of the chondrocytes during the development of the lesion has been facilitated by the techniques of quantitative cytochemistry. The activity of glucose-6-phosphate dehydrogenase (G6PD) has been investigated as indicative of the NADPH-generating pentose-phosphate pathway; the activities of glyceraldehyde-3-phosphate (G3PD) and lactate dehydrogenase (LDH) have been studied as indicators of glycolytic activity. In young STR/ORT mice the G6PD activity of the lateral tibial cartilage was greater and more variable than in the control mice of the CBA/HT6 strain. The activity in the medial cartilage, relative to that in the lateral cartilage, decreased with age; this change was not reflected in the activities of the other enzymes. In the lateral cartilage, the expected relationship was found between the G6PD and the G3PD activities and between the LDH and the G3PD activities. In the medial cartilage, the G6PD activities were not related to the G3PD activities.

The decreased proportionality of the G6PD activities in the medial cartilage as against that in the lateral cartilage was detected in mice as young as 9 weeks; by 27 weeks of age nine of the 13 mice showed marked depression of medial as against lateral G6PD activities. In contrast, only four of the 13 mice showed any overt histological change until up to the age of 28 weeks.

Keywords: osteoarthritis, cartilage, quantitative cytochemistry, glucose-6-phosphate dehydrogenase

The timing and the histological changes associated with the natural development of osteoarthritis in the males of the STR/ORT strain of mouse have been studied in detail by Walton (1977a, b; 1979). He showed that there were few male mice over the age of 6 months who had not developed an arthropathy of the knee joint as assessed histologically. He suggested that the development of osteoarthritis in this strain of mouse might be related to the displacement of the patella that is a marked feature of these animals. However, in a study of 78 mice of various ages he showed that 40 mice had osteoarthritis and a displaced patella but 19 showed no displacement even though they had deve-

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loped osteoarthritis. It was therefore considered advisable to test whether, in addition, metabolic abnormalities might underlie the very high incidence of osteoarthritis in this strain of mouse.

A striking feature of the development of osteoarthritis in this mouse, as recorded by Walton (1977a, b; 1979) was that, initially at least, it affected specifically the medial tibial articular cartilage. It seemed relevant to examine the glucose-6-phosphate dehydrogenase (G6PD) activity, which is the controlling step of the pentose-phosphate pathway, since the dehydrogenases of this pathway are the main source of NADPH which is essential for many of the biosynthetic mechanisms that are required for the maintenance of tissues (Chayen & Bitensky 1982). For comparison, the activity of glyceraldehyde-3-phosphate dehydrogenase (G3PD) has been tested, since the activities both of the early part of the Embden–Meyerhof pathway and of the pentose-phosphate pathway link with this enzymatic activity (Krebs & Kornberg 1957); lactate dehydrogenase (LDH) activity has been assessed as an indicator of maximal glycolytic activity.

The medial tibial cartilage, in which the osteoarthritic lesion begins, is very small (up to 120 μm deep). Moreover, the cells of the different zones, from the surface to the bone, may have different metabolic activities as has already been shown for canine articular cartilage (Dunham *et al.* 1986). Consequently, recourse was made to the techniques of quantitative cytochemistry which, at the level of the individual cell, have yielded results in other tissues that are quantitatively comparable to those that are achieved by conventional biochemical procedures (Chayen 1984).

Methods and materials

Seventeen mice of the STR/ORT strain aged between 4 and 52 weeks and 22 mice of the CBA/HT6 strain aged between 7 and 60 weeks were killed by asphyxiation with nitrogen. The knee joints were dissected out with the associated muscle. The patella was marked to facilitate correct orientation of the joint. They were soaked in a 5% aqueous solution of polyvinyl alcohol (PVA) for 5 min and were then chilled to −70°C by precipitate immersion in n-hexane (BDH grade low in aromatic hydrocarbons) surrounded by a mixture of industrial spirit and crushed CO_2 ice. The joints were then mounted onto microtome chucks with the patella uppermost, using 5% PVA as the adhesive. Sections were cut at 10 μm thickness in a Bright's cryostat with the microtome fitted with a tungsten-carbide knife (Johnstone 1979). Care was taken to ensure that the sections contained full depths of medial and lateral tibial cartilages. The sections were reacted for the three enzymatic activities (as below). Histological observations were made on sections stained with toluidine blue and by phase contrast microscopy of unstained sections.

For assaying the activity of glucose-6-phosphate, lactate and glyceraldehyde-3-phosphate dehydrogenases (G6PD, LDH and G3PD respectively), the reaction medium consisted of 30% polyvinyl alcohol (PVA grade G04/140, Wacker Chemicals Ltd.) in 0·05 M glycyl glycine buffer and 3·0 mM nitroblue tetrazolium. This use of PVA has been shown to retain these 'soluble' enzymes within such sections (Chayen 1978). For measuring total dehydrogenase activity, phenazine methosulphate (0·7 mM) was added just before use. For glucose-6-phosphate dehydrogenase activity the medium contained 5 mM glucose-6-phosphate monosodium salt and 3 mM NADP (pH 8·0); for lactate dehydrogenase activity, 5 mM sodium lactate and 2·5 mM NAD (pH 8·0). To assay glyceraldehyde-3-phosphate dehydrogenase activity, 5 mM fructose-1, 6-diphosphate (Boehringer) was used as the primary substrate; 10 U/ml aldolase were added to the reaction medium containing 1·5 mM NAD at pH 8·5 and left at 37°C for 20 min before use (Henderson 1976). Unless otherwise specified, all reagents were

obtained from Sigma; coenzymes were obtained from Boehringer.

Measurement

The coloured enzyme reaction product was measured with a Vickers M85A scanning and integrating microdensitometer with a ×40 objective; spot diameter of 0·5 μm in the plane of the section; and at a wavelength of 585 nm (Butcher & Altman 1973). A mask which encompassed individual cells was selected and the results were expressed in absolute units of extinction (MIE × 100) per cell. The enzyme activities were all shown to be linear with time. For expressing the mean activity of either medial or lateral tibial cartilage, 20 cells in duplicate sections were measured in zone 2 (that zone that lay below the outer three cells that lined the articular surface). The measurements were made across the whole width of the cartilage. The correlation coefficient (Snedecor & Cochran 1967) was determined to define the probability of correlation between the measurements.

Results

Histology

In the articular cartilage of the knee joint of the STR/ORT mice the zones 1, 2 and 3 (Dunham *et al.* 1986) were discernible in the lateral cartilage although zone 3 became less clear, with few cells, with increasing age. A similar change was found in CBA mice of equivalent ages. As regards the medial cartilage in the STR/ORT mice, the zonation was less clear, with only zone 1, which occupied the superficial 10–15 μm, being clearly definable.

The full depth of both the lateral and medial cartilages of the STR/ORT mice was consistently about 120 μm. This could be used as a criterion of the angle of sectioning, to avoid diagonal sectioning.

In the STR/ORT mouse the osteoarthritic lesion appears initially on the medial cartilage close to the junction with the cruciate ligament. In our studies two out of ten animals showed some histological changes by 21 weeks, but in the age groups from 28 to 52 weeks this was present in three of the four animals. In the CBA mice only one animal (aged 36 weeks) showed any significant cartilage fibrillation.

The initial histological change seen in the medial cartilage of the STR/ORT mice was cracking of the articular surface. With increasing age the fibrillations progressed to deep fissures and the number of histologically intact cells within the matrix decreased. Severe fibrillations were seen from around 30 weeks onwards. In some animals almost total erosion of the medial cartilage was seen at 12 months of age.

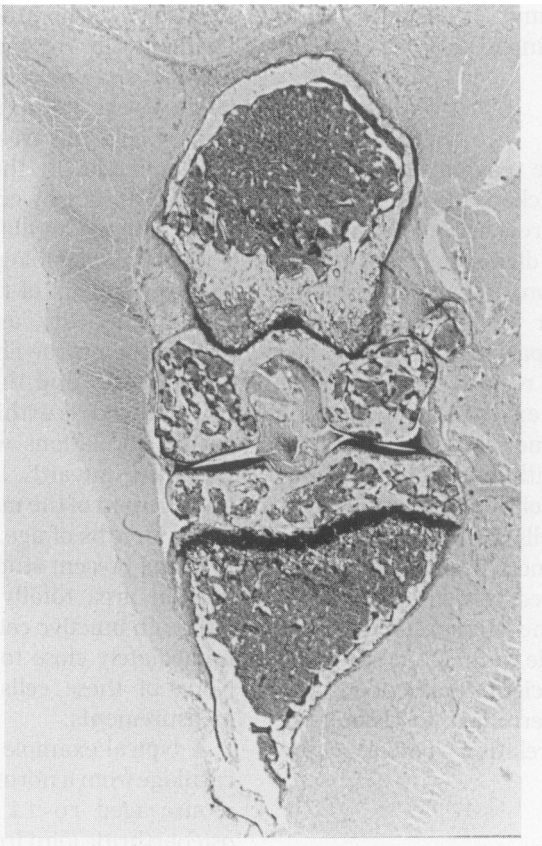
In the present studies, only one specimen had an area totally devoid of active cells although inactive cells were found generally immediately close to an area of fibrillation. None of these cells was included in the measurements.

A typical example of the medial articular cartilage from a normal joint, in an STR/ORT mouse aged 10–12 weeks, and one of an osteoarthritic joint from an STR/ORT mouse, 34 weeks of age, are shown in Fig. 1.

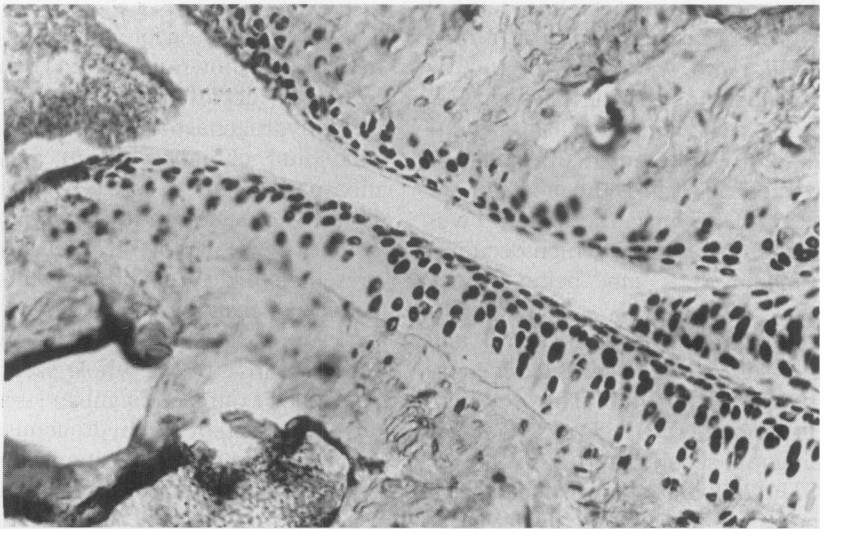
Method of measuring enzymatic activities

The procedure used for measuring enzymatic activities is shown in Fig. 2. The amount of formazan, deposited as the consequence of the dehydrogenase activity, was measured in individual chondrocytes in zone 2 at a measured distance (between 70 and 80 μm) from the outside rim to the tibial notch; the activity for each of the measured cells is presented in Fig. 2; in other results the mean activity of each dehydrogenase is presented. In general, cells of this zone were fairly representative of the whole section. In this particular example, the mean (±s.e.m.) glucose-6-phosphate dehydrogenase activity was 39±5·3 microdensitometric units per cell in the lateral cartilage as against 16±1·9 in the medial cartilage. The equiva-

(a)



(b)



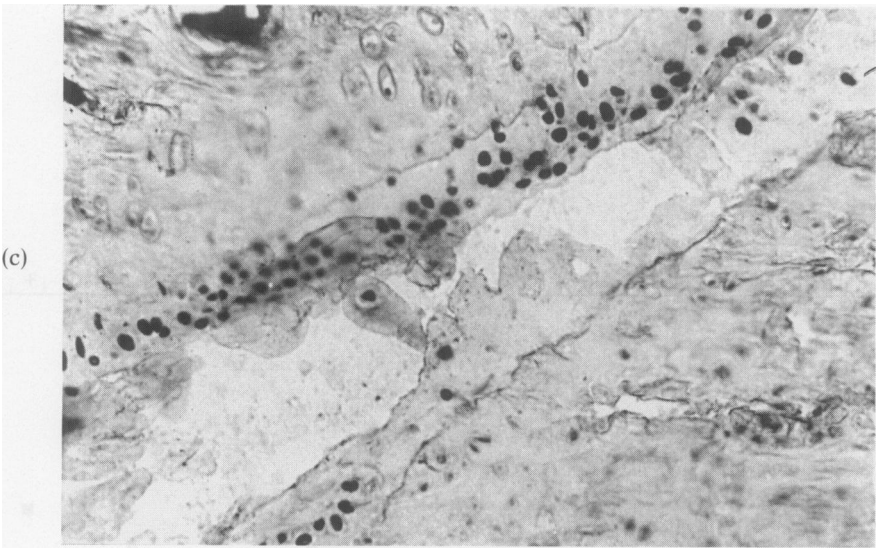


Fig. 1. (*a*) Low power view (× 12) of a 10 μm section of an intact knee joint of an STR/ORT mouse (11 weeks of age); stained with toluidine blue. The tibial articular cartilage is well stained; the tibial growth plate just below this is even more intensely stained. The growth plate and articular cartilage of the femur are cut at an angle due to the natural flexure of the joint. (*b*) Higher power view of a serial section to that in Fig. 1a, showing the medial tibial articular cartilage, and also the femoral articular cartilage (× 180). This section had been reacted for lactate dehydrogenase activity. (*c*) Section of the knee joint of an STR/ORT mouse of 34 weeks, reacted for lactate dehydrogenase activity (× 180). The chondrocytes of the femoral articular cartilage show good activity; the medial tibial cartilage has been largely eroded.

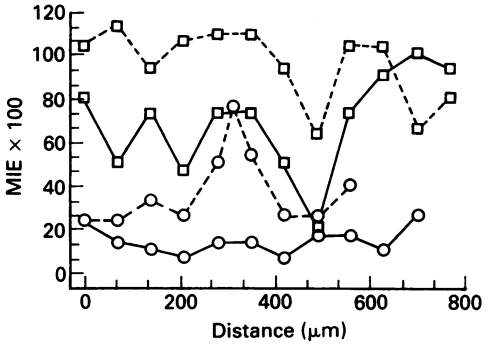


Fig. 2. Activity (MIE × 100) of lactate dehydrogenase (□) and of glucose-6-phosphate dehydrogenase (○) at specified distances from the outside rim to the tibial notch in chondrocytes of the lateral (broken lines) and the medial (solid lines) articular cartilages of a 14-week STR/ORT mouse.

lent values for lactate dehydrogenase activity were 96 ± 4·4 and 73 ± 4·6 units.

Enzymatic activities

Glucose-6-phosphate dehydrogenase (G6PD) activity. In the CBA strain of mouse the G6PD activity per cell in both the medial and lateral cartilages of the tibial plateau remained fairly constant, with some suggestion of a rise in activity in the cells of the lateral cartilage with increasing age (Fig. 3). Up to the age of 32 weeks the activity in the medial cartilage was greater than that found in the lateral cartilage of the same tibial plateau.

In contrast, in the STR mice, the activity in the lateral cartilage was very variable and showed no rise with age. But the striking

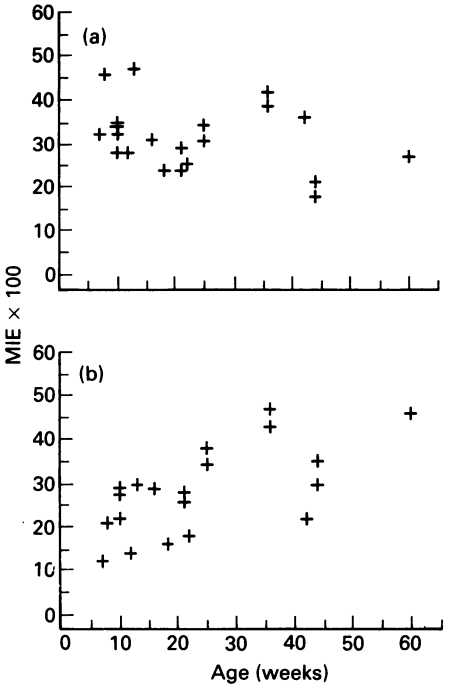


Fig. 3. Mean activity per cell of glucose-6-phosphate dehydrogenase (MIE × 100) of chondrocytes in (a) the medial and (b) the lateral cartilages of CBA mice of various ages.

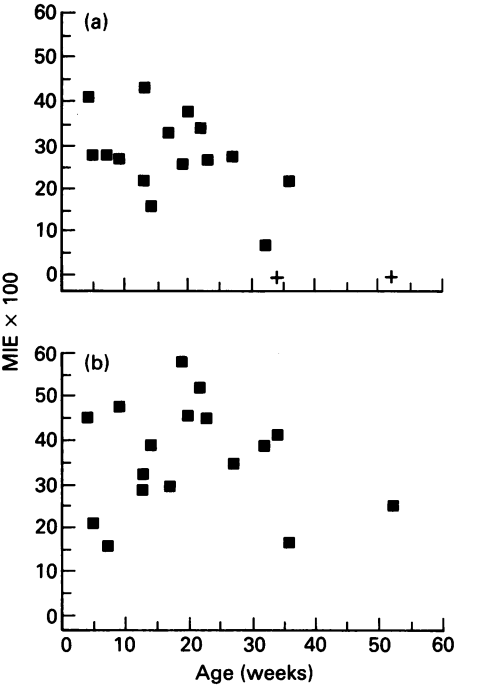


Fig. 4. Mean glucose-6-phosphate dehydrogenase activity (MIE × 100) of chondrocytes in (a) the medial and (b) the lateral cartilages of STR/ORT mice of various ages. The two medial cartilages, marked by +, were severely eroded.

feature was the decline with age in the cartilage of the medial tibial plateau (Fig. 4). In the STR mice the mean activity of the lateral tibial chondrocytes was higher than in the CBA mice: $38{\cdot}2 \pm 3{\cdot}2$ (±s.e.m.; $n = 14$ mice) units of activity as against $24{\cdot}1 \pm 1{\cdot}9$ ($n = 16$ mice) in the CBA mice (comparison of Figs 3 and 4). In contrast, the corresponding mean activity in the medial chondrocytes of the STR strain was $28{\cdot}4 \pm 2{\cdot}6$ as against $30{\cdot}9 \pm 1{\cdot}9$ in the CBA strain of mice. After 32 weeks of age, the medial cartilage of some STR mice was already eroded; the G6PD activity in the remaining chondrocytes was very low.

Because the activity in the STR mice could vary considerably (e.g. Fig. 4*b*) it seemed best to relate the activity in the potentially affected medial cartilage to that found in the lateral cartilage of the same mouse seeing that it is generally held that this cartilage is relatively free from osteoarthritic changes (Walton 1977a, b). Such an evaluation (Fig. 5) indicated a time-related decline in the proportionality between the G6PD activities in the medial as against the lateral cartilage over the first 32 weeks ($n = 14$; $r = -0{\cdot}638$; $0{\cdot}01 > P > 0{\cdot}001$).

A time-related decline in this proportionality was also found in the CBA mice but in these, the decline was due to elevation of the activity in the lateral cartilage; that of the medial cartilage remained relatively unchanged.

Lactate dehydrogenase (LDH) activity. In the CBA mice the LDH activity in the cells of the medial cartilage was higher than in those of the lateral cartilage, the proportionality

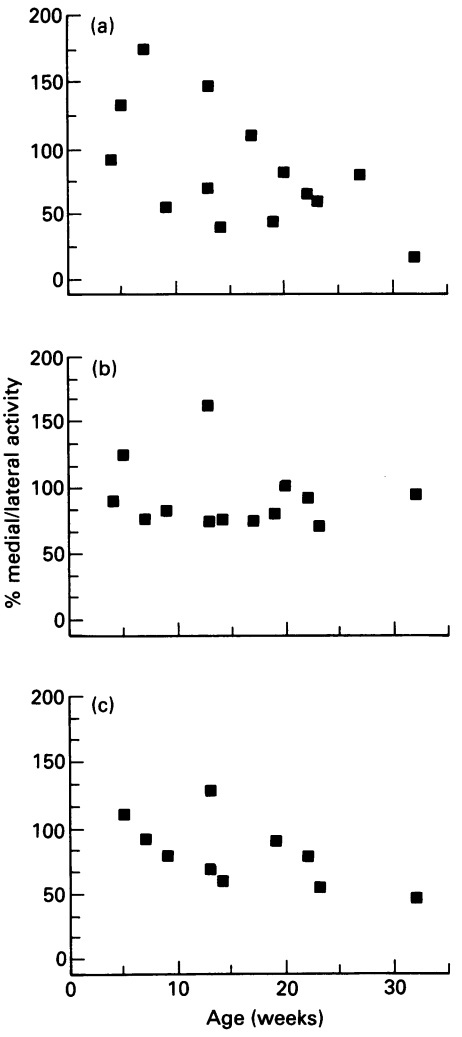


Fig. 5. Relative activities (expressed as a percentage) of chondrocytes of the medial as against the lateral cartilages, in STR/ORT mice of various ages up to 32 weeks: (*a*) glucose-6-phosphate dehydrogenase; (*b*) lactate dehydrogenase; (*c*) glyceraldehyde-3-phosphate dehydrogenase.

dropping from about 1·25:1 to close on 1:1 with age. In contrast, in the STR mice, the activity in the cells of the medial cartilage was generally lower than that of the lateral cartilage (Figs 2 and 5) but did not decline with age ($r=-0{\cdot}146$; $n=13$).

Glyceraldehyde-3-phosphate dehydrogenase (G3PD) activity. The results in both the lateral and medial cartilages were very similar in the STR/ORT mice of all ages of up to 40 weeks. Most specimens had activities of between 30 and 50 units (mean integrated extinction per cell in unit time). Of the ten mice, three specimens of lateral cartilage, and one of medial cartilage had higher values; lower values (20–30 units) were found in four medial cartilages. When these activities were calculated as a proportion of that in the medial as against that in the lateral cartilage, there was a decline with age ($r=-0{\cdot}619$; $0{\cdot}05>P>0{\cdot}02$) which was less striking than that found for results with the equivalent G6PD activities (Fig. 5).

Relative activities in the STR/ORT mice. G3PD is the central enzyme linking the Embden–Meyerhof and the pentose-phosphate pathways. Moreover, its activity was relatively constant for both cartilages. To overcome variations in total amount of the dehydrogenases that could occur in the cartilage of different mice, and to obtain some assessment of the glucose metabolism, it seemed reasonable to assess the G6PD and the LDH activities relative to the G3PD activity in each cartilage of each mouse.

In the lateral cartilages, there was good positive correlation ($r=0{\cdot}85$; $n=11$; $P=0{\cdot}001$) between the G6PD and the G3PD activities, as might have been expected. The correlation between these activities in the medial cartilage was less clear (Fig. 6; $r=0{\cdot}596$; $n=11$; $0{\cdot}05>P>0{\cdot}02$). The results in Fig. 6 seemed to fit to two populations: the first included two specimens that had activities of above 30 units of activity and two with activities of below 20 units of G6PD activity, which appeared to correlate with the equivalent G3PD activities; and the second in which the G6PD activity was relatively constant irrespective of the G3PD activity (from 20 to 60 units).

As regards LDH activity, in both the medial and lateral cartilages this correlated quantitatively with the G3PD activity indi-

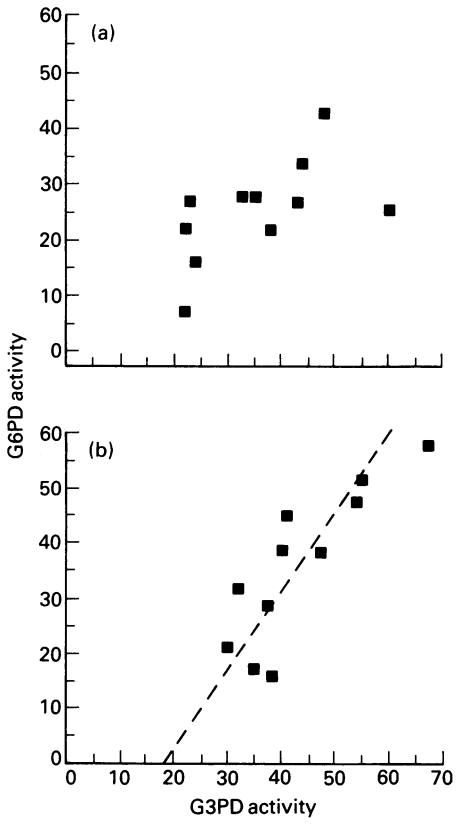


Fig. 6. Relationship between the activities (MIE × 100) of glucose-6-phosphate and of glyceraldehyde-3-phosphate dehydrogenase in (*a*) the medial and (*b*) the lateral cartilages of STR/ORT mice.

cating that this part of the Embden–Meyerhof pathway was normal in the medial cartilage ($r=0{\cdot}75$ for both cartilages; $0{\cdot}01>P>0{\cdot}001$).

Comparison of enzymatic activities and histology in the cartilages of CBA and STR/ORT mice. The CBA mice of ages up to 21 weeks showed no histological changes in the tibial plateau. In contrast, two out of the ten STR/ORT mice had some degree of fibrillation in the medial articular cartilage and some apparent loss of cellularity. In one, this occurred as early as at 9 weeks of age.

In the CBA mice of up to 30 weeks, the G6PD activity in the medial cartilage ($30{\cdot}9\pm1{\cdot}9$) was higher than that in the lateral cartilage ($24{\cdot}1\pm1{\cdot}9$). Thereafter, the activities in the two cartilages were relatively equivalent. In contrast, ten out of 14 STR/ORT mice showed depressed G6PD activity in the medial cartilage relative to that in the lateral cartilage in the same joint (Fig. 5).

Discussion

In the STR/ORT mice, enzymatic abnormalities were found long to precede obvious histological change. Of these enzymatic changes, the most consistent was decreased G6PD activity in the medial cartilage relative to that found in the lateral cartilage.

In the CBA mice, initially the G6PD activity in the medial cartilage was greater than that in the lateral cartilage. With increasing age, the activities in the two cartilages became more equivalent (Fig. 3). In the lateral cartilage of mice of the STR/ORT strain, the G6PD activity per cell was generally higher than in the equivalent CBA mice, and had a wider degree of variation between mice of the same age (Fig. 4*b*). In contrast to what pertained in the CBA mice, the G6PD activity in the medial cartilage of the STR/ORT mice was generally lower than that of the lateral cartilage (Fig. 4*a*). Thus the medial/lateral G6PD activity in each mouse showed a downward trend with age (Fig. 5; $0{\cdot}01>P>0{\cdot}001$). There was no equivalent trend with the activities of LDH and the decline of G3PD with age was less striking (Fig. 5).

In the STR/ORT mice up to and including 36 weeks of age, there was a good linear relationship between the LDH activity and that of G3PD. This pertained both to the medial and the lateral cartilages. A similar relationship was found between the G6PD and the G3PD activities in the lateral cartilages (Fig. 6). In this graph it is noticeable that the G3PD activity could be as high as 15–20 units even when the G6PD activity was zero. This level of G3PD activity may correspond to the amount of activity related

to the Embden–Meyerhof pathway alone. In contrast, this relationship between G6PD and G3PD activities was less clear cut in the medial cartilages (Fig. 6).

These results imply that in the medial cartilage, early in the develpment of any overt osteoarthritic change, and long before histological damage can be discerned, there is a metabolic defect involving the G6PD activity. This activity, and the related pentose-phosphate pathway, are essential for the normal functioning of cartilage. They provide pentose sugars for nucleic acid biosynthesis, other sugars for the formation of normal cartilage, and NADPH for many biosynthetic pathways (Wood 1986). Consequently, it seems fair to surmise that this defect of the G6PD activity may be a contributory factor in the development of osteoarthritis.

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